## Autoradiographic Analysis of Growth and Proliferation in Human Breast Cancers Using Tritium Thymidine in Vivo

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We have studied kinetics of cellular proliferation in human neoplasm in vivo, using tritiated thymidine local labeling technique. In this paper we report the result of analysis of growth and cell proliferation of two breast cancer cases.

Two cases with carcinoma simplex were used for this experiment, case I being a 49-year-old woman and case II a 38-38-year-old one. We assumed that the growth pattern in human cancer was exponential growth and the volumes of tumor were measured during the clinical course of these cases. The following doubling time $t_D$ and growth rate $\delta$ of cases were estimated with the use of the formula, $V=V_0e$ , $V=2V_0$ $V=V_0e$ , $t'=t_D$.

Case I;$t_D$ is equal to 169 days and equal to 0.0041.

Case II;$t_D$ is equal to 34.3 days and equal to 0.0202.

For kinetic analysis of cell proliferation, we repeated injections of tritiated thymidine into the tumor in vivo (in vivo local cumulative labeling method). This method was applied to these two cases. Results obtained in this experiment were as follows.

Case I; labeling index (LI) counted in flash label autordaiographs was 19%, at 4 days continuous labeling 53%, at 6 days 83%, and at 8 days 93%. In the same way, case II; LI at flash labeling was 18%, at the 5 days 59%, and at the 7 days 70%.

From these data a proliferation curve was drawn, from which generation time $t_G$ of case I was equal to 10.2 days and DNA synthetic time $t_S$ 2 days. These values were very similar to those of human squamous cell cancer (Ashihara 1967).

Suppose p is the probability for a cell to become the generative cell after the cell division and be q the probability for a cell to flow from the generative cell to CLLS. i.e. cells of limited life span, p and q can be calculated by $p=2\frac{t_G}{t_D}-1$ and q-1-p.

The pattern of the cancer cell proligeration is quantatively expressed as the decrease of q or increase of p. Thus, case I; $p=0.52$ and $q=0.48$, case II; $p=0.63$ and $q=0.57$.

## Autoradiographic Studies on Cytokinetics of Human Uterine Cancers in Solid form and in Ascitic Form Using $^3$H-Thymidine

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The generation time and the DNA synthetic time of the cell population of human uterine cancers, grown both in solid form and ascitic form, was studied by $^3$H-thymidine autoradiography in vivo. Materials we studied for kinetic analysis of cellular proliferation were three cases of uterine cancers. Case 1 is a 63-year-old woman, Case 2 is a 70-year-old woman, both bearing cervical cancers in the solid form. For the kinetic analysis of cell

proliferation of the solid tumor, we applied the "in vivo local cumulatice labeling method" using $^3$H-thymidine. Namely in the Case 1 or Case 2, one locus on the tumor received repeated injections of 20 $\mu$Ci of $^3$H-thymidine for 3 or 4 days every 24 hours and the another locus received single injection just before operation. Thus we obtained a specimen labeled continuously for 3 or 4 days and a flash labeled specimen. Case 3 is a 54-year-old woman bearing corpus cancer with numerous cancer cells proliferating in the ascites. For the cytokinetic analysis, 2 mCi of $^3$H-thymidine was injected intraperitoneally and after that smeared specimens were repeatedly obtained from the aspirated ascites during 6 days. After fixation, all the specimens were autoradiographed by dipping into SAKURA NR-M2 nuclear emulsion.

Results—Case 1; Labeling index (LI) counted in flash label autoradiographs was 24% and at 3 days of continuous labeling 64%. Plotting these labeling indices against time, a proliferation curve was drawn, from which generation time ($t_G$) =6.8 days and DNA synthetic time ($t_S$) =36 hours were estimated. Case 2; LI at flash labeling was 25% and at 4 days 76%. From the proliferation curve, $t_G$ was estimated at 7.8 days and $t_S$ at 44 hours. Case 3; Percentages of labeled metaphases among the mitoses was plotted against time and the labeled mitoses curve was drawn. From this graph, the following calculations were made; $t_G$=60 hours, $t_S$=18 hours, $t_2$ (mean) =9 hours, $t_M$=1.5−3.5 hours, and $t_1=t_G-(t_S+t_2+t_M)=31.5-29.5$ hours.

The generation time and the DNA synthetic time in the ascitic tumor were shorter than those in the solid tumor, but it is not clear whether difference is due to environmental changes or to the peculiarity of this particular strain of the carcinoma.

## Technique of $^3$H-Thymidine Labeling Via Artery and Analysis of Proliferation in Human Cancers in Vivo

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We performed $^3$H-thymidine autoradiographic studies on the cell proliferation of human tumors in vivo by the "arterial labeling method" as modified local flash labeling.

Stomachs of rabbits were used as an experimental material. Through the vinyl tube inserted into the left gastric artery, 100$\mu$Ci of isotonic $^3$H-thymidine diluted by 5% glucose solution mixed with several drops of patent blue was injected very slowly by an infusion pump. About 30 to 60 minutes after the infusion, the tissue was fixed and autoradiographs were prepared by dipping the sections into Sakura NR-M2 emulsion. Autoradiographically, the labeled cells of the normal rabbit stomach were found in the area between the surface epithelium and the glandular cell zone. Its labeling index was about 20–25% in various gastric areas. This result is almost equal to that obtained previously by means of the "local labeling method". From the experimantal study, the injected solution colored with the patent blue was found to spread on the entire mucosal surface of the stomach and on a part of the duodenum, but the distribution of the labeled cells were limited in much narrower area. The grain counts were decreased in the peripheral area.

This arterial labeling method was also applied to 8 cases of human gastric carcinomas. Three hundred microcurie of the isotonic solution of $^3$H-thymidine was slowly infused