

THE TRIALS IN THE CYTOLOGICAL DIAGNOSIS OF GASTRIC CANCER

PART II USE OF TETRACYCLINE FLUORESCENCE TECHNIQUE IN EXFOLIATIVE CYTOLOGY

by

YOSHIYUKI YOSHIDA

From the 2nd Surgical Division, Kyoto University Medical School
(Director: Prof. Dr. YASUMASA AOYAGI)
Received for publication Mar. 10, 1962

INTRODUCTION

The fact that tetracycline fluorescence may be detected in various tumors and bones following administration of tetracycline was reported by RALL¹⁾, MILCH²⁾ and McLEAY³⁾, and has been confirmed by many examiners. Present study was undertaken to investigate if the tetracycline fluorescence technique may aid the cytodiagnosis of gastric cancer and promise increased insight into cytochemical changes leading to malignancy.

CLINICAL MATERIALS AND METHODS

Four patients with gastric carcinoma and one patient of carcinosis from gastric carcinoma were subjected to this study.

A total doses of 6 000 mg of Cosa-tetracycn* (tetracycline with glucosamine) was administered perorally 11-12 hours prior to the examination of gastric exfoliative cytology or the collection of ascites or blood. Two thousand mg of Cosa-tetracycn daily was given to each patient in divided doses at 6 hour interval and was continued over 3 days. Gastrectomy was performed at 34 hours after the last administration of the drug.

Surgical materials and smears from gastric cytological specimens, ascites and capillary blood obtained from these patients were subjected to the fluoroscopic examination. Surgical materials were collected immediately after gastrectomy, and examined grossly under a 200 watt-ultraviolet light (3100~5400 Å), and then tissue sections were cut in

* Cosa-tetracycn, supplied by Pfizer Taito Co., Ltd., Tokyo, Japan.

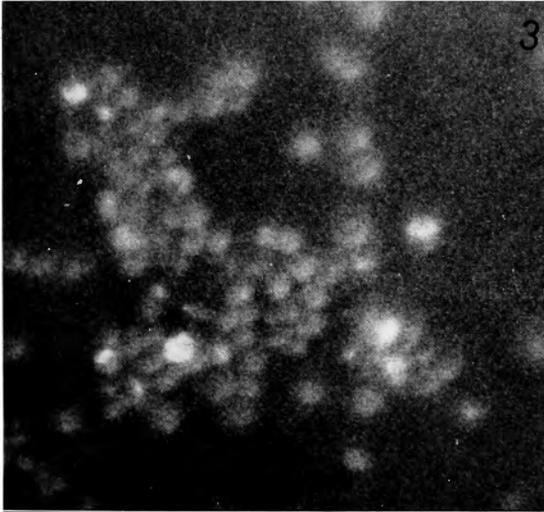
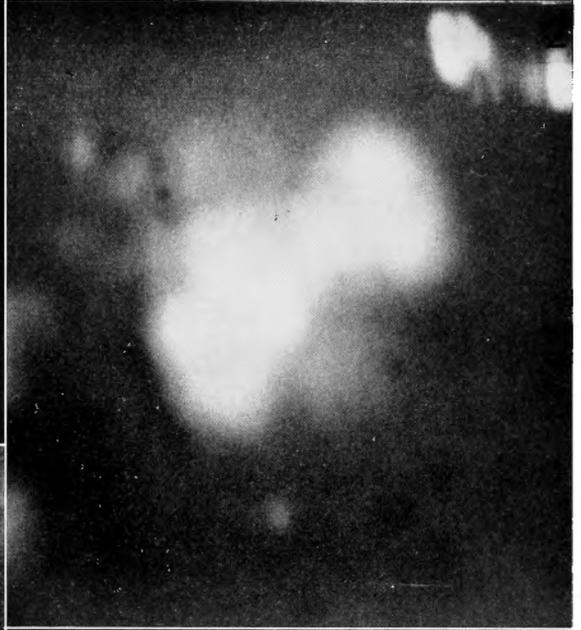
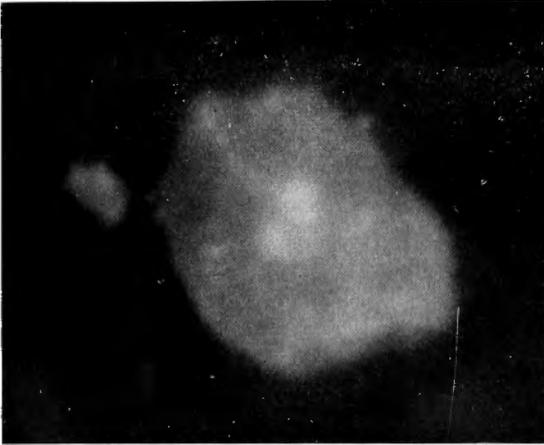
Fig. 1 : Normal squamous epithelial cell in the cytologic specimen showing the brilliant whitish yellow nucleus and faint yellow cytoplasm. $\times 400$.

Fig. 2 : Nuclei of cancer cells and inflammatory cells in the cytologic specimen showing the brilliant gold-yellow tetracycline fluorescence. $\times 400$.

Fig. 3 : Cancer cells in ascites from the patient with carcinomatous peritonitis showing the gold-yellow tetracycline fluorescence of various grades. $\times 80$.

Fig. 4 : The section of surgical material showing the brilliant white-bluish auto-fluorescence in the serosa membrane and connective tissue. $\times 80$.

Fig. 5 : The section of surgical material showing the brilliant white-bluish auto-fluorescence in the connective tissue and the vessel wall. $\times 80$.



a cryostat in about 15μ thick, mounted on glass slides in glycerin and covered with glass cover slips.

These smears and tissue sections were examined in dark field with a Carl Zeiss microscope equipped with a photographic attachment. The ultraviolet source was an Osram HBO 200 ultra high-pressure mercury lamp and the 3100 to 5400\AA lines were obtained with a piece of KG-1 filter and two of BG-12. Magnification of microscope was 8×10 or 40×10 . Photographs were taken with Fuji SSS film using 35 mm Olympus PM-6 and an OG-5 filter attached to the microscope. Exposure time varied from 10 seconds to 10 minutes.

RESULTS

Most of the cancer cell obtained in gastric exfoliative cytology and ascites yielded a tetracycline fluorescence. These cells showed brilliant gold yellow fluorescence in nuclei with intensely yellow granules and faint yellow fluorescence in cytoplasm. Normal squamous epithelial cells from the oesophageal tract showed the brilliant whitish yellow nuclei and the faint yellow cytoplasm. Leucocytes in blood and inflammatory cells in gastric cytological specimens showed brilliant yellow fluorescence in the nucleus and faint blue in the cytoplasm.

As to surgical specimens, macroscopically, normal mucosa surface presented diffuse yellow fluorescence, and tumor surface and metastased lymphonodes showed brilliant gold-yellow fluorescence.

And microscopically, strikingly brilliant white-bluish auto-fluorescence was appeared in serosa membrane, connective tissue and in the vessel wall. Normal cells of the gastric mucosa showed orange-yellow fluorescence, the most brilliant in body chief cells. Nuclei of carcinoma cells, inflammatory cells and fibroblasts presented a brilliant orange-yellow fluorescence. Very faint yellow fluorescence was noted in the cytoplasm of these cells.

Tetracycline fluorescence diminished rapidly during the observation under exposure of ultraviolet light. Therefore, the observation and photographing should be made as quickly as possible, otherwise correct findings would hardly be obtainable.

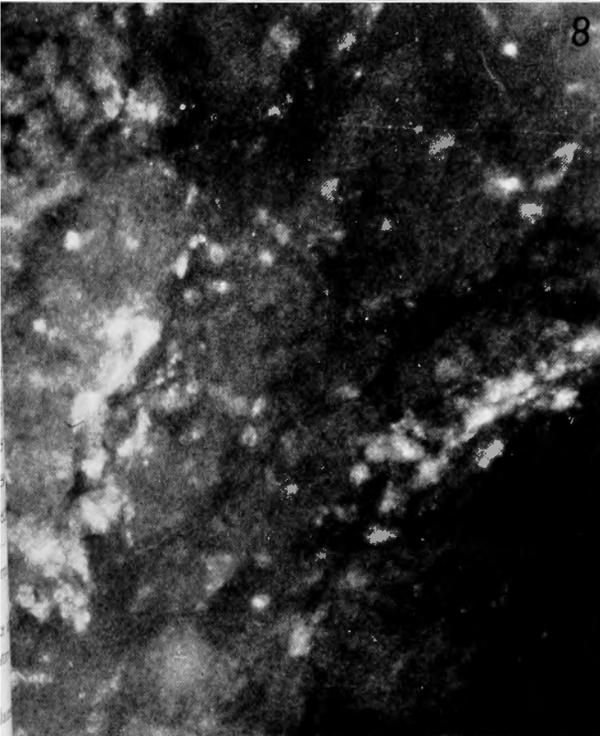
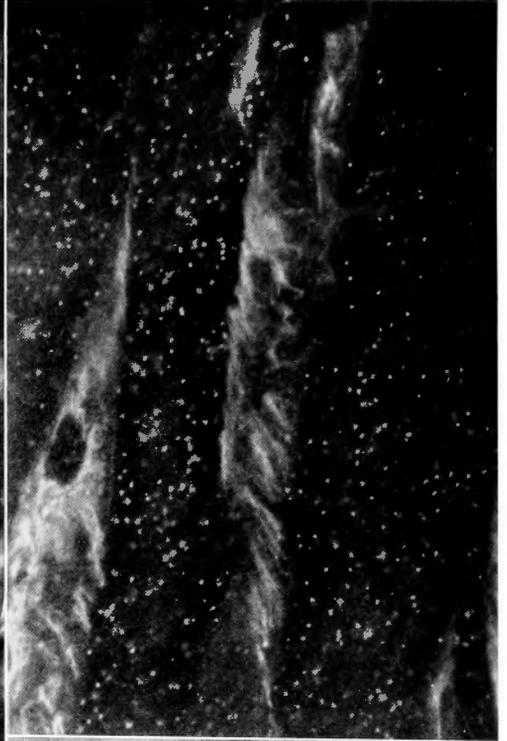
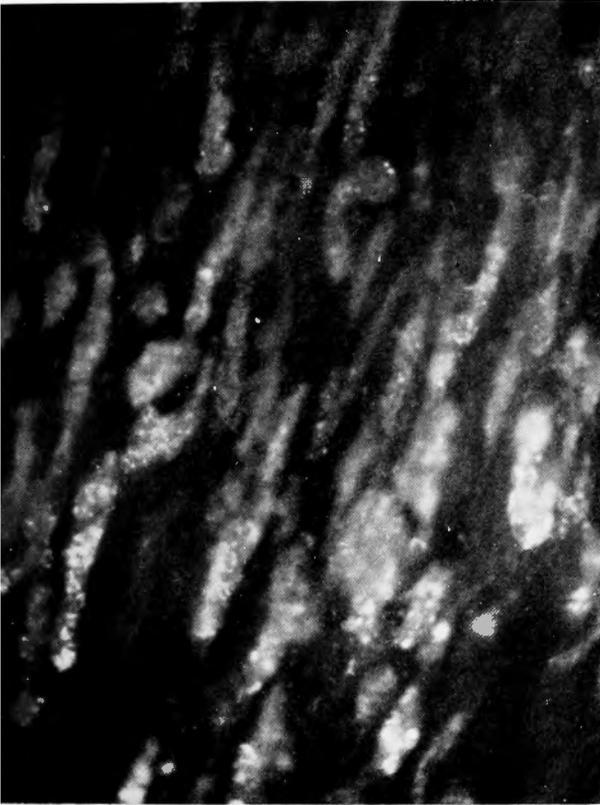
DISCUSSION

The attempts have been made by FRIEDMAN⁵⁾, MELLORS^{6,7)} and BERTALANFFY⁸⁾ to utilize the various fluorescent dyes to the identification of cancer cells in the fixed specimen in exfoliative cytology. Recently many investigators (CRAMER⁹⁾, STICH¹⁰⁾ and RASSMUSSEN-TAXDAL¹¹⁾) recognized the affinity of the various fluorescent dyes to the tumor tissue of the human being, and the mechanism of selective uptake of these drugs has been discussed. However, on account of several drawbacks, the fluorescence techniques

Fig. 6 : The section of surgical material showing the orange-yellow tetracycline fluorescence in normal cells of the gastric gland and the most brilliant fluorescence in body chief cells. $\times 80$.

Fig. 7 : The section of surgical material showing the brilliant white-bluish auto-fluorescence in the connective tissue and the brilliant gold-yellow tetracycline fluorescence in inflammatory cells scattered in the muscle layer. $\times 80$.

Fig. 8 and 9 : The section of surgical material showing the brilliant orange-yellow tetracycline fluorescence in cancer cells, inflammatory cells and fibroblasts in submucosal layer. $\times 80$ and $\times 400$.



with these drugs have not yet contributed to the diagnosis of cancer.

Tetracycline has the benefit of prominent concentration in malignant tumor tissue without giving toxicity to living body after administration of this drug. Therefore, its application to exfoliative cytology should be highly expected.

Appearance and persistence of fluorescent material in tumor tissue after tetracycline administration were demonstrated macroscopically by RALL et al. Author also observed the same phenomenon in gastric cancer specimens. Moreover, microfluorometric study of the gastric cytological specimens and the resected stomach after administration of this drug disclosed tetracycline fluorescence in malignant cells.

The precise mechanism whereby localization of induced fluorescence occurs remains unelucidated. MILCH et al explained this mechanism with formation of a complex with new bone matrix and calcium by tetracycline. McLEAY suggested that cancer tissue had the ability to bind tetracycline, possibly through an enzyme factor in metabolism; glutamate has been suspected. HELANDER & BÖTTIGER¹²⁾ found terramycin concentrated in the reticuloendothelial system and in the liver and kidney. In the present study, author observed prominent localization and persistence of tetracycline in cancer cells, inflammatory cells, fibroblasts and normal cells of the gastric mucosa, particularly in body chief cells, but not in areas adjacent to the blood vessel, suggesting that the localization of fluorescence in these cells is closely related to prosperous metabolism and not to blood supply.

For the screening test in the exfoliative cytology, this tetracycline fluorescence technique has drawbacks such as the necessity of long interval administration of the drug before the examination, and of speedy observation on account of rapid diminution of the fluorescence in the cytologic specimens. Furthermore, it is difficult to identify the malignant cells, because the identification of the cell is to be made only through the intensity and dimension of the fluorescence, and because the fine nuclear structure is hard to obtain by this procedure. Therefore, it is not adequate to use this technique as the routine screening test of exfoliative cytology.

If a sufficient saturation of the drug in tumor cells could be obtained in a short period, and disparity between malignant cells and the other cells in their fluorescences could be increased, this technique would obtain the position of excellent screening test in exfoliative cytology.

SUMMARY

The investigation of tetracycline fluorescence was made with the gastric cytological specimens, ascites and surgical materials obtained from the patient with gastric carcinoma or carcinosis of gastric carcinoma following the administration of Cosa-tetracycline.

Malignant cells in these specimens showed very intense tetracycline fluorescence, but normal cells of the gastric mucosa, particularly body chief cells, inflammatory cells and fibroblasts also showed fairly intense fluorescence. Moreover, the fine cellular structure is not presented by this method, so that it is difficult to distinguish malignant cells from the other cells.

This fact and complicated procedure of this examination may not permit the

application of tetracycline fluorescence technique as the routine screening test in exfoliative cytology.

The author wishes to express sincere gratitude to Dr. RYO INOUE, an instructor of the Surgical Department, for many valuable suggestions and helpful discussions in this investigation. The author's grateful thanks are due to Dr. KITASU SUZUE, Prof. of the Pathological Division of Kyoto University, for lending the fluoromicroscope and the cryostat, and also due to Dr. MASAHISA KYOCOKU and Dr. SHIGEHISA AOKI in this division, for their kind guidances in the course of the work.

References

- 1) Rall, D. P., Loo, T. L., Lane, M. & Kelly, M. G. . Appearance and persistence of fluorescent material in tumor tissue after tetracycline administration. J. Nat. Cancer Inst., **19**, 79, 1957.
 - 2) Milch, R. A., Rall, D. P. & Tobid, J. E. . Bone localization of the tetracyclines. J. Nat. Cancer Inst., **19**, 87, 1957.
 - 3) McLeay, J. F. . The use of systemic tetracyclines and ultraviolet in cancer detection. Am. J. Surg., **96**, 415, 1958.
 - 4) * Kozukue, H. & Amaki, I. : Practical application of microfluorometry. Rinsho-byori, **6**, 105, 1958.
 - 5) Friedman, H. P. : The use of ultraviolet light and fluorescent dyes in the detection of uterine cancer by vaginal smear. Am. J. Obst. & Gynec., **59**, 852, 1950.
 - 6) Mellors, R. C. & Silver, R. . A microfluorometric scanner for the differential detection of cells. Science, **114**, 356, 1951.
 - 7) Mellors, R. C., Glassman, A. & Papanicolaou, G. N. . A microfluorometric scanning method for the detection of cancer cells in smears of exfoliated cells. Cancer, **5**, 458, 1952.
 - 8) Bertalanffy, L., Masin, F. & Masin, M. . Use of acridine-orange fluorescence technique in exfoliative cytology. Science, **124**, 1024, 1956.
 - 9) Cramer, H. u. Brilmayer, C. : Verwendbarkeit von Fluorescein-Natrium und Atebrin in der Tumordiagnostik. Münch. Med. Wschr., **45**, 2233, 1951.
 - 10) Stich, W. . Biochemische Krebsdiagnostik. Med. Klin., **38**, 1522, 1954.
 - 11) Rassmussen-Taxdal, D. S., Ward, G. E. & Figge, F. H. J. . Fluorescence of human lymphatic and cancer tissues following high doses of intravenous hematoporphyrin. Cancer, **8**, 78, 1955.
 - 12) Helander, S. & Böttiger, L. E. . On the distribution of terramycin in different tissues. Acta med. scandinav., **147**, 71, 1953.
- (* Written in Japanese)

和 文 抄 録

胃癌細胞診の診断適中率向上を目的とする二、三の試み 第二編 胃癌細胞診へのテトラサイクリン蛍光法の応用

京都大学医学部外科学教室第二講座 (指導: 青柳安誠教授)

吉 田 良 行

4例の胃癌及び1例の胃癌による癌性腹膜炎の患者に、コサ・テトラシン(グルコサミン添加テトラサイクリン)を経口的に合計6,000mgを3日間に亘つて投与し、一定時間後、これらの患者から採取した胃細胞診試料、腹水及び手術材料のテトラサイクリン蛍光について、蛍光顕微鏡学的観察を行なった。

これらの試料中の癌細胞は極めて強いテトラサイクリン蛍光を発することを認めた。併し、正常粘膜細胞特に主細胞、炎症性細胞並びに線維芽細胞もまた可成り強い蛍光を示したし、さらに、この蛍光を以てしては、細胞、特に核の微細構造が得られなかつたので、癌細胞を判別するのが困難であつた。