UTILIZATION OF LYMPHOCYTIC DNA BY THE UROTHELIUM OF URINARY BLADDER TUMORS

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The role of lymphocytic infiltration of tumors is not yet clear [10]. Meanwhile the possibility that many regenerating organs in mammals can utilize lymphocyte DNA has been demonstrated [3]. This is a manifestation of the "trophic" function of lymphocytes, i.e., their ability to penetrate into foci of active tissue proliferation, where they are destroyed, thus supplying DNA fragments required as "building material" by multiplying cells [1, 4]. The existence of a similar mechanism of maintenance of proliferative activity can therefore be postulated in relation to tumor tissue also. The aim of this investigation was to study this possibility.

EXPERIMENTAL METHOD

Four instillations, each consisting of 1.5 mg nitrosomethylurea in physiological saline (pH 6.5) were given through a catheter into the urinary bladder of 80 noninbred albino rats weighing initially 150-180 g. Of these rats 52 survived until the end of the experiment, and 35 of them developed urinary bladder tumors. At the 33rd week of the experiment 24 rats were anesthetized with thiopental and a rubber band was applied to the neck of the urinary bladder through a laparotomy incision in the lower third of the abdomen, and tied. In this way the urinary bladder and the tumor in it were completely isolated from the general circulation. Immediately after the rubber band had been tied the rats were given an intraperitoneal injection of [^{3}H]thymidine (specific activity 1540 TBq/mmole, in a dose of 1 μCi/g). The rubber band was removed 1.5 h after being applied and the circulation in the organ restored. The rats were killed by decapitation 20 and 30 min and 9, 12, 17, 36, and 72 h after removal of the band. Ten animals, not undergoing the operation and receiving [^{3}H]thymidine by the intraperitoneal injection in a dose of 1 μCi/g 1 h before sacrifice served as the control. The bladders were fixed in Carnoy's fluid and embedded in paraffin wax. The sections were coated with type M emulsion, exposed for 1 month at 4°C, developed, and stained with toluidine blue. The autoradiographic investigation was confined to epithelial bladder tumors. Cells with at least 6 grains of silver above the nucleus were taken to be labeled.

EXPERIMENTAL RESULTS

In neoplasms in the control animals the isotope was found in many epitheliocytes, in some of the fibroblasts, and among lymphocytes infiltrating the stroma and parenchyma of the tumors. Absence of a blood supply to the tumor for 1.5 h was not significantly reflected in their morphology, with the exception of temporary disturbances of the hemodynamics and lymph flow. Free [^{3}H]thymidine and its breakdown products are removed from the blood stream by about the end of the first hour after injection [2]. As would be expected, therefore, during the first 20 min after removal of the rubber band and restoration of the circulation in the bladder, cells labeled with [^{3}H]thymidine were found neither in the stroma nor in the parenchyma of the tumors (Table 1). However, by the 9th hour the first labeled cells began to be found in the tumor stroma; these were lymphocytes. Their number gradually increased to reach a maximum by the end of the first day after the operation (Fig. 1a); later their number fell appreciably. Labeled lymphocytes were found next to the epithelium, and some of them penetrated into it (Fig. 1b). By the 36th hour single labeled epitheliocytes appeared in the tumors and were usually

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TABLE 1. Number of Labeled Cells in Tumor at Different Times after Removal of Rubber Band from Neck of Bladder

Labeled cells	Time after removal of band, h						
	1/3	1/2	9	12	17	36	72
Stromal lymphocytes	—	—	+	++	++++	+++	++
Intraepithelial lymphocytes	—	—	—	—	+	+	++
Tumor epitheliocytes	—	—	—	—	—	+	+++

Legend. Number of labeled cells indicated by symbols ranging from absence (—) to presence in the largest number (++++).

located in the basal row of cells of the parenchymatous layer; the degree of labeling (the number of grains of silver) above the nucleus of an epitheliocyte and lymphocyte, moreover, was about the same (Fig. 1c). The number of labeled epithelial cells was appreciably increased 72 h after restoration of the blood flow in the bladder, and accordingly they were found not only in the basal row, but also in parabasal rows of the epithelium, although the number of grains of silver above their nuclei was reduced somewhat (dilution of the label). The isotope was detected only in certain areas of tumor tissue: in foci of squamous-cell metaplasia, which as the control data showed, take up [^{3}H]thymidine more intensively than the surrounding transitional-cell epithelium; moreover, the degree of labeling was greater (Fig. 1d). All these facts are evidence of active proliferation of labeled tumor cells.

Cells of the tumor urothelium thus did not begin to take up the isotope until 36 h after injection of [^{3}H]thymidine. This delay indicates reutilization of the label by the tumor epithelium. The question arises: What is the "donor" of this label; a number of candidates for this role can be suggested: 1) any dying labeled body cells whose nuclear material enters the blood stream and reaches the various organs and tissues including bladder tumors; 2) labeled cells penetrating into the stroma of the neoplasm from the blood, and disintegrating close to the tumor urothelium, which then takes up the labeled DNA; 3) lymphocytes labeled with [^{3}H]thymidine which are in direct contact with the urothelial tumor cells and transmit radioactive DNA to them.

If labeled DNA of cells which died (whole or in the form of low-molecular-weight precursors) were spread by the blood, simultaneous appearance of label would be expected both in lymphocytes and in epitheliocytes in the S-phase of the cell cycle. However, labeled lymphocytes appeared first of all in the tumor stroma, and only about 24 h later were epitheliocytes containing radioactive material first recorded (Table 1). On this basis, the first claimant for the role of donor of labeled DNA can be rejected. The second candidate likewise is unsuitable for performing this function, for the isotope was taken up by single epitheliocytes and not by groups of them, which would happen if the label spread via interstitial fluid. The degree of labeling above the nucleus of the lymphocyte and epitheliocyte 36 h after the operation also was similar, and this is not in harmony with views on reutilization of DNA of dying lymphocytes [5].

All the facts mentioned above can be satisfactorily explained only on the assumption of direct contact of a living lymphocyte, labeled with [^{3}H]thymidine, with a tumor cell and transmission of part of the lymphocytic DNA to that cell, as several workers have suggested [6]. These contacts are effected by intraepithelial and stromal lymphocytes, and the latter may be in close contact with pseudopodia of epitheliocytes which have penetrated through the basement membrane [9]. One of the results of these interactions is maintenance of the proliferative activity of the tumor urothelium. This is a manifestation of the trophic function of lymphocytes. Such intercellular contacts are of general biological character, for they take place

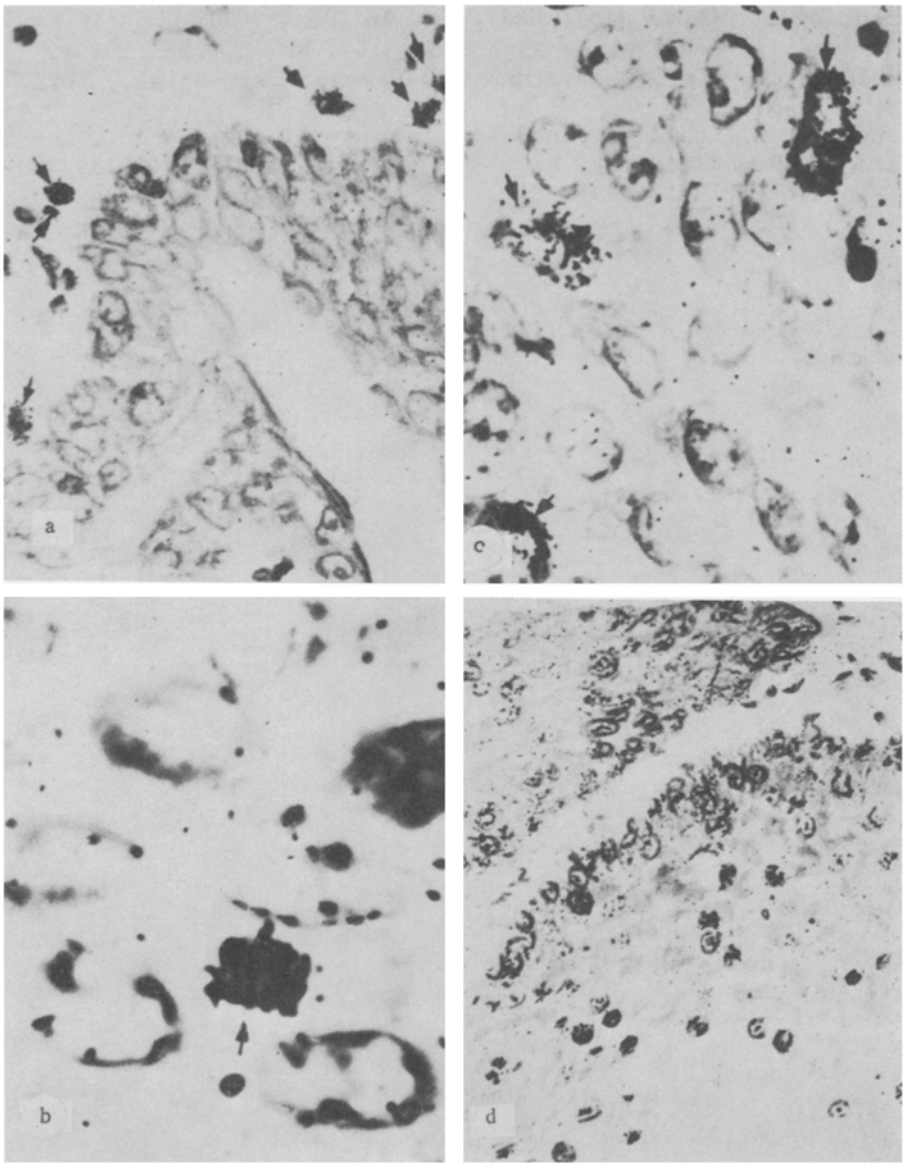

Fig. 1. Distribution of label in tumors at different times after removal of rubber band from bladder neck: a) lymphocyte labeled with [^{3}H]thymidine (arrows) located in tumor stroma. 17 h after removal of band from bladder neck. Toluidine blue, 500 ×; b) labeled lymphocyte (arrow) inside epithelial layer. 17 h after operation. Toluidine blue, 1250 ×; c) labeled tumor cells (arrows) arranged isolated from each other in basal row of parenchymatous layer. Toluidine blue. 36 h after removal of band from bladder neck, 1000 ×; d) focus of squamous-cell metaplasia of tumor contains labeled epithelial cells through thickness of layer. 72 h after operation. Toluidine blue, 280 ×.

under both normal and pathological conditions, and lymphocytes can evidently transmit their DNA not only to epitheliocytes, but also to connective-tissue cells [8] and muscle cells [7].

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