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Tetracycline fluorescence in cancer detection

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TETRACYCLINE FLUORESCENCE IN CANCER DETECTION

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Tumor tissue fluorescence by ultraviolet light was discovered by accident by Rall, et. al. at the National Cancer Institute in 1957. (1) These individuals discovered the occurrence while subjecting autopsy specimens of metastatic cancer of the breast to ultraviolet light. A yellowish fluorescence shown from the tumor tissue while surrounding tissue, normal in nature, appeared only auto-fluorescent, i.e., demonstrating a bluish direct reflection of light from the surface of the tissue. When the yellowish fluorescence was noted a review of medications of the patients being examined was made. Tetracycline was suspected due to the yellow color it made in solution.

Further experimental study was done after this initial discovery. Tetracycline was given intraperitoneally for five days in doses of 50 mgm./kgm. to mice with six day old implants of sarcoma 37. At varying intervals these mice were autopsied after administration of the drug was stopped. Fluorescence was noted in all tissues except the brain after six hours, and retained in only bone and tumor tissue after twenty-four hours, this remaining present up to twenty days later.

McLeay in 1958 made a preliminary report of his work on tetracycline fluorescence. (2) Specimens from controls and tetracycline prepared patients were used, these being subjected to ultraviolet light immediately after surgery. An ultraviolet

light source of a 9 watt, 3,660 Angstrom unit, Mineralight was used and placed at a distance of six inches from the tissues being examined. Cancer specimens that had been tetracycline prepared gave a fluorescence of a yellow-orange nature over the entire lesion and allowed sharp demarcation of bordering auto-fluorescent normal tissue. Using Terramycin^{R} as the source of oxy-tetracycline, McLeay reviewed literature on dosage administration and found that an oral dose of 500 mgm. of terramycin every six hours gave a blood level of 3 mgm./ml.; as compared to an intravenous dose of 500 mgm. every twelve hours which gave a blood level of 12 mgm./ml. Using intravenous tetracycline at the above level placed in 100 c.c. of 5 per cent glucose solution via slow drip, McLeay supplemented the oral dosage. The drug administration was cessated at least twelve hours prior to surgery to allow sufficient time for the normal tissue to flush out the tetracycline while retaining it in the cancer. The specimens recovered surgically included the following: 1,000 normal tissue specimens, i.e. non-cancerous which showed only auto-fluorescence; 42 cases of carcinoma specimens without tetracycline treatment which showed only auto-fluorescence; a control group of eleven with suspected tumor preoperatively showing only auto-fluorescence after four days of tetracycline administration to a blood level of 5 mgm./ml. or higher, which postoperatively were diagnosed as adenoma of thyroid (two cases), bronchiectasis,

diverticuli, fibrocystic disease of breast (two cases), benign gastric ulcers (two cases), rectal polyp, and ulcerative skin lesions (two cases); and a final group of eleven patients with cancer consisting of adenocarcinoma of colon, rectal sigmoid, sigmoid, rectum, cecum, intraductal carcinoma of the breast, and osteogenic sarcoma of right femur. This last group was evaluated visually by the author with the impression that seven showed good fluorescent intensity, one showed fair, two were excellent, and one was poor. All eleven specimens demonstrated usually quantitative fluorescence, and only in lymph nodes examined did a rating of none, negative or questionable have to be used. McLeay indicated that he found no false positives, i.e. tetracycline fluorescence in pathologic conditions other than cancer. Tetracycline (degree of) fluorescence was always present in his series in carcinoma patients although to varying degrees of intensity. More prominent fluorescence seemed to be related to blood levels, and to more anaplastic type of tumor, and was not found in necrotic tissue or normal tissue. Fluorescence was never found in specimens not prepared by tetracycline administration, and in tissues prepared and displaying fluorescence it was destroyed by placing specimen in formalin.

Malek and Kolc in 1959 attempted to study tetracycline penetration into tissues involved in the following processes:

regeneration, necrosis, inflammation, tumor; by the use of fluorescence (3). This was attempted since various studies had shown tetracycline accumulates in the reticuloendothelial system, in bone, and in tumor tissue and remains there for increasing amounts of time, the latter being the longest. For study, dogs, guinea pigs, and rabbits were used. Cutaneous wounds were formed on rabbits by incision and suture, and bone fracture inflicted to hind extremity. Skin necrosis was produced in rabbits by intradermal injection of 0.2 ml. of staphylococcal toxin. Acute pancreatic necrosis was caused in dogs by ligature of pancreatic duct, and thoracic duct. Rabbits were used to form areas of sterile inflammatory process by injection of turpentine into areas on the back. Female dogs were used which had spontaneous tumors of the mammary gland.

The cutaneous wounds fluoresced only slightly in early stages of healing (three days) and none could be shown later (seven to fourteen days). After bone fracture (one week), fluorescence could be demonstrated in entire fractured bone, but primarily under periosteum; there was no fluorescence at the broken ends. In later stages fluorescence was found primarily at bone callous, and other areas of bone showed less fluorescence. Cutaneous necrosis by staphylococcal toxin revealed no tetracycline fluorescence. There was, however, rapid accumulation of tetracycline in experimental necrosis of the

pancreas. In areas of sterile inflammation fluorescence appeared along a tree-like formation thought to be lymphatic channels and vessels. Spontaneous tumors in female dogs always demonstrated fluorescence, of varying degrees of intensity and never over the entire tumor but rather in certain parts only. Histologically it was found that this fluorescence occurred only in tumorous tissue itself, particularly where calcification was present. There appeared to be a direct ratio between hardness of the tumor and fluorescent activity.

These results appear to establish that the degree of tetracycline penetration, and thereby fluorescence, is influenced greatly by the pathologic process involved. Certain authors hold that bone incorporates tetracycline in an indissoluble complex with calcium. Other authors feel tetracycline absorbtion is taken up by the substance of fibrous tissue that is involved by inflammation or tumor production. Others feel that both calcium and tetracycline are bound by certain peptides. Malek and Kolc after review of the above theories and their own studies feel that inflammatory hyperemia allows increased penetration and an increased resorbtion of tetracycline by the lymphatic system. Tetracycline binding of the proteins of inflammatory exudate may be the reason tetracycline is drawn to lymphatic systems. Stasis in lymphatic tissue around inflammation may have a bearing here also. Penetration into tumorous tissue

is due to affinity of tetracycline for substances with which it may create different complexes, however, further study is required for the precise determination of these substances.

Following these reports of tetracycline fluorescence, Vasser, et. al. undertook a study of ultraviolet light fluorescence in patients with malignant tumors and non-specific skin ulcerative lesions. (4) They also studied experimental animals with skin ulcerations, intradermal abscesses, and subcutaneous non-neoplastic proliferative tissue reactions. Of primary interest is the group containing: nine cases of carcinoma, including three cases of infiltrating squamous cell carcinoma of the cervix, all with eventual hysterectomy; one case of infiltrating squamous cell carcinoma of the skin of the leg due to depilatory X ray fourteen years prior; one case of squamous cell carcinoma of the penis; one case of testicular chorionepithelioma metastatic to skin of groin; one case of adenocarcinoma of the pancreas studied at autopsy four weeks after tetracycline administration; two cases of undifferentiated bronchogenic carcinoma studied at autopsy eight and fourteen weeks after tetracycline administration. Other groups studied included five patients with chronic nonspecific skin ulcers, fifteen rats with hydrochloric acid induced skin ulcers, five rats with subcutaneous absorbable gelatin sponge, five rats with intradermal Staphylococcus aureus and Escherichia coli

abscess, along with control groups, and routine gross and microscopic fluorescence surveys of numerous surgical and autopsy tissues not previously prepared by tetracycline administration. All groups were examined for gross fluorescence with 3,660 A ultraviolet Mineralite lamp for surface and cut-surface tetracycline fluorescence. Ultraviolet light fluorescence microscopy was carried out also in a manner previously described by Vassar and Culling. (5) Tetracycline dosage was 500 mgm., orally, twice daily, for two days, in humans. In experimental animals, a single dose via stomach tube corresponding to 100 mgm./kgm. of body weight was used.

Patients studied with carcinoma showed typical fluorescence, bright yellow color, in every case, in both gross and microscopic preparations. Fluorescence was confined invariably to the tumor area and was often seen as a narrow band just inside the advancing edge. Interval and length of the duration of fluorescence varied with two patients with carcinoma of the skin, showing minimal gross fluorescence twenty-four hours after drug administration, and increasing intensity after an interval of forty-eight to seventy-two hours. This fluorescence then decreased slightly over twenty-four to forty-eight hours and then showed no appreciable change for up to twenty-one days. Autopsy specimens showed brilliant fluorescence up to fourteen weeks after last recorded tetracycline administration. Micro-

scopic technic of smears and cyrostat-cut serial sections showed fluorescence confined invariably to macrophages and tissue debris in tumor stroma, and in no case were malignant cells seen to fluoresce. The group of non-specific skin ulcers showed fluorescence for forty-eight to seventy-two hours which then diminished rapidly in two cases. Three others developed less intense fluorescence which faded rapidly twenty-four hours after drug administration was stopped. Hydrochloric acid induced skin ulcers revealed minimal gross fluorescence which faded rapidly twenty-four hours after administration. Subcutaneous absorbable gelatin sponges showed no fluorescence in granulation tissue around the sponges. Intradermal abscesses revealed only minimal fluorescence at scab formation site. In no member of control groups without tetracycline was there gross or microscopic evidence of fluorescence.

These findings confirm tetracycline induced fluorescence, localization and persistence in human malignant tumors and their metastases. Of notable interest is the finding of fluorescence not in the malignant cells but invariably within the tissue stroma in malignant tissues. There exists the possibility that materials persist and localize in neoplastic stroma due to tissue destruction, repair, and increased histocytic activity. The mechanism suggested by the above findings and theory may be initiated by transudation or exudation of free and protein

bound tetracycline into areas of tissue activity. Localization and binding of tetracycline in this manner after possible peptide complex linking and/or calcium precipitation may occur. To corroborate this theory, Lacko in 1959 found that precipitation of lipoprotein tetracycline complex occurs in the presence of calcium ions. (6)

As previously noted tetracycline ultraviolet fluorescence occurs in a great variety of malignant tumors, as it also does in many types of bone tumors and bone conditions. Since calcium appears to aid the precipitation of the tetracycline peptide complex, and calcium content and its influence on bone formation is very important, research was done on bone lesions and their fluorescent properties after tetracycline administration. McLeay and Walske conducted such a study and reported in 1960. (7) Terramycin was used as a source for oxytetracycline in doses of 15 mgm./kgm. of body weight, per day, intravenously, in divided doses added to 100 c.c. of saline solution, for three or more days prior to surgery. The drug was discontinued at least twelve hours prior to surgery, preferably twenty-four hours. In this instance an ultraviolet light of 3,660 Angstroms was used, with 100 watt power rather than 9 watt as the only source of light in the operating room after bone exposure. Also, a bacteriologic assay system was used to determine levels of tetracycline concentration in surgical specimens removed. Patients with the following bone

conditions were studied: osteitis deformans; five tumors including Ewing's tumor; periosteal osteogenic sarcoma, osteogenic sarcoma, (two cases); chondrosarcoma; and control groups containing osteomyelitis (three cases); non-union, (two cases); and one fracture of one weeks duration. In all cases of bone tumor a brilliant yellow fluorescence was noted in tumor, marrow and extensions along periosteal surface. This area was surrounded by a yellow fluorescence in normal reactive bone, and finally the normal blue auto-fluorescence of mature bone was noted. All members of the control group showed the yellow fluorescence of reactive bone only, without the brilliant yellow fluorescence as described above. The malignant conditions had an average level of 25 mgm. of tetracycline per gram of tumor. The control levels were 1-4 mgm. of tetracycline per gram in the reactive new bone. It was pointed out that the amount of fluorescence by above methods allowed ease in demarcating and localizing neoplastic tissue for purposes of biopsy and excision.

Phillips and others, in 1960, attempted fluorescence in a large group of malignant conditions. (8) Twenty-one cases of various adenocarcinomas were studied along with hepatoma (1), osteogenic sarcoma (1), squamous cell carcinoma (5), reticulum cell sarcoma (2), transitional cell carcinoma (2), and ductal carcinoma of the breast (2). A control group included fibroadenomas, cystic disease and ductal hyperplasia of breast,

thyroid adenomata, mixed parotid gland tumor without histologic evidence of malignancy, duodenal and benign gastric ulcers, benign rectal polyps, diverticulitis, villous adenoma of rectum, and hemorrhoids. ~~Actinomycin~~ Achromycin was the source of tetracycline by oral, intramuscular, and intravenous routes, in various dosages and time intervals to determine effect on fluorescent intensity. In no case did a benign condition show more than normal auto-fluorescence, as did carcinoma specimens not prepared by tetracycline. In this study some malignant conditions did not show yellow fluorescence but this appeared to be related to inadequacy of size of dose administered. This is felt to be true since every malignant condition that did not fluoresce in low doses of tetracycline did fluoresce when higher doses had been given. Total fluorescent intensity in tumor tissue was suggested as a direct relationship and function of tetracycline dosage. Furthermore, anaplastic tumors fluoresced more strongly than those well differentiated. An observation was made that reticulum cell sarcoma had more fluorescent intensity than adenocarcinoma, and both more than squamous cell carcinomas after comparable dosages of tetracycline. Although study of regional lymphatics and metastatic lesions was not specifically undertaken, fluorescence was noted present in many instances. However with all the above results, various evaluations made at the operating table showed that even

relatively thin layers of normal tissue interposed between tumor and ultraviolet source seemed to nullify efforts to localize tumor. It was felt that visual and tactile abilities of the surgeon were superior.

In late 1960 M. I. Grossman related in a personal communication to Klinger and Katz that fluorescence could be seen after tetracycline administration in the gastric sediment of patients with cancer of the stomach. Further study was undertaken at that time. (9) A total of eighteen patients with gastric carcinoma were studied along with forty-one controls consisting of gastric ulcers (nineteen cases), duodenal ulcers (five cases), hepatic cirrhosis (five cases), iron deficiency anemia (two cases), rheumatoid arthritis (three cases), gastro-intestinal bleeding of unknown etiology (three cases), pyloric syndrome secondary to acute cholecystitis (two cases), and pernicious anemia (two cases). Of the cancer patients, eleven diagnoses of gastric carcinoma were confirmed by co-ilieotomy, four were resected and the remainder were inoperable. The seven cases remaining were not operated upon because of metastases. Gastric lavage was done after a twelve hour fasting period. The aspirate was centrifuged, and supernatant fluid decanted and spread over filter paper. After drying it was examined for fluorescence under ultraviolet light. Tetracycline in doses of 250 mgm., three times daily, was then administered

for five days, and gastric lavage centrifugation, and sedimentation repeated, followed by ultraviolet examination. Of the eighteen carcinoma patients observed, seventeen gave a positive test showing a yellow fluorescence. Among the forty-one control patients, no tetracycline fluorescence was observed. The one carcinoma patient failing to give a positive test was noted to have a Bormann type III, Dukes C, adenocarcinoma. One patient with the same histologic lesion did give a positive result with no alteration in technic or procedure. The procedure studied, although on a limited group of subjects, appears simple, easy, and useful in diagnosis of gastric carcinoma.

Later in 1961, Berk and Kantor confirmed the preceeding results. (10) Using similar technics to those of Klinger and Katz, a study of fifty-eight patients with a variety of benign and malignant disorders of the stomach was made. All ten patients having cancer, confirmed by histologic examination, and two patients with benign gastric ulcer showing mucosal atypism, gave a positive fluorescent test. Of forty-six patients with normal stomachs or benign lesions, only one gave a positive test. It was felt that the results were significant and that the procedure was a simple one to perform. Suggestions made included that pH levels of stomach content had a bearing on the amount of fluorescence and regulation of degree of acidity should be considered. Also

d.p.

specimens should be examined within a short time after their preparation. Thirty hours should be allowed to pass after last administered dosage of tetracycline before lavage procedure is undertaken.

To this point nearly all studies reported near perfect correlation of fluorescence when malignancy was present. Ackerman and McFee reported otherwise in 1962. (11) Tetracycline fluorescence was noted in some, but not all of primary cancers of lymphatic tissues, breast, female genital tract and alimentary tract. No pattern of prediction of fluorescence was noted, and correlation of fluorescence with dose, histology, or route of administration could not be made. Tumor fluorescent distribution appeared to be sporadic. Tumorous lymph nodes failed to fluoresce and metastatic cancer showed only varying degrees of affinity. No reasons for uneven distribution were given, nor for negative results obtained. Confirmation was made, however, that fluorescent material was localized in necrotic tissue and stroma rather than within the cells. It was felt that further study was needed with regard to understanding of tetracycline deposition in human tissues.

In 1963, Sherman and others reported further study of gastric carcinoma fluorescence. (12) Sixteen of seventeen cases of gastric carcinoma gave a positive gastric sediment fluorescence. The seventeenth case showed fluorescence microscopically. Of

twenty-nine cases of non-cancerous gastric pathology, twenty-seven showed no fluorescence, with two cases reported as false positive. Sixty-four control patients showed no fluorescence. Oral administration had a lesser percentage of error (5.9%) than intramuscular administration (8.5%). Five fluorouracil chemotherapy was reported to interfer with fluorescent test causing negative results in two patients even with repeated attempts. Three others receiving 5-fluorouracil showed positive tests initially but turned negative after a seven to twenty-one day course of 5-fluorouracil. Fluorescent microscopy on frozen tissue sections was attempted on eight patients with gastric pathology. Six of these showed golden brown diffuse fluorescence in unstained frozen sections, and positive confirmation of carcinoma was made by routine stained permanent slides. Two patients gave negative tests, i.e. no fluorescence, and were later reported as benign gastric ulcer, and benign gastric polyp. Further study on frozen section investigation was thought to be important.

Mahn-Pederson and Jesting in 1963 attempted to reproduce gastric sediment fluorescence in Denmark. (13) Six cases of gastric carcinoma revealed only two positive results. One of six benign gastric ulcers also showed a positive test. Six of twelve patients with duodenal ulcer gave positive results. These results brought a conclusion that gastric sediment test for

fluoresence was not an adequate method for estimating malignancy of gastric ulcer for these authors.

Late in 1963 Berquist and others reported that tetracycline fluorescence was noted to occur in the beta globulin electrophoretic position. (14) This was noted after addition of tetracycline to pooled human serum in vitro. Tetracycline and calcium in excess caused patterns with less globulin than untreated serum controls. Thus tetracycline would appear to be complexed with beta globulin or lipid-bearing globulins which are active in cell permeability and ion transport. Such decreased globulin patterns indicate a precipitation of tetracycline with calcium in a lipo-protein complex. This further corroborates early theory.

Berk and others in 1963, presented, in a discussion at the American Society for Gastrointestinal Endoscopy, a fluorometer for use in the gastric sediment fluorescent tests. (15) The equipment consisted of an ultraviolet light source and various multi-layers of filters, with a set of six zinc sulphide comparison slides. The equipment was designed to eliminate the subjectivity of the filter paper observation technic previously used.

Further study of tetracycline administered tumor fluorescence by Cabrera in 1964 showed positive fluorescence in primary carcinoma of breast, metastatic carcinoma of the

lung and kidney, and carcinoma of the colon in the abdominal wall. (16) Along with this addition to the growing list of tumors showing tetracycline uptake and fluorescence, the authors studied homogenized, dialized, fluorescent tumor tissue with a fluorescent microscope. It was observed by this method that fluorescence appeared to be present in the cytoplasm of cells which had tetracycline uptake. Also reported was again a confirmation that tetracycline does not specifically bind to tumor tissue but also attaches to the surrounding non-cancerous tissues. An attempt at using tetracycline fluorescence to deliniate oral tumors at time of surgery proved unsuccessful as the entire mouth fluoresced along with the surgeon's gloves.

Sandlow and Necheles added further uses to tetracycline fluorescent studies in search and detection of malignancy in 1964. (17) Tetracycline treated patients (25) had secretion tests performed with duodenal contents aspirated. Twelve patients were normal controls without evidence of pancreatic disease, and normal secretion test results. Six had chronic pancreatitis and seven had pancreatic carcinoma, proved by autopsy in two and surgery in five. No tetracycline fluorescence was seen in twelve controls or six with chronic pancreatitis. All seven carcinoma patients showed fluorescence. Twenty-four patients demonstrating pleural effusions were studied. Twelve patients had tuberculosis, heart failure, bacterial pneumonia,etc.

None of these demonstrated fluorescence. Carcinoma involving the pleura was found in the other twelve patients, all of whom had positive bright yellow fluorescence in the effusion. Ascitic fluid showed positive fluorescence in twelve patients with carcinoma involving the peritoneum. These authors also attempted to clarify previously noted research which gave differences of opinion regarding efficiency and reliability of tetracycline fluorescence. It was felt that better results were being found in larger groups being studied. Also the following technical pointers were listed: drugs such as riboflavin should not be administered prior to test, milk products and aluminum hydroxide decrease tetracycline absorbtion, a total dose of less than 3 1/3 to 4 grams of tetracycline is not adequate for consistent positive results.

Burrows in 1964 assessed the use of tetracycline fluorescence as a test for skin malignancy. (18) Following the example of Lipnik who described a rapid screening test in 1963, Burrows painted lesions with the following mixture: tetracycline 1% and cyanocobalmin 0.1% in water. Lesions were first examined under Wood's light for fluorescence and then painted with 4.9% trichloracetic acid and ten seconds later residual fluorescence was re-examined. (19) Fluorescence visable after this time period was considered a positive test. Lipnik reported a false negative result rate of 8% and a false positive test rate of about 2%.

Burrows in attempting to reproduce these results reported 43% false negatives and 54% false positives. Malignant conditions included basal cell epithelioma and squamous cell epithelioma, along with benign conditions of keratosis, sebonbolic wart, common wart, molluscum, scar, papilloma, scar and painful nodule of the ear. These results caused Burrows to feel that this particular examination was not sufficiently accurate for routine diagnostic usage. Neither individual attempted study after use of systemic tetracycline administration.

Another possible use of tetracycline induced ultraviolet fluorescence was reported by Whitmore in 1964. (20) After oral administration of tetracycline, 250 mgm. every six hours for two to six days, with the drug discontinued from twelve to seventy-two hours before observation, patients were examined cystoscopically. The bladders of these patients were examined by routine incandescent light and by a specially designed ultraviolet cystoscope. Nine carcinomas were identified by these methods in six bladders that revealed no mucosal abnormality by routine cystoscopy. Five of these lesions proved later to be early invasive epidermoid carcinomas, and four were in situ carcinomas. Two other areas were noted to show fluorescence that later proved to have no microscopic abnormality. Seventeen patients with previously grossly visable bladder carcinoma were also studied. Of these, thirteen tumors

showed a positive degree of fluorescence, two were questionable and two were negative. The authors felt that examination of the bladder for tetracycline fluorescence in this manner was a valuable test for earlier detection of bladder cancer, and also emphasized the limitations present in conventional incandescent light bladder visualization in routine cystoscopy. Better results and easier examinations could be made by refinement of the ultraviolet endoscopic system used.

Hiduchenko, in 1965, attempted to apply the use of tetracycline as a diagnostic tool in bronchogenic carcinoma. (21) Twenty-three patients with proved bronchogenic carcinoma and twenty-three patients with a variety of other pulmonary diseases including pneumonia, tuberculosis, emphysema, and congestive heart failure were studied. Pre-test sputum specimens were obtained and examined for fluorescence. Five patients showed presence of yellow fluorescence and were subsequently excluded. The remaining patients were given tetracycline in divided doses of one gm. per day orally for four days. After a twelve hour fast, thirty-thirty-six hours after last dose of tetracycline, fresh sputum specimens were obtained, pH was adjusted to 7-8, small amounts of the specimens were spread over Whatman #3 filter paper and allowed to dry over night. Ultraviolet examination was carried out the following day. Positive results were considered present when definite bright yellow fluorescence

was present, whether in small flecks or diffuse areas. Positive results were found in sixteen of nineteen carcinoma patients, and three of twenty-two controls. No reasons could be found for false negatives or false negatives found. Further investigation of microscopic and biochemical levels would help to explain these findings. Although the test could not be considered a reliable method of diagnosis of bronchogenic carcinoma, it once again had proved a valuable adjunct.

One other new use for tetracycline fluorescence was discussed by Ayre in 1965. (22) Tetracycline in doses of two grams daily was given to patients previously studied by both Papanicolaou and ~~acridine~~ acridine orange fluorescence techniques and shown to have cervical lesions of dysplasia and in situ carcinoma. Cytology smears were made with Ayre cervical spatula, immersed in normal saline, and examined under a Zeiss fluorescence microscope. Fluorescence was noted to be present by this technic in both hyperactive malignant and pre-malignant cells in the human cervix. Besides the fact that fluorescence occurs, it was noted to be present in this study within the cancerous or pre-cancerous cells. This is particularly significant because in this state no tumor or noeplastic ulcerative tissues exists. Therefore, the test allows identification before ordinary visual observation of the lesions is possible and also indicates that the tetracycline is present, in these instances, within the neoplastic cells themselves.

Finally, in 1965, a comparison of the previously noted gastric sediment fluorescence test was made with results of exfoliative cystology studies on gastric content. (23) Sixty-seven patients, fourteen with gastric malignancy were studied after administration of 250 mgm. of tetracycline orally four times a day for a period of five days. This dosage was altered to a dose of 500 mgm. four times a day for two days during the last ten months of the study. After discontinuation of the medication for thirty-six hours, Levine tube lavage was performed, with pH adjustment to 7.5. Centrifugation was performed for 15 minutes, the supernatant fluid poured off, and the sediment spread and dried on Whatman #1 filter paper. Examination was then carried out under an ultraviolet light in a darkened room. The authors reported only five positive results in the fourteen patients with gastric adenocarcinoma and false negatives in the remainder. Exfoliative cytology by comparison gave seven positive results. Tetracycline fluorescence was noted to be present and falsely positive in fourteen of the fifty-five patients with benign lesions. No false positive results were found in this group by exfoliative cytology. Thus the fluorescent sediment test proved to be inferior to cytologic studies at this time. In only one case did the combined use of both tests serve to diagnose a previously undiagnosed case of adenocarcinoma. A statistical

compilation of all studies using gastric sediment fluorescence was made, revealing 68.6% positive tests when malignant conditions were present in the stomach. A total of 16.6% positive tests were recorded in benign conditions, these recorded, therefore, as false positives. Reasons for false negative results could not be explained, while false positives could be attributed possibly to previous vitamin administration containing riboflavin and obstruction causing tetracycline retention.

SUMMARY

Discovery has been made that the antibiotic tetracycline has strong fluorescent properties when examined under ultraviolet light of 3,600 Angstroms, causing a bright to brilliant yellow fluorescence. By this technic and others tetracycline is noted to be picked up by most tissues of the body, and leaves nearly all tissues except bone and malignant tissues within twenty-four hours after administration is stopped. Bone and malignant tissues retain tetracycline and thereby their ability to fluoresce for much longer periods of time up to several weeks or longer. This feature has been used to detect malignancy of nearly all types of tissue as well as malignant exfoliated cells in sputum and cervical secretions, pleural, and ascitic fluids; and also to delineate gross and microscopic lesions.

Attempts to localize the tetracycline either within tumor tissue or its connective stroma have yielded controversial results.

Mechanism of tetracycline uptake in tumor tissue remains to be studied. Current thoughts indicate a complex of tetracycline, lipid (lipo-proteins) and calcium is formed and deposited within tumor tissue or its connective stroma and cellular debris.

Tetracycline induced fluorescence of gastric sediment in gastric lesions has been the most thoroughly studied examination to this point.

Various specialized instruments have been constructed to allow visualization of tetracycline fluorescence within bladder, stomach, colon. Comparisons of positive findings by tetracycline fluorescence have been made with microscopic cytologic technics, Papanicolaou technic, and relative success or failure of tetracycline studies evaluated.

CONCLUSION

Tetracycline induced fluorescence under ultraviolet light has been shown to be a relatively ~~in-expensive~~ inexpensive, simple technic for aiding in localizing, delineating and discovering locations of various malignant conditions within the human body. It does not require highly trained personnel and appears to be

valuable for clinical usage in various forms of examination. The methods are relatively rapid and inexpensive and further perfection of ultraviolet light endoscopy equipment may render its use even easier. Results of studies show that the specificity of the tests are not perfectly reliable, nor will they be until more is known about the mechanism of localization of tetracycline in various tumors and tissues. However, use of such tests appear to be of value for screening technics, or as aids in arousing suspicion of presence of malignancy and its localization. Increased research and further study of factors affecting tetracycline fluorescence may help to eliminate false positives or negatives from the results. Careful technic is also essential in performing tetracycline induced fluorescent examinations.

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