Viability of Cancer Cells Penetrating Tissues during Operations for Cancer of the Rectum*

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RECENTLY the operability rate for cancer of the rectum has increased 70 to 90 per cent and the mortality rate has lessened 3 to 8 per cent. However, according to statistical data, the results are not reassuring. Almost 50 per cent of patients live five or more years after operation; the remainder die of recurrence and metastasis. Local recurrence is especially common in the suture line on the anterior wall of the abdomen and in the perineum, in the anastomosis and in the colostomy stoma.

Reports in medical literature suggest that cancer cells are torn away from the primary lesion during operation and penetrate injured surfaces, causing metastases and recurrences. However, they provide no information regarding the viability of the cancer cells.

The objective of this investigation was to determine the viability of cancer cells and to discover their potency in the causation of recurrence and metastasis by examining the cells obtained in washings made at operations for cancer of the rectum. Our report is based on clinical observation of 198 patients with cancer of the rectum who underwent operations in the Scientific Laboratory on Proctology with the clinic of the Ministry of Health of the R.S.F.S.R.

During 130 operations for cancer of the rectum, 3,680 smears were made from washings and were studied cytologically. To determine the viability of the cancer cells, the following experiments were performed: 42 experiments were performed with cancer cells grown from washings, and heterotransplantation of the cells was made, from washings, to newborn white rats that had been treated with cortisone in 26 experiments performed on 470 rats.

Washings from surgical instruments, gauze packing used to mobilize the rectum, surgeons' gloves, hands of the surgeons and their assistants, the pelvic cavity (after removal of the rectum), the anastomosis, the serous surface, and portions of the resected rectum were studied.

Cancer cells were found in 160 (34.7 per cent) of 460 washings. Most were recovered from the surface of the rectum, instruments, gauze packing, and the surgeons' hands. A great number were found within the lumen of the rectum, about 10 cm from the lesion. Cancer cells were found in 50 per cent of abdominoperineal operations and low anterior resections. Conglomerate groups of closely adjoined cells of various sizes and forms were seen among large fields of erythrocytes and other blood elements. There were large nuclei which sometimes occupied the entire cell (Fig. 1).

A characteristic feature of the malignant cells was disturbance of nucleocytoplasmic correlation with enlarging nuclei, and "naked" nuclei without protoplasm were also seen (Fig. 2). Often we observed hyperchromic nuclei with others that were hypochromic. Mitotic change was a characteristic feature. All features that we have mentioned were observed in cancer cells obtained from the washings. If even one of the signs of malignant involvement of the

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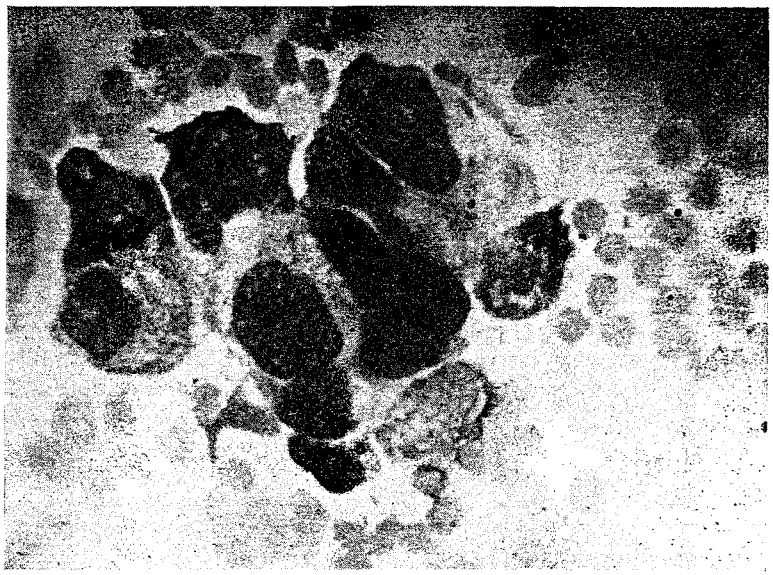

FIG. 1. Cancer cells in washings obtained from hands of surgeons.

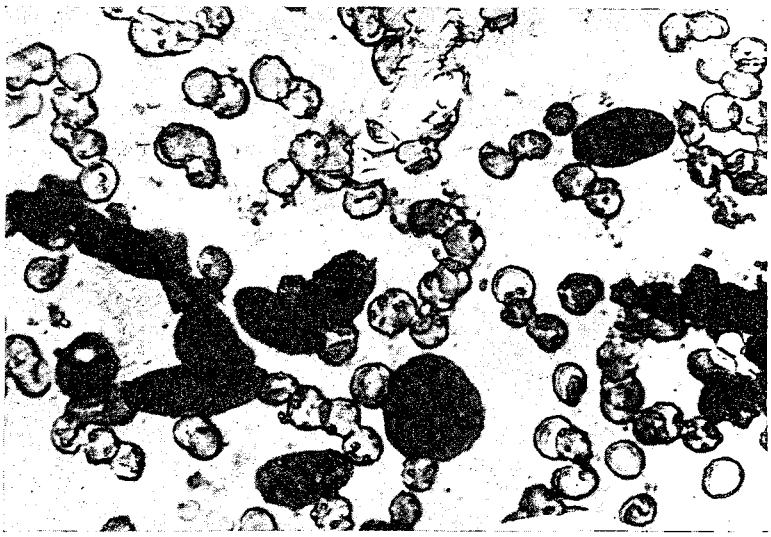

FIG. 2. "Naked" polymorphous nuclei.

cells was missing, results were considered doubtful. We have concluded that tearing away of cancer cells often takes place during an operation for cancer of the rectum. The cancer cells serve as a source of dissemination in the wound. Probably the reason for their dissemination is injury to the lesion while it is being removed.

There is no unanimity of views regarding viability and the possibility of inoculation of cancer cells that are torn away from the tumor. A. N. Rygick and S. A. Kholdin insist upon the implantation theory of metastasis, but other authors disclaim this theory.

Our study of follow-up results after operations for cancer of the rectum revealed that 25 of 117 patients (21.3 per cent)—the

only group in which cytologic analysis was made—had local recurrence after periods of one to three years. Usually, the recurrences involved the site of the operation, the anastomosis, scars in the anterior abdominal wall, perineal tissues adjacent to the colostomy stoma, and the anal ring after pull-through operations.

Implantation recurrences are characterized by the promptness of their appearance after operation, localization in typical areas, and the absence of lymphatic and hematogenous metastases.

Utilizing heterotransplantation and tissue cultures, we obtained direct proof of

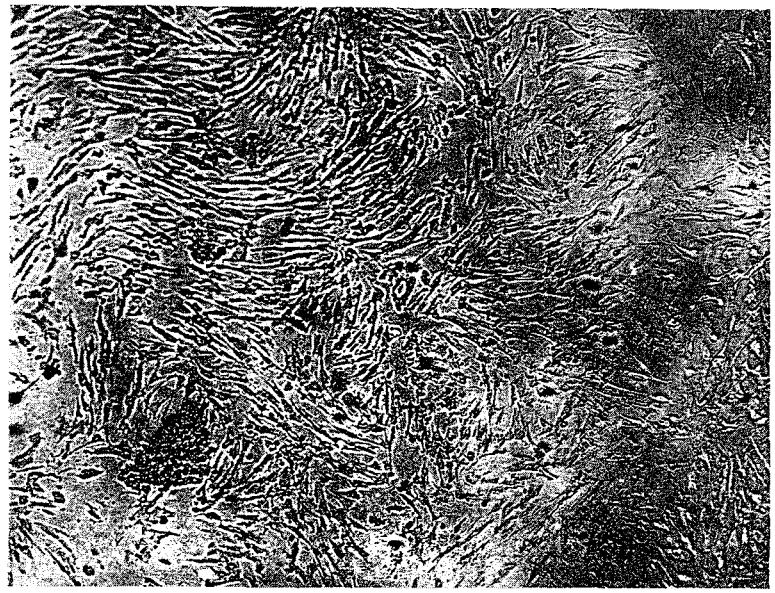

FIG. 3. Cancer cells after sowing.

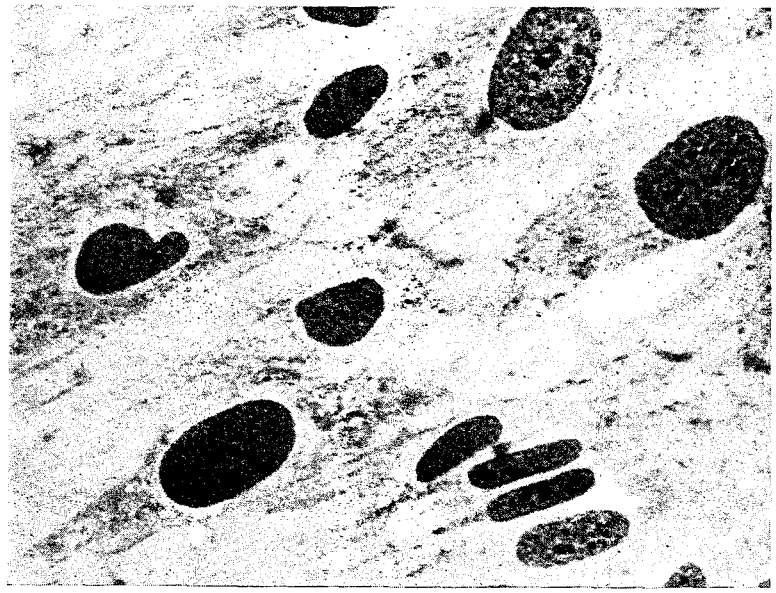

FIG. 4. Cancer cells in mixed culture.

the viability of cancer cells that are torn away from cancers.

We cultured cells obtained from washings on the artificial media "Eagle" and observed their reproduction at various periods of time. We grew diploid cancer cells, using diploid cells as a substrate.

In 15 (35.7 per cent) of 42 experiments results were positive. By coloring the washings with trypan blue, we found that the number of living cells fluctuated from 36.7 to 91.2 per cent. Fifteen to 20 minutes after sowing cancer cells on the surface of a monolayer culture of diploid cells, colonies and islands of 35 to 40 cells were formed (Fig. 3).

Tumor cells were distributed along the entire surfaces of the culture media by

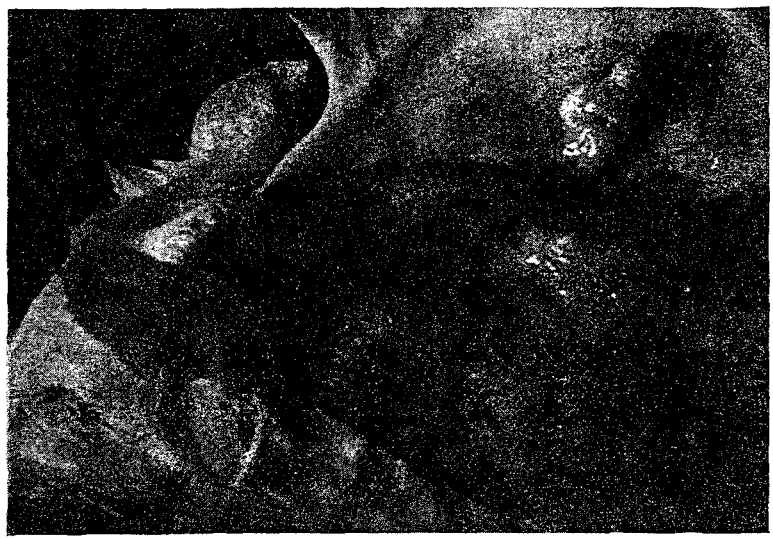

Fig. 5. Tumor growth after injection of cancer cells.

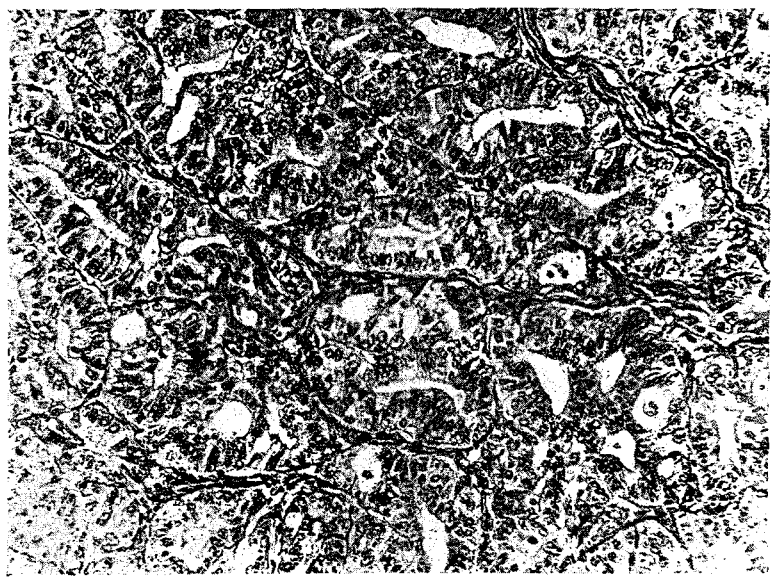

FIG. 6. Adenocarcinoma.

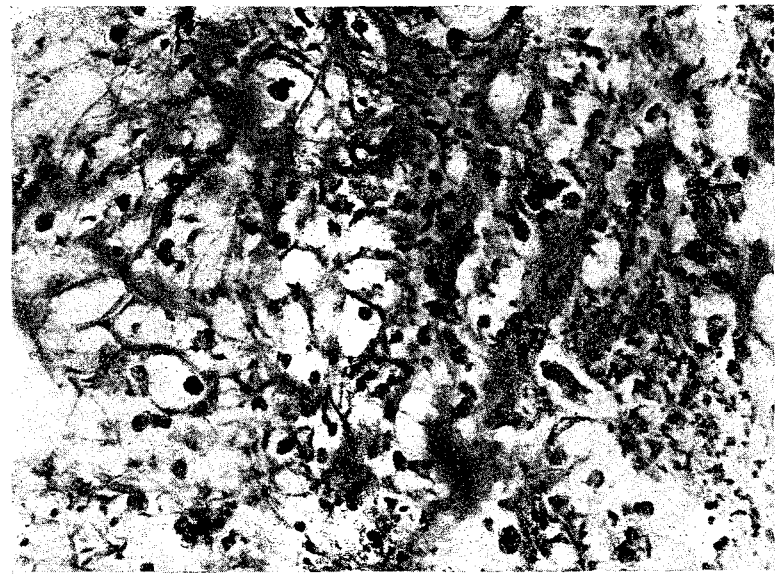

FIG. 7. Mucous cancer.

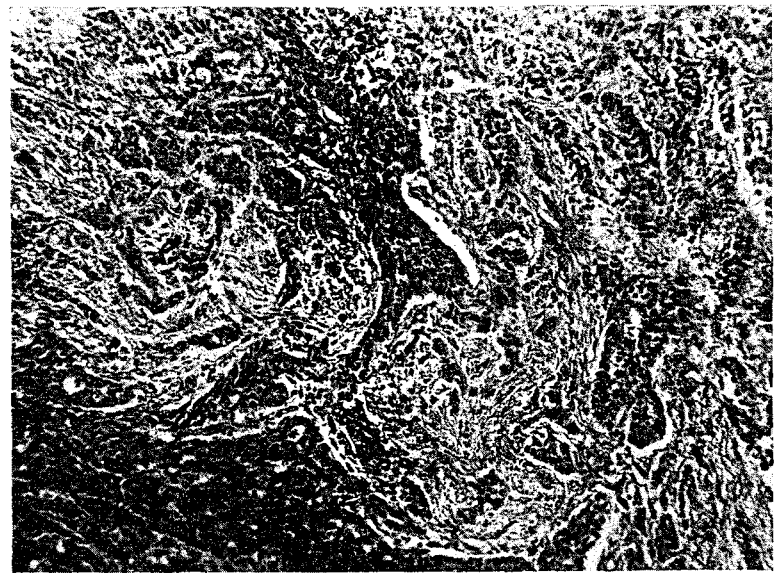

FIG. 8. Solid cancer.

groups, or one by one. They were transparent and refracted light well, and we observed polymorphism in their form and color. The structures of the cells growing in tissue culture corresponded to those of cells in the primary lesion. We also discovered that tumor cells can grow for periods as long as three months, going through two to 12 passages (Fig. 4).

The sediment after centrifugation of washings was introduced subcutaneously into newborn white rats treated with cortisone. This experiment was performed on 26 of 470 rats. In six of 41 rats, heterotransplantation was successful. Tumor nodules of various sizes and forms appeared on the back under the skin and in the subcutaneous cellular tissue (Fig. 5). Usu-

ally the tumors appeared two to four weeks after implantation, and histologically they corresponded with the primary tumor.

Many difficulties were encountered with bacterial flora in the washings. At the place of injection, abscesses appeared often and led to the death of the rats.

Two tests on each of 15 rats showed adenocarcinoma, and histologic analysis of heterotransplants gave positive results (Fig. 6). Three tests on each of 21 rats revealed mucosal cancer (Fig. 7) and one test on each of five rats revealed "solid" cancer (Fig. 8). Undoubtedly the tumors described arose from cells obtained from washings which were implanted in the subcutaneous cellular tissue of rats, and their structure corresponded to the cellular structure of the primary tumor.

Similar experiments using human beings cannot be performed. However, the fact that development in humans of metastatic tumors which are histologically identical to their primary tumors supports the point of view that cancer cells which are torn away from the primary lesion are viable. Effective methods of prophylaxis against implantation metastasis have not been developed.

We hope our investigation and the experimental data obtained may induce further study and that new and effective methods of prophylaxis against implantation metastasis and recurrence may be developed.

Summary

Conglomerate groups of cancer cells were found in cultures made from washings obtained from the hands of surgeons, surgical instruments, the pelvic cavity and the serous surface of the rectum in 34.7 per cent of cases.

Cancer cells were cultured by special methods on a monolayer of diploid cells in tissue culture. Fifteen to 20 minutes after culturing, the cancer cells, with their high potency for growing together with diploid cells, became attached to the substrate.

Cancer cells torn away from the primary lesion at operation preserve their ability to grow in tissue cultures.

Cancer cells showed activity in heterotransplants to newborn white rats; histologically, the tumors were identical to the primary lesions in humans.

As a result of experimental data concerning carcinogenic properties of cancer cells torn away from primary lesions, and according to clinical observations of local recurrences in the anastomotic zone, around the colostomy stoma and in postoperative scars, we believe that cancer cells can cause implantation metastases after operations performed for cancer of the rectum.