The Cytological Effect of Chemicals on Ascites Sarcomas, VI. The Action of γ-L-glutamyl Isonicotinic Acid Hydrazide on Living Tumor Cells of the Yoshida Sarcoma[1)]

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(With 5 Text-figures)

Quite recent biochemical studies carried out on amino acids in normal and malignant tissues of various animals have dealt with the solution of glutamine metabolism in the cells. The findings are of particular interest because there is significant difference in the level of glutamine and its requirement between tumor and normal tissue cells (Roberts *et al.* 1949, 1950, 1956, Eagle 1955). Especially, the findings with ascites tumors in mice and rats have strongly suggested that gultamine plays an essential part in the growth of tumors (Roberts *et al.* 1955, 1956). It was considered that the attempt selectively to disturb the protein metabolism of tumors may be feasible by interference with glutamine metabolism. This possibility led first to investigations into the inhibitory effects against the growth of microorganisms using a large series of compounds structurally related to glutamine and glutamic acid (Ayengar and Roberts 1953).

Subsequent experiments were made in order to examine the cytological effect of those compounds upon Yoshida sarcoma *in vivo* (Tanaka and Roberts, unpubuished). It was found that, of the compounds used, γ-L-glutamyl isonicotinic acid hydrazide (GINH) caused considerable damage to the tumor cells. The present study with the aid of a phase microscope deals with supplementary experiments on the inhibitory effects of this drug.

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Material and methods

The Yoshida sarcoma used here was maintained by inoculation of tumor ascites approximately 0.5 ml. into the Wistar rat by 5 days-interval transfers. Samples for the present experiment were obtained from 3 and 4 days old tumors.

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The procedure of chemical treatment is as follows : γ-L-glutamyl isonicotinic acid hydrazide (GINH) was dissolved in physiological saline at pH 7.2, or in the diluting fluid (Makino and Kanô 1955) at a concentration of 10 mg/cc. With the use of micropipettes, 0.01 ml. of either solution thus prepared was mixed promptly with 0.09 ml. of tumor ascites. Therefore, 1ml. of tumor was exposed to 1mg of GINH. Hanging drop preparations were made of a droplet of this mixture according to Makino and Nakahara's method (1953, 1955).

Control observations were run with the tumor cells by applying physiological saline or the diluting fluid alone. Several slides were prepared with both control and treated material at each time of the preparation.

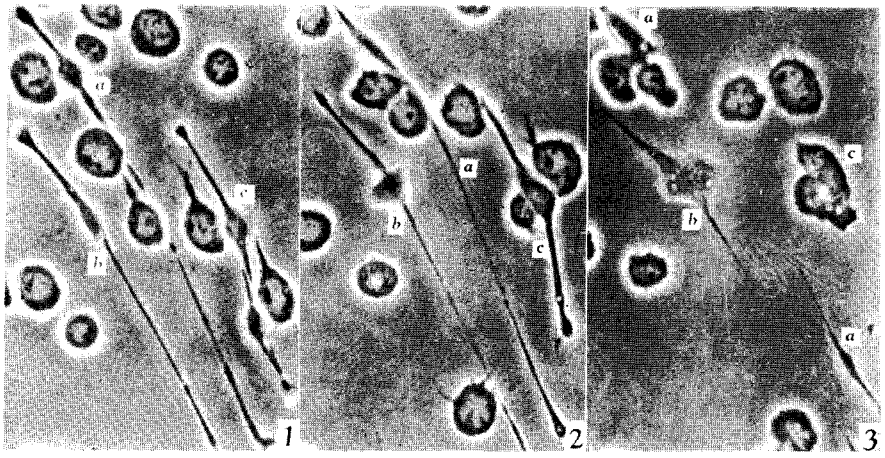
The observations were carried out using a phase microscope (Zeiss), in the optical combination of a dark-medium contrast 40× objective and a periplan 10× eye-piece. The experiment was run at a temperature of approximately 36°C.

Observations

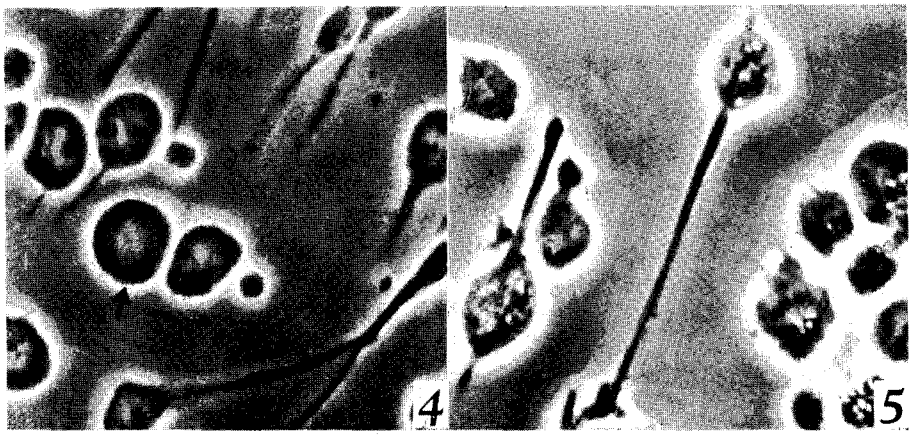
In the control sample, the Yoshida sarcoma cells were generally rounded and showed occasional or many tiny spicules in their cytoplasmic surface. The cytoplasm contained numerous mitochondria showing active movement; they varied in form from spheres to slender rods and filaments. The nucleoli were large and always prominent. Cells in division were often observable in either control media; no harmful effect was seen in the tumor cells for a period of 3 to 4 hours in the hanging-drop preparation.

Tumor ascites taken from the abdominal cavity on the 3rd or 4th day of transplantation contained such native cells of the peritoneal fluid as monocytes, macrophages and lymphocytes. The large monocytes or macrophages seem to be characterized by the following features : size smaller than tumor cells, the nucleus with a thin but well-defined nuclear membrane, small and indistinct nucleoli varying in number, and several highly refractile droplets and granules. Nevertheless they are actually very difficult to distinguish from tumor cells under the living condition.

The morphological changes of tumor cells in response to GINH were observed immediately after preparations, at least within 5 minutes. General pattern of response was an appearance of extremely long, fine, spine-like projections of the cytoplasm in the resting cells, though it was variable in frequency (Fig. 1). Most of the affected cells showed straight elongation of cytoplasmic projections in two opposite directions, as if they were polarized; the projections ran nearly parallel with the neighbouring cells. A few cells with three or more protrusions were occasionally visible. Their stretching became striking with time, and at 30 to 40 minutes after preparations were made such tumor cells usually reached over 100 μ in length. The ends of the projections were pointed or well-spread. A small number of mitochondria, vacuoles and some other granules were observed having migrated into the projections. After stretching, the projections began to degenerate (Figs. 2–3); top ends of the protrusions gradually rounded, and then they showed clumped vacuoles and mitochondria. In the meanwhile, the tumor



Figs. 1 to 3. Photomicrographs of Yoshida sarcoma cells under influence of γ-L-glutamyl isonicotinic acid hydrazide (GINH), taken by phase microscopy. 1-3 : the successive siries of a course of cell damage in response to GINH : *a*, *b* and *c* indicate three cells representing stretched-out, long cytoplasmic projections. *a* and *c* are tumor cells, and *b* is probably monocyte. × 800. 1, 10 minutes after exposure. 2, 38 minutes after exposure. The cells begin to degenerate. Top ends of cytoplasmic projections begin to round, and a few vacuoles are seen in the projection in *b* and *c* cells. 3, 60 minutes after exposure. The projections pull off from the cell body in *a* and *b* cells, while they shorten and withdraw into the main body in *c* cell. The vacuolization of cells becomes visible.



Figs. 4 to 5. Photomicrographs of Yoshida sarcoma cells under influence of γ-L-glutamyl isonicotinic acid hydrazide (GINH), taken by phase microscopy. 4 : 30 minutes after exposure. A cell at early metaphase indicated by arrow remains unaffected. × 1200. 5 : 90 minutes after exposure. Vacuolization of cells and degeneration of cytoplasmic projections are remarkable. × 1400.

cells underwent shrinkage of cytoplasm; their projections became shortened as a whole with time. Later, there sometimes followed the formation of two or three cytoplasmic pieces constricted off from the cell body in affected cells (Figs 1–3a), or the rounding up of cells by means of withdrawal of the projection into the main body in some others (Figs. 1–3c, 5). The small cytoplasmic pieces thus produced were often found to contain clumped vacuoles and mitochondria.

A group of cells with no cytoplasmic stretching, on the other hand, showed cytoplasmic blebbing in their surface resulting in the formation of cytoplasmic buds. As a whole, consequent reduction in size and irregularity in shape of the cell body were induced following the above cytoplasmic changes, with a remarkable vacuolization of the cytoplasm (Fig. 5). The disintegration of tumor cells thus proceeded and their death seemed to result, at two hours or more.

Mitotic tumor cells remained without being affected by GINH (Fig. 4) : no cytoplasmic projections as occurred in the resting cells were found. They proceeded to regular mitosis even in two hours or more after preparation.

An additional evidence to be mentioned here is a remarkable response of monocytes and macrophages to this chemical. These cells produced the cytoplasmic projections nearly similar in appearance to those of the tumor cells (Figs. 1–3b); their sensitivity to the drug appeared to be higher, since their projections showed remarkable elongation and remained *in situ* for a longer time than in the case of the tumor cells.

Discussion

Cytological studies on the effects upon tumors of a number of agents that are of potential interest in a program of experimental cancer chemotherapy have been carried out by many investigators. There are many chemicals which influence mitosis. Among the effective agents, some have produced spindle inhibition of the colchicine type and some others have exerted an effect similar to that of ionizing radiation causing chromosomal bridging and breakage. Certain antimetabolites are also regarded as chemotherapeutic agents. Biesele and his co-workers (1951 to 1955) have made extensive observations with purine and related substances that might intervene the nucleic acid metabolism in culture of various mammalian tissues; they have discovered that the effective agents among them caused mainly chromosome abnormalities. γ-L-glutamyl isonicotinic acid hydrazide in this study was found to be neither a mitotic poison nor a radiomimetic agent. This chemical exerted a selective toxicity on the cytoplasm of the resting cells but did not effect cells in mitosis. The most interesting evidence was that affected tumor cells developed long, straight cytoplasmic projections immediately after treatment. So far as the author is aware, there is no paper which reports a similar cytological feature. In the tissue culture material, there is a certain type of cells which show morphological features fairly identical with those of the GINH-treated tumor cells. However, it should be taken into consideration here that a reversible or temporary

transformation of healthy cells may occur due to the change of the culture media (Ludford 1951), or of the temperature (Nakahara 1955). Such a transfromation differs from the change which results from a chemical as shown in the present study.

The cells native to the peritoneal cavity occurring with the tumor ascites indicated also a marked response to GINH. This seems to suggest the considerable toxicity of this chemical.

In contrast to the results with GINH in this study, *in vivo* experiment has revealed that the Yoshida sarcoma cells undergo no particular cytoplasmic damages but show unusual stickiness of the chromosomes (Tanaka and Roberts, unpublished). Biochemical experiments have demonstrated a rapid uptake and utilization of glutamine by the Yoshida sarcoma cells (Roberts and Tanaka 1956). It is highly probable from these findings that GINH may act in a complicated action upon cells *in vivo*. Possibly the tumor cells are affected *in vitro* in some way other than an antimetabolic action. It remains questionable at present whether a rapid and characteristic response of the cytoplasm of the malignant and nonmalignant cells to GINH is primarily related to the glutamine metabolism inhibition.

Summary

Cytological effects of γ-L-glutamyl isonicotinic acid hydrazide (GINH) on living tumor cells of the Yoshida sarcoma were studied with a phase-contrast microscope.

GINH exerted a characteristic influence upon the cytoplasm of the resting cell, but not upon that of the cell nor of the nucleus in process of division. The affected cytoplasm of the resting cell shows long, spine-like projections and then undergoes disintegration. The monocytes or macrophages, native to the peritoneal cavity, present a similar morphological change in response to this chemical.

It is the author's great pleasure to dedicate this article to Professor Tohru Uchida in memory of his 60th birthday.

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