INTERCELLULAR COMMUNICATION AND TISSUE GROWTH

III. Thyroid Cancer

A. JAMAKOSMANOVIĆ and W. R. LOEWENSTEIN

From the Cell Physics Laboratory, Department of Physiology, College of Physicians and Surgeons of Columbia University, New York 10032

ABSTRACT

Intercellular communication was examined in normal and cancerous isolated thyroids with an intracellular electrical technique. The cells of normal thyroid (rat, mouse, hamster, man) communicate, within any given follicle, through permeable junctions. The cells of a wide variety of thyroid cancers (rat, hamster) do not communicate to any detectable degree and have resting membrane potentials lower than those of normal cells.

In an earlier study from this laboratory it was found by intracellular electrical techniques that cells of cancerous liver, unlike cells of normal liver, do not communicate through membrane junctions (Loewenstein and Kanno, 1966, 1967; Loewenstein and Penn, 1967). We have now extended this study to another set of tissues, normal and cancerous thyroids, and have found a similar situation there.

MATERIALS AND METHODS

Normal, goitrous, and adenomatous human thyroids were obtained from surgical patients anesthetized with Pentothal, halothane, nitrous oxide, or cyclopropane. A piece of the resected gland was cut off and placed in a chamber of Krebs' solution (pH 7.1) equilibrated with a mixture of 5% CO_2 and 95% air; electrical measurements were done in this chamber on cells well away from the cut edges. The measurements were begun within about 30 min after thyroidectomy (including ligature of blood vessels); the measurements themselves took generally about 30 min.

Normal thyroids of rat, mouse, and hamster were isolated intact together with portions of trachea and larynx. Thyroid tumors of rat and hamster were isolated generally when they had reached the size of a pea. Such tumors lacked necrotic regions and could be isolated intact. A few tumors were larger and contained necrotic and surgically damaged regions. Electrical measurements were then made well away from such regions. The normal thyroids or thyroid tumors were removed under ether anesthesia and set up for electrical measurements in Krebs' solution (23°–25°C). The removal and setting up took about 5 min; the electrical measurements took 5–45 min.

The thyroid tumors were of the following types: Fischer rat No. 1-1D, 1-C2, 1-3, 1-4, 1-5A, 1-5E, 1-6, 1-7, 1-8, 16-1, 16-4 (Wollman, 1963), and Syrian Golden Hamster No. Thy-2 (Fortner, J. G., Data unpublished). The rats with subcutaneous cancer transplants (flanks) were kindly provided by Dr. S. Wollman, National Cancer Institute, and the hamsters by Drs. J. G. Fortner and G. Sichuk, Sloan-Kettering Institute. The normal thyroids were taken from nonselected laboratory animals and from animals of the same genetic strain as the carriers of the cancerous material.

The technique for measuring intercellular communication was that described in the first paper of this series (Loewenstein and Kanno, 1967), except that one intracellular current source instead of two was used (Figs. 2 and 4, insets). Most measurements were made on cells at the surface of the organ or tumor, generally on an edge where the outlines of

follicles and cells could be seen in transillumination (Fig. 1). This facilitated microelectrode location. The intracellular position of electrodes was recognized by membrane potentials (at zero current) and resistances (Table I). In intraluminar positions the electrodes recorded potentials from 0 to −2 mv (see also Woodbury and Woodbury, 1963). In the experiments in which a single microelectrode connected to a bridge was used for both current passing and voltage recording (Fig. 4, inset), bridge balance was checked over the working range of the current before entry of the electrode into the cell and after retraction. Cases showing imbalance after retraction were rejected. The microelectrodes used for bridge measurements were specially selected for their stability of resistance in the range of current used; in general, only a very small percentage out of a batch of ordinary microelectrodes was of sufficient stability. All measurements were performed at temperatures of 23°–25°C.

The regions of tumor and goiter tissues on which electrical measurements were made were examined, in all cases, by standard histopathological techniques. Dr. R. Lattes, from the Department of Surgical Pathology, kindly provided the diagnoses.

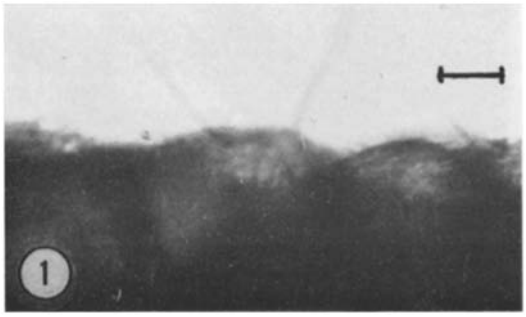

FIGURE 1 Photomicrograph of normal thyroid edge in transillumination (rat). Two microelectrodes are seen on center follicle. Calibration 50 μ.

RESULTS AND DISCUSSION

Normal Thyroid

Normal thyroid cells communicate through permeable junctions. Fig. 2 illustrates an example of intercellular communication in rat thyroid. An ion current (i) injected into a cell produces membrane voltages (v) detectable inside cells several junctions away. The main route of the current here is clearly from cell interior to cell interior; the corresponding voltages at extracellular locations in the tissue are much smaller than at intracellular ones and are of the same order as in the bathing medium (Fig. 2). This result is quite typical for normal thyroid of rat, man, mouse, and hamster. In all four thyroids, this kind of communication is detectable over distances encompassing several cells (Fig. 3), and the ratios of membrane voltage, v_{II}/v_I (*communication ratios*), are about 0.25 (Table I). Communication occurs between cells of a given follicle, but not between cells of different follicles. It was not feasible to record systematically from all cells in a follicle; but from partial mapping of the voltage fields, we got the impression that most cells within a given follicle, if not all, are interconnected. The cells of thyroid follicles are known to have junctions of the occluding kind (Farquhar and Palade, 1963).

Goitrous and Adenomatous Thyroids

Cells of human thyroid with diffuse toxic and nontoxic nodular goiter or with benign follicular adenoma communicate as well as those of normal thyroid. The communication ratios and the resistances between cell interior and exterior (*cell input resistance*) are indistinguishable from those of normal thyroid (Table I).

Cancerous Thyroid

Cells of thyroid cancer (rat, hamster) present an entirely different picture. These cells do not appear to communicate to any detectable degree (Fig. 4). The various cancer types examined differ widely in growth rate and differentiation. For instance, cells of rat cancer type 1-1C2 grow slowly and resemble normal thyroid cells relatively closely in follicular arrangement and in ability of making of thyroglobulin and thyroxin. On the other extreme are the fast growing and far deviated cells of rat cancer type 16-1 or 1-3 which are not arranged in follicles and do not make thyroglobulin or thyroxin (Wollman, 1963, and Personal communication). But regardless of their growth rates and the extents of morphological and functional deviation, all of the cancer types examined lacked

TABLE I

Electrical Parameters of Thyroid Cells

Thyroid preparation	Communication ratio	Cell input resistance	Resting potential*
	v_{II}/v_I	$10^6\ \Omega$	*mv*
Normal			
Rat	0.28 ± 0.03	2.70 ± 0.09 (14; 6)	47.2 ± 0.6 (30; 13)
Mouse	0.25 ± 0.05	2.10 ± 0.12 (7; 3)	48.5 ± 0.8 (16; 5)
Hamster	0.25 ± 0.07	3.04 ± 0.19 (7; 4)	38.5 ± 0.7 (19; 4)
Man	—	2.54 ± 0.13 (3; 1)	36.6 ± 1.7 (3; 1)
Goitrous			
Man			
Diffuse toxic	0.25 ± 0.06	2.75 ± 0.46 (8; 3)	39.2 ± 1.5 (12; 3)
Nodular nontoxic	—	2.78 ± 0.17 (12; 4)	40.0 ± 1.3 (12; 4)
Adenomatous (Benign)			
Man	—	2.83 ± 0.31 (6; 2)	37.3 ± 1.8 (6; 2)
Cancerous			
Rat			
Type 1-1C2	<0.002	12.40 ± 0.73 (10; 2)	31.7 ± 0.9 (10; 2)
1-1D	<0.002	26.50 ± 0.50 (14; 4)	26.8 ± 1.2 (14; 4)
1-8	<0.002	10.51 ± 0.60 (8; 2)	26.4 ± 1.7 (8; 2)
1-4	<0.002	28.2 ± 0.35 (35; 2)	26.6 ± 0.5 (35; 2)
1-5A	<0.002	26.2 ± 0.83 (11; 2)	24.4 ± 0.7 (11; 2)
1-5E	<0.002	26.50 ± 0.55 (12; 3)	23.6 ± 0.6 (12; 3)
1-6	<0.002	25.20 ± 0.46 (11; 2)	31.7 ± 0.8 (11; 2)
1-7	<0.002	27.40 ± 0.48 (11; 3)	21.7 ± 0.6 (11; 3)
16-1	<0.002	14.98 ± 0.86 (5; 1)	23.8 ± 1.4 (5; 1)
16-4	<0.002	11.80 ± 0.16 (23; 3)	25.9 ± 0.6 (23; 3)
1-3	<0.002	10.60 ± 0.39 (14; 2)	26.6 ± 0.8 (14; 2)
Hamster	<0.002	12.5 ± 0.94 (10; 4)	22.6 ± 0.5 (23; 4)

Mean values with their standard errors. The differences in communication ratio, input resistance, and resting potential between normal and cancerous thyroids of rat and hamster are in all cases significant at a level of significance of 0.001 or better, except for the difference in input resistance of hamster thyroid which has a significance level of 0.01. In parentheses are the number of cells followed by the number of different preparations on which the measurements were taken (together for the columns Communication ratio and Cell input resistance).

Method's limit of resolution of communication ratio, 0.002.

* Potential between cell interior and bathing medium at zero current, after subtraction of electrode tip junction potential (electrode tip junction potential in all cases <5mv).

detectable cellular communication; the communication ratios were below 0.002, the limit of resolution of the method, and the input resistances were 4–10 times higher than those in normal thyroid (Fig. 1; Table I). This picture is essentially the same as that in the four forms of liver cancer studied previously, and the same comments and conclusions concerning junctional membrane permeability and its possible role in tissue growth and differentiation apply here (Loewenstein and Kanno, 1967; Loewenstein, 1966, 1968).

An additional cautionary comment is in order. We know now that intercellular communication (in normal tissues) is labile. It appears to depend upon, among other factors, cell surface- and perijunctional insulation, cytoplasmic calcium concentrations, cell adhesion, and cellular metabolism. Alterations in any of these factors can rapidly lead to interruption of cellular communication (Loewenstein et al., 1967; Loewenstein, 1967; Politoff et al., 1967). The question thus arises whether the failure of communication found in cancer is caused by the isolation of the tissue or by the measuring procedure itself. Both the cancerous and normal tissues were isolated, and measurements were done in both under identical conditions. The question then becomes whether the cancerous tissue is more susceptible to interruption

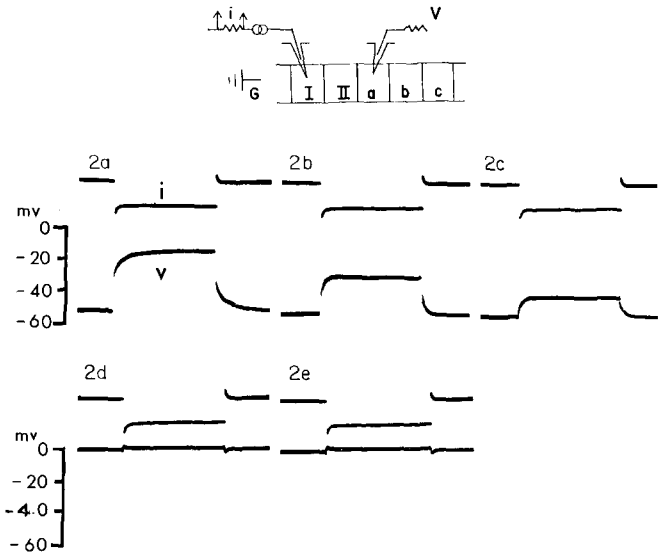

FIGURE 2 Intercellular communication in normal thyroid (rat). Current ($i = 5 \times 10^{-8}$ A; 40 msec duration) is passed between a microelectrode inside a cell and the bathing medium; the resulting changes in membrane voltage (v) are recorded with a roving microelectrode placed inside other cells of the same follicle at distances of about: *a*, 20; *b*, 30; *c*, 35 μ from the current passing electrode. *d*, the voltage recording electrode is in an extracellular location between cells, after pushing it from intracellular position *b* obliquely towards *a*. *e*, voltage-recording electrode in the bathing medium. Scale gives potential of recording electrode (including 1–2 mv electrode tip junction potential) with respect to bathing medium. *G*, ground electrode in bathing medium in common for current passing and voltage recording circuits.

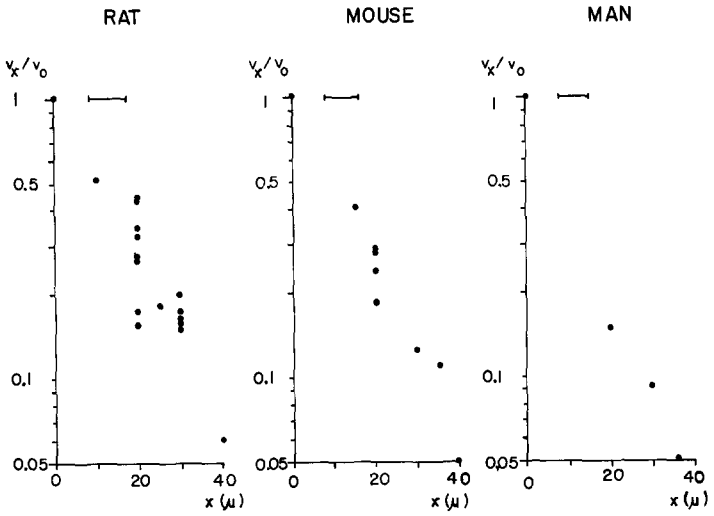

FIGURE 3 Spatial decrement of membrane voltage in normal thyroid of rat, mouse, and man. Abscissa: distance, x, from intracellular current source. Ordinate: intracellular voltage; v_0, voltage at x = 0. Data from various preparations; current 5–15 $\times$ 10^{-8} A. Horizontal bars on top of graphs subtend the mean cell diameter parallel to follicular surface; standard deviations of the mean diameter are below 12 percent in all three species.

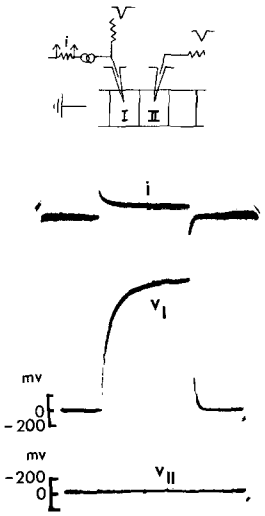

FIGURE 4 Lack of intercellular communication in cancerous thyroid (type 1-4, rat). Current ($i = 5.10^{-8}$ A; 40 msec duration) is passed from inside to outside of a cell (*I*) and the changes in membrane voltage are recorded in this cell and simultaneously in a contiguous cell (*II*). The electrode in cell *I*, connected to a balanced bridge circuit, serves for both current passing and voltage recording. The resting potentials of cells *I* and *II* are 25 and 26 mv, respectively. Oscilloscope trace polarities v_I and v_{II} are in opposite directions. In this particular experiment, the lower limit of detectable communication at maximal voltage gain (not illustrated) corresponded to a communication ratio of 10^{-4}, and even at this level there was no communication detectable. In most other measurements on cancerous material (Table I), the current used was at least one order of magnitude lower (and v_I correspondingly smaller) than in the example illustrated, and the limit of detectable communication was a ratio of 2×10^{-3}. Erratum: the two calibration scales should read -140 instead of -200.

of communication than the normal tissue. We can safely discard surface injury as a possible cause of interruption of communication, because the cell input resistance is high. (See Loewenstein and Kanno, 1967, for a discussion of this point.) But we have no way to ascertain whether, in regard to the other factors mentioned, the safety margins for communication in cancerous tissues are as high as in their normal counterparts.

The cells in any given type of rat and hamster cancer were quite uniform histologically. Thus the probability is high that the electrical measurements were all taken on cancer cells. In the case of the human goitrous and adenomatous materials, there is no certainty as to the nature of the cells on which the measurements were made; these materials contained also histologically normal cells, and no effort was made to correlate the electrical and histological results on a cell-to-cell basis.

Aside from communication, the cancerous cells differ from normal ones also in their membrane potentials at zero current (resting potentials). Cancerous thyroid cells have consistently lower resting potentials than do their normal counterparts (Table I). This result is in line with Latmanisowa's (1962) finding of subnormal resting potentials in epithelial cells of cancerous stomach and intestine.

The question which then immediately presents itself is whether there is a relationship between the alterations of cellular communication and resting potential. The present results provide no clue. But another set of results from this laboratory may possibly have some bearing; communication has just been found to depend on metabolic energy supply (Politoff et al., 1967). Since the resting potential also depends upon such energy supply, it may now perhaps be worthwhile to look for a metabolic defect as a common cause of the alterations of resting potential and communication in cancer cells (see Loewenstein, 1967, 1968).

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Dr. Jamakosmanović is a National Institutes of Health International Fellow in Physiology from the Department of Physiology, University of Sarajevo Medical School, Yugoslavia.

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