Tetracycline fluorescence and cytological procedures compared for the detection of malignancy

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EDITORIAL SYNOPSIS  The usefulness of the tetracycline fluorescence test for gastric carcinoma is limited by the proportion of false positive results obtained. This paper suggests a method by which this proportion may be reduced, and also that the test is particularly useful in the diagnosis of pancreatic carcinoma.

In recent years many publications have appeared on the use of tetracycline fluorescence for detecting malignancy, especially of the stomach (Klinger and Katz, 1961; Sandlow, Allen, and Necheles, 1963; Berk and Kantor, 1962; Berk and Kantor, 1963; Sherman, Chryssanthou, Sukoff, Mininberg, Beckman, and Weingarten, 1963; Vassar, Saunders, and Culling, 1960; Ackerman and McFee, 1963; Berk, 1963; Sandlow and Necheles, 1964a). While most of these reports have been favourable, some have been discouraging (Vassar et al., 1960; Ackerman and McFee, 1963; Rugtveit and Hope, 1964). Recently a report has appeared (Cummins, Compertz, and Kier, 1964) comparing the results of tetracycline fluorescence and gastric cytology which indicated that while tetracycline fluorescence was not of much value, cytology was of much value either. When combined, the results were more encouraging, but the authors did not dwell on this point.

In reviewing the tetracycline fluorescence test for malignancy performed in our own laboratory, we have had encouraging results with 90% accuracy in stomach washings and over 80% accuracy in duodenal drainage. Comparison of our results with those obtained by cytology on the same patients indicates clearly that in our laboratory and with our techniques, the tetracycline fluorescence test is more accurate than cytology.

MATERIALS AND METHODS

All patients in whom the diagnosis of malignancy was confirmed, and in whom both cytology and tetracycline fluorescence tests were performed, are included in this report.

Our procedure has already been described in detail (Sandlow et al., 1963). Tetracycline, 500 mg., was administered orally four times a day for two days. If gastric retention was suspected, tetracycline, 250 mg., was administered intramuscularly twice a day for two days. After the fluid was obtained, it was kept cold and a sufficient amount of 5% sodium bicarbonate solution added to bring the pH up to 8.3. The aspirate was centrifuged for 10 minutes in a refrigerated centrifuge at approximately 3,000 r.p.m. The supernatant was decanted and the precipitate spread thinly over Whatman no. 3 filter paper and allowed to dry in the dark. The dry specimen was examined immediately and 24 hours later in a dark box with ultra-violet light of 3,600 Angstroms by two trained observers, independently and before the diagnosis had been established. The smallest pinpoint of bright yellow fluorescence was considered positive for malignancy. In the first 130 gastric washings, fluorescence examinations were made 36 hours after the last dose of tetracycline. In the last 130 washings, whenever yellow fluorescence was noted at 36 hours, the washing was repeated 24 hours later (60 hours after tetracycline). Only if fluorescence was noted in the 60-hour specimen, were these washings called positive.

GASTRIC WASHINGS  Two-hundred and sixty patients had washings done for both cytology and tetracycline fluorescence tests. The washings for the two tests were done independently by our department and by a separate cytology laboratory at Michael Reese Hospital. The method used by the cytology laboratory for collecting gastric specimens was a modification of Raskin's technique (Raskin, Kirsner, and Palmer, 1958) and staining and examination were according to Papanicolaou's method. Forty-five of the 260 patients had gastric malignancy proven by surgery, biopsy, or necropsy. In the remaining 215 patients no malignancy was found. In this group, gastric ulcers were considered benign when complete healing had occurred as shown radiologically and/or by gastroscopy, or when surgery and histology had revealed absence of malignancy. The remainder of the patients had normal upper gastrointestinal x-ray findings in addition to normal findings by gastroscopy.
SECRETIN TESTS One hundred patients have had secretin tests in which both cytology and tetracycline fluorescence tests were done on the duodenal aspirate. This series included 15 patients who were proven to have pancreatic carcinoma by surgery and/or necropsy, and 85 patients in whom no malignant lesion presented itself within an average follow-up of one year.

Secretin tests were done 60 hours after tetracycline had been given, as it is difficult to repeat the test on consecutive days.

RESULTS

GASTRIC WASHINGS The 260 patients included in this series constitute the majority of patients in whom gastric washings have been studied with tetracycline fluorescence. Of the 45 with gastric malignancy, cytology was positive in 31 and negative in 14. The tetracycline fluorescence test was positive in 42 and negative in three. Benign lesions, including controls previously reported, such as gastric ulcers, pernicious anaemia, achlorhydria, and gastritis, made up the remaining 215 patients; in these, cytology was negative in 212 washings and positive in only three. Tetracycline fluorescence tests were negative in 197 and positive in 18. Follow-up in the latter group has revealed no malignancy within a period of one to two years. Since we have started repeating positive tests after 60 hours the number of false positives has decreased significantly.

Figure 1 shows that among malignant gastric lesions cytology had 69% accuracy, whereas tetracycline fluorescence tests had 93.5% accuracy. In benign lesions cytology had 99% accuracy while tetracycline fluorescence had 92%. In summary, in only 7% of the cases did tetracycline fluorescence reveal false negative results, whereas cytology had 30% false negative results. At the same time tetracycline fluorescence had 8% false positive results and cytology only 1%.

SECRETIN TESTS In the patients proved to have carcinoma involving the pancreas, cytology was negative in all 15, whereas tetracycline fluorescence tests were positive in 13 and negative in two. Eighty-five patients had benign lesions and cytology was negative in all of them, whereas tetracycline tests were negative in 82 and positive in three. Thus, tetracycline fluorescence was 80% accurate for malignancy but cytology was 0% accurate. For the benign lesions, tetracycline fluorescence tests had 96% accuracy while cytology had 100% accuracy, since in no case was positive cytology found.

DISCUSSION

We have previously reviewed the pertinent literature of tetracycline fluorescence (Sandlow and Necheles, 1964a; Sandlow and Necheles, 1964b), but some recent studies should be mentioned. The application of tetracycline fluorescence as a diagnostic procedure has been widened to include detecting malignant tumours of the stomach, pancreas, and lung, malignant cells in ascitic fluid and pleural exudate, and most recently tumours of the urinary bladder. Berk (1964) now reports 86% accuracy in patients with gastric carcinoma and 97% in benign gastric lesions. The most interesting work is that of Busch and his associates for diagnosing carcinoma of the bladder with an ultra-violet fibre-optic cystoscope (Busch, Whitmore, and Esquivel, 1965). We have constructed a similar instrument for examination of the stomach.

Kaplan and DeLosReys (1965) have confirmed our earlier work on the usefulness of the tetracycline fluorescence test for pancreatic carcinoma. They report 12 patients with carcinoma who all had positive tests, while eight patients without carcinoma were all negative.
Lester, Sevelius, Jimmerson, and Colmore (1965) have studied 30 patients by tetracycline fluorescence tests for bronchogenic carcinoma. Fifteen had cancer and 15 had acute or chronic pulmonary disease. Of the 15 patients with carcinoma, 12 had positive gastric washings while only seven had positive sputum cytology; 13 of the 15 controls were negative for fluorescence. The authors felt that this technique was superior to that of cytology.

Not all studies are as favourable. Rugtveit and Hope (1964) reported that 17 of 18 patients with gastric carcinoma had positive fluorescence tests, but nine out of 19 patients with benign lesions also had positive tests. In most studies where results were not satisfactory, techniques varied greatly from those used by investigators who obtained satisfactory results. The patients of Rugtveit and Hope received only 3 g. of tetracycline, and most received it intravenously; the aspirations were done at varying times from 24 to 96 hours after the dose of tetracycline. Their ultra-violet light source also seems to have been different from ours.

Much of the controversy over the usefulness of this procedure should be resolved if we knew more about the binding of tetracycline within cells. Some work has been done on a relationship of the binding of tetracycline to calcium (Finerman and Milch, 1963). Tetracycline localization in the cells of certain malignant tumours may be attributed to the intracellular presence of hydroxyapatite on ribosomal particles. In powdered bone, both calcium phosphate and calcium carbonate bind tetracycline. When the calcium was removed by E.D.T.A., no binding occurred. Finerman and Milch (1963) feel that persistence of tetracycline fluorescence in biological tissue is probably related to its calcium content.

Other investigators (Riley, 1963) have transplanted adenocarcinoma of the human lung into cheek pouches of Syrian hamsters. Following administration of tetracycline, fluorescence was noted in the healthy tumour tissue, but not in necrotic or infected regions. Calcium was found in the cancer cells by staining techniques and micro-radiography; gross calcification was never seen. E.D.T.A. caused a loss of the fluorescence.

In our own laboratory, recent work on S-180 tumour mice has shown that, grossly, there is no question that tetracycline is retained in tumour tissue, while necrotic areas did not contain any fluorescence. Dr. M. Berg, in our laboratory, is examining the microscopic aspect of this problem; he has demonstrated that fluorescence is found in the cytoplasm of 'healthy' tumour cells. Berk (1964) comments that in his work on mouse gastric carcinoma, fluorescence has been noted in the non-necrotic areas of tumours.

TECHNICAL PROBLEMS Although the technical aspect of the fluorescence test has been discussed many times it appears necessary to re-emphasize certain points.

Dose Antibiotics and dosage used by different investigators vary. We have found that the patient must receive not less than 3-5 g. orally or 1 g. intermuscularly. Our best results have been obtained using 4 g. of tetracycline orally in divided doses over a two-day period.

Timing We have found that in most patients the tetracycline has left normal cells in 12 to 24 hours, and performance of the test at 36 hours has generally given good results. We have eliminated some false-positive tests by repeating the test at 60 hours. All patients who are negative at 36 hours are not tested at 60 hours. However, when positive, it is the 60-hour specimen that is interpreted rather than the 36-hour one. In the case of secretin tests, we have found that running the test at 60 hours is more reliable; most patients do not tolerate two duodenal intubations performed successively in two days.

Light source In the past, we have not commented on lighting except to say that we use a source of 3,600 Angstroms. Further description is obviously necessary, for we have noted that interpretation of the yellow fluorescence varies with the conditions for observing it. Our results are based on observations made through a viewer with $2 \times$ magnification. The specimen is situated 27 cm. from the viewer and 15 cm. from two ultra-violet hand lamps$^1$ with no. 18 Wratten filters built into a dark box. We have recently obtained an ultra-violet titration assembly$^2$, which we have modified. When comparing the interpretations, we find some difference between the two instruments, and we are trying to adapt the Fisher viewer. We also are in the process of adapting the spectrofluorophotometer for more objective measurements of fluorescence. Berk (1964) and Berk, Ibsen, and Morimoto (1965) have been trying for some time to make the interpretations of this procedure more standardized and objective. At first they used a modified fluorometer with varying results. They then attempted to quantitate the amount of tetracycline that could be extracted from the washings. More recently they have tried paper and column chromatography, with encouraging preliminary results. Paper chromatography seems to be good as a screening procedure, while column chromatography is a quantitative procedure.

It should be noted at this point that the reliability of individual observers to discriminate the bright yellow fluorescence from other yellow and white colours seems to be one of the major problems in the

$^1$Ultra Violet Products Corp; Palo Alto, California.

success of this procedure. Klass and Sklar (1965) noted that in 48 cases they had accurate results in only 61%, while they had false positive results in 31% and false negatives in 8%. They felt that it was important to watch technical details minutely, but the main problem was in discrimination of the colours. Berk et al. (1965) emphasize that there are many shortcomings, especially in techniques. Lavage, pH alteration, waiting for the specimen to be dry, the ultra-violet light source, were all problems which they mention, in addition to the difficulty of interpreting colours.

NOTE ON PERNICIOUS ANAEMIA A special note must be made about patients with pernicious anaemia. At least one author has found that this was an area in which false positives occurred. Early in our work we studied a series of 20 patients with achlorhydria, with and without pernicious anaemia. None of these patients had positive fluorescence in gastric washings. The only patient who had pernicious anaemia and a positive fluorescence test had carcinoma of the stomach.

In conclusion, we have now performed the tetracycline fluorescence test in 350 gastric washings, in over 175 secretin tests, and for smaller numbers in fluids from the lungs, pleura, and abdominal cavity. We still feel that the procedure is essentially simple to perform, safe, and reliable. However, it has some shortcomings which should be investigated further. As with any laboratory procedure, results must be considered in the light of the total clinical and laboratory picture.

SUMMARY

The results of the tetracycline fluorescence test and of cytology in the diagnosis of cancer of the stomach and pancreas were compared.

Both the tetracycline fluorescence test and cytology tests were performed on gastric washings from 260 patients. In the 45 patients with gastric carcinoma, cytology was positive in 31 and the tetracycline fluorescence test was positive in 42. Of the 215 benign lesions, there were three false-positive cytological results and 18 false-positive fluorescence tests.

One hundred patients had secretin tests performed for both cytology and tetracycline fluorescence examinations. Of 15 patients with pancreatic carcinoma, none had positive cytology, while 13 had positive fluorescence. Of the 85 patients without carcinoma, none had false positives with cytology but three had false-positive fluorescence tests.

The recent literature is quoted, emphasizing the broad scope of this procedure, and some work on calcium binding and localization of tetracycline in the cell.

The technical aspects of the test are emphasized. After continued work on this procedure for four years the test appears to be simple, safe and reliable. Further refinements are obviously necessary to improve uniform accuracy of this procedure.

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REFERENCES


