TETRACYCLINE FLUORESCENCE OF MALIGNANT TUMOURS

by

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CONTENTS

ABBREVIATIONS

Throughout the thesis the following abbreviations are used:-

TC	=	Tetracycline
OTC	=	Oxytetracycline
CTC	=	Chlortetracycline
DMTC	=	Demethylchlortetracycline.

Section I

FLUORESCENCE

HISTORICAL ASPECTS

The phenomenon of fluorescence was first reported by Sir David Brewster, being mentioned in a paper "On the Colours of Natural Bodies" (Brewster, 1833) read before the Royal Society of Edinburgh and described at length before the British Association at Newcastle (Brewster, 1838). Particular reference was made by him to the striking colour to be seen in a particular variety of fluor-spar on illumination with an invisible part of the spectrum of sunlight. Sir John Herschel presented his observations "On a Case of Superficial Colour presented by a Homogeneous Liquid Internally Colourless" (Herschel, 1845) which was followed by a further paper by Brewster (1846) on the subject of "The Epipolic Dispersion of Light".

The detailed paper by Professor G.G. Stokes "On the Change in the Refrangibility of Light" reported the role of ultraviolet light in relation to quinine, chlorophyll and other organic materials with the optical phenomenon being christened "dispersive reflexion"

although in a footnote he wrote, "I confess I do not like this term. I am almost inclined to coin a word and call the appearance fluorescence from fluor-spar, as the analagous term opalescence is derived from the name of a mineral". Thus although the term fluorescence was first coined by Stokes (1852) to whom is usually attributed the discovery of the phenomenon, the credit should be that of Sir David Brewster (1781 - 1868) whose life-long research into the science of optics culminated in his appointment as Vice-Chancellor of the University of Edinburgh in 1860, and as President of the Royal Society of Edinburgh in 1864.

CURRENT THEORY OF FLUORESCENCE

Current theories of the absorption and emission of radiation combine the classical and quantum theories of optics with the quantum mechanical theory of atomic and molelecular structure.

Light is a form of electromagnetic radiation (energy), the propagation of which may be regarded as a wave phenomenon, being characterised by a frequency (ν), a wavelength (λ), and, in a vacuum, a constant velocity (c). The relationship between these three

factors being

$$v = \frac{c}{\lambda}$$

When light enters matter, it may pass through with only little absorption taking place, the material then being considered essentially transparent. There is a little loss of energy, but the velocity of the light is diminished appreciably. The refractive index of a substance is the ratio of the velocity of light in a vacuum to the velocity in that substance.

Alternatively, light on its passage through a medium may be absorbed either entirely or in part, a process which involves a transfer of energy to the medium. This absorption is a highly specific phenomenon dependent on the particular energy of the radiation and the molecular structure of the medium. According to quantum theory, energy from light is absorbed in integral units, called quanta. The energy of a quantum is given by the expression,

$$E = h\nu$$

or

$$E = h\frac{c}{\lambda}$$

where h is Planck's constant, 6.62×10^{-27} erg-seconds, c the velocity of light, ν the vibration frequency (second $^{-1}$), and λ the wavelength. Wavelength is commonly quoted in Angstrom units or millimicrons (mμ.).

1 Angstrom unit = 10^{-8} cm. = 10 millimicrons (mμ.)

Each molecule possesses a series of closely spaced energy levels and can pass from a lower energy level to a higher one by absorbing an integral quantum of light which is equal to the difference between the two energy states. All of the light which is absorbed, at any one instant, by a liquid solution containing many molecules is taken up by only a few molecules, and only those few are therefore promoted to an "excited" state and are capable of fluorescing or undergoing photochemical change. The relationship between energy and wavelength of light is shown in Table 1.

Wave-length (mu)	Frequency ν (second $^{-1}$)	Chemical units of energy (K/cal/mole)	Colour	Molecular Effects
1000	3×10^{14}	29	Infra red	Stimulation of molecular vibration.
800	3.8	36	Visible Limit	Absorption in the visible and ultra-violet regions produces electronic excitation.
700	4.3	41	Red	
620	4.9	46	Orange	
580	5.2	49	Yellow	
530	5.8	54	Green	
470	6.4	60	Blue	
420	7.2	68	Violet	
400	7.5	71	Visible Limit	
300	10.0	95	Ultra-violet	
200	15.0	143	Ultra-violet	

TABLE 1. THE RELATIONSHIP BETWEEN ENERGY AND WAVELENGTH OF LIGHT.

The emission of light by molecules following selective absorption of energy is referred to as luminescence. Heat, electricity and chemical reaction, as well as light, can bring about molecular excitation leading to luminescence. Fluorescence and phosphorescence are the two types of luminescence following excitation due to the absorption of any form of electromagnetic radiation. Phosphorescence differs from fluorescence by the persistence of the luminescence following excitation by the light source and by a marked enhancement and increase of the emission time with a lowering of temperature.

The molecule, capable of fluorescence, absorbs a photon of light, being left in an "excited state". If the molecule does not decompose as a result of the increase in energy, and if all the energy is not dissipated by subsequent collisions with other molecules, then after a short period of time, 10^{-8} to 10^{-7} second, which is characteristic of the atom or molecule, the electron returns to a lower energy level, emitting a photon in the process. The difference between the energy of the initial state and the final state determines

the energy of the emitted radiation, which we call fluorescence. The emitted fluorescence has a greater wavelength or lower energy than the light which is absorbed (Stoke's Law). A small amount of energy is dissipated as heat (thermal deactivation) in the overall process.

Fluorescence is characterised by specific excitation (or activation) spectra and fluorescence (or emission) spectra. The excitation spectrum will differ from the fluorescence spectrum as a result of instrumental artefacts. As an approximation the fluorescence spectrum has been considered by some as the mirror image of the absorption spectrum. There are many examples, such as anthracene, which makes this plausible. However, most polyatomic molecules in aqueous solution have only one fluorescent band which is associated with the absorption band of longest wavelength. When such molecules are excited by light coincident with an absorption band at shorter wavelength, the excited molecules undergo internal conversion with loss of sufficient energy so as to pass over into the state corresponding to the longest wave absorption band.

The two requisites for fluorescence are the ability of the molecule to absorb light and the presence on the resonating molecules of at least one electron-donating substituent. Thus while benzene is non-fluorescent, phenol and aniline are strong fluorophores (substances capable of fluorescence). Acetylation of aromatic amino or phenolic groups results in total loss of fluorescence, while alkylation has little or no effect, indicating that the substituent requirement is for a group capable of increasing the electron density of the aromatic molecules. Polycyclic aromatic systems and aliphatic conjugated polyenes comprise a second general classification of fluorophores which require no additional substituents. Examination of the formula of the tetracyclines given later (Fig.2) show that they fulfil the requirements of a fluorophore in that they are polycyclic aromatic systems and in addition have electron-donating substituents (Udenfriend, 1962).

ULTRAVIOLET LIGHT

Although any electromagnetic radiation may induce fluorescence, the most usual wavelengths utilised are in the ultraviolet range.

The International Commission on Illumination defined visible radiation for practical purposes as the range of wavelengths between 3,800 and 7,800 Angstrom (380 and 780 mμ.). Ultraviolet radiation is usually defined as electromagnetic radiation of wavelength between 40 and 4000 A. (4 & 400 mμ.). It bridges the gap between the longest wavelength X-rays and the shortest wavelengths of light visible to the human eye, although the limits of the visible spectrum are not very clearly defined, varying with age and from one individual to another.

The most efficient and useful sources of ultraviolet are the arcs which occur when a current of electricity is passed between electrodes separated by a gas or vapour. The passage of current through the gas results in various phenomena, one of which is the emission of radiation. The amount of radiation which lies in the ultraviolet varies with the nature of the

arc. The commonly available source of artificial, as opposed to solar, ultraviolet light is the enclosed mercury vapour lamp. Such lamps are usually to be found in any hospital, being used for therapy in the physiotherapy departments and by the dermatologists for the diagnosis of ringworm. Although the usual mercury vapour lamp with a Wood's filter is readily available it is not the most efficient source of ultraviolet light for the purpose of exciting TC fluorescence (see later). The emission spectrum of a mercury-vapour arc is of the band type as shown in Fig.1. For ultraviolet fluorescent work, a filter glass in front of the mercury vapour lamp cuts out practically the whole of the visible spectrum, although it is transparent to most of the ultraviolet region. Thus the induced fluorescence contrasts with the faint blue light emitted from the ultraviolet source.

Convenient lamps (of which a range are made by Engelhard Hanovia Lamps of Slough) provide a medium-pressure mercury-vapour arc enclosed in a quartz envelope and emitting the full characteristic mercury spectrum as shown in Fig.1. A filter glass, Chance's

OX 1, is designed to transmit the longer ultraviolet rays and to exclude as far as possible all visible radiation, thus its main transmission is from 300 to 400 mμ. with the major spectral line of mercury being in the region of 366 mμ. Such a portable lamp was used in the gross demonstration of TC fluorescence as described later.

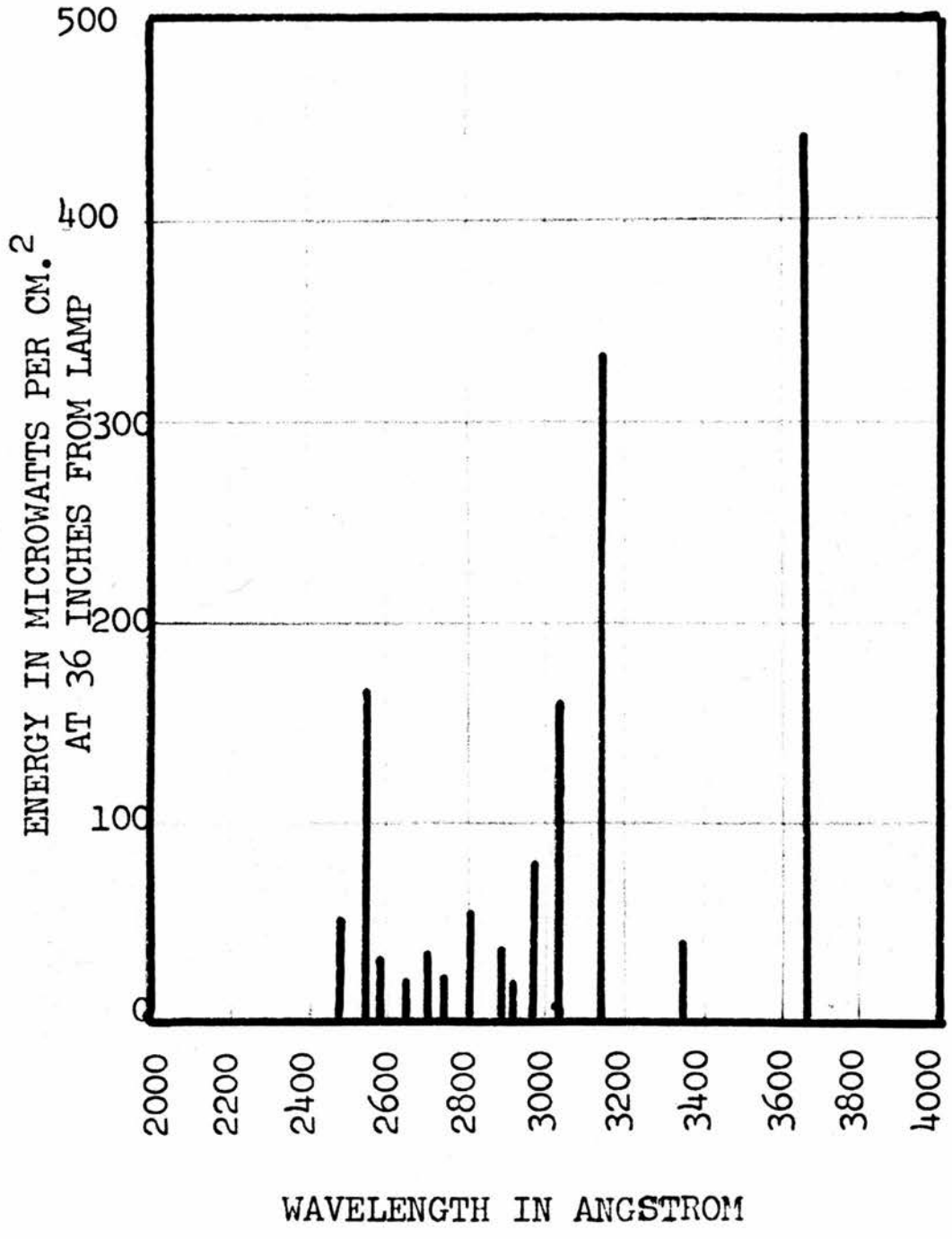


Fig. 1. ENERGY DISTRIBUTION IN THE VARIOUS LINES OF THE SPECTRUM OF A TYPICAL MEDIUM PRESSURE QUARTZ MERCURY ARC.

Section II

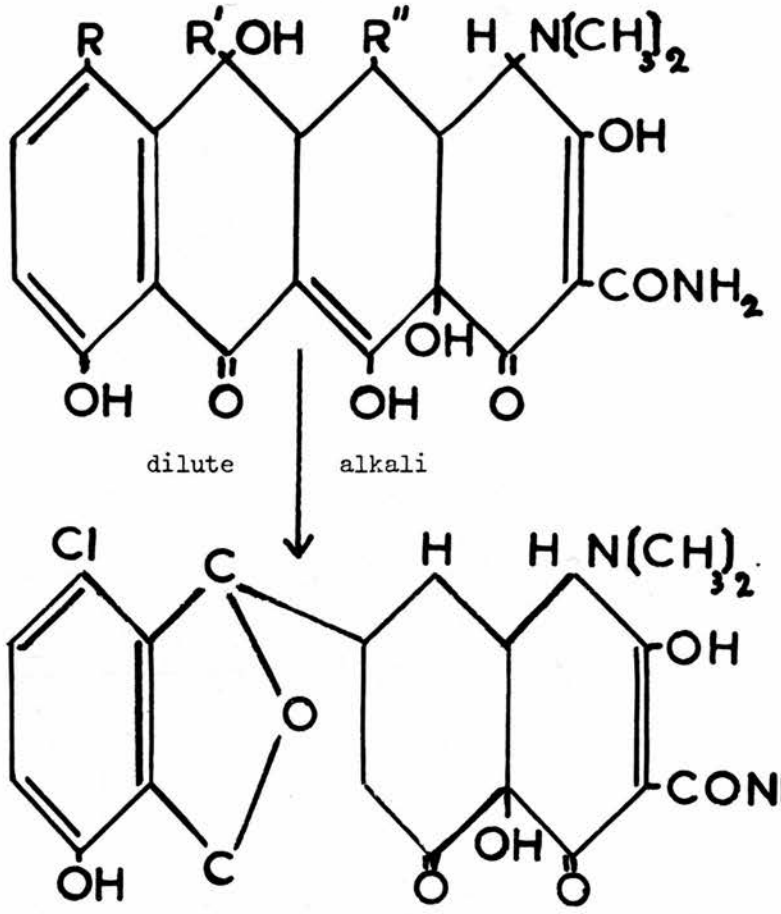
THE TETRACYCLINE GROUP OF DRUGS

Tetracycline (TC), the most important of the broad spectrum antibiotics, was first referred to by Stephens *et al*. (1952). These authors demonstrated the chemical structures of "Terramycin" and "Aureomycin" and proposed the name *tetracycline* for the basic structure common to both compounds. They assigned the name *oxytetracycline* (OTC) to "Terramycin" because of the presence of an hydroxy group in position 5 of the basic molecule. "Aureomycin" having a chloride group in position 7 and lacking an hydroxy group in position 5 was later assigned the name *chlortetracyline* (CTC).

The TC drugs became available commercially in November, 1953 and were later produced by a direct fermentation process.

There have been many modifications of the basic TC molecule but the commonly used antibiotics include TC, OTC, CTC and, more recently, *demethylchlortetracycline* (DMTC). The formulae of these drugs is shown in Fig.2.

The antibacterial action of this group of drugs does not concern this thesis, suffice it to say



ISOCHLORTETRACYCLINE

	R	R′	R″
TETRACYCLINE	H	CH_3	H
CHLORTETRACYCLINE	Cl	CH_3	H
OXYTETRACYCLINE	H	CH_3	OH
DEMETHYLCHLORTETRACYCLINE	Cl	H	H

Fig. 2. CHEMICAL STRUCTURE OF FOUR TETRACYCLINE

ANTIBIOTICS

that their action is mainly bacteriostatic and that the wide antibacterial range of action is remarkably similar for the group of drugs.

ADMINISTRATION

From the voluminous literature it is difficult to obtain any clear idea of the blood levels achieved after a given dose of any of the TC group of antitiotics. The difficulty is that the four major TC drugs, although possessing virtually identical antimicrobial spectra, differ in their relative activity against different organisms. The usual methods of assay are against the staphylococcus and bacillus cereus either by broth dilution or agar diffusion. These assay methods tend to minimise the effects of protein binding, the degree of which varies with each of the drugs. Furthermore, maximum antimicrobial activity is dependent on pH of the culture mediums used. Consequent upon these difficulties, comparisons between the blood levels achieved by the different antibiotics is extremely difficult to assess, although the fluorometric methods of assay recently introduced should give more accurate measurements (Ibsen et al., 1963; Kohn, 1961a).

As the antibiotic used in the current investigation is TC, figures will be given for blood levels of this drug given by different routes. The derivatives OTC and CTC usually follow the same pattern, but in the case of DMTC the blood levels tend to remain higher for a longer period due to differences in protein-binding, absorption and excretion.

Oral

Combining the results of several workers, Dowling (1955) reports serum levels in adults receiving 250 and 500 mg. by mouth at 6 hour intervals. With the smaller dosage regime there was a gradual rise to between 2 and 4 µg./ml. by 5 days, whereas with 500 mg. dosage a level of 4 µg./ml was achieved within 3 or 4 days. At about this level, even though the drug be continued, there is no further build-up in serum levels. After a single dose of 500 mg. TC an average serum level of about 3.5 µg./ml. is achieved in 3 hours, which is higher than with the other 3 derivatives (Kunin & Finland, 1961) a feature which may be of importance in the choice of antibiotic for uptake by tumours as described later.

Intravenous

After a single intravenous infusion of 500 mg. of TC Kunin et al. (1959) found a mean level of 8 µg./ml. and by 24 hours the concentration was under 1 µg./ml., whereas DMTC was appreciably higher. It should be stressed however that there was an extremely large scatter in the serum levels, which was even more marked after an infusion of 1000 mg. (Finland et al., 1954) but of the order of 16 to 32 µg./ml.

Intramuscular

Intramuscular injections of TC appear to be fairly unsatisfactory for therapeutic purposes if given in the usual doses of about 2.5 mg./kg. body weight, but, if 10 mg./kg. doses are used, levels of between 0.6 and 5.0 µg./ml. can be achieved. There is however fairly considerable injection site pain with the higher dosage regime. (Spies et al., 1955).

DISTRIBUTION

TC can be found in all body fluids after oral or parenteral administration including pleural and ascitic fluids, although there is some difficulty in diffusion into the cerebrospinal fluid unless high blood concentrations are achieved (Dowling, 1955).

EXCRETION

Some 20 to 60% of a dose of TC is excreted in the urine within 24 hours (Maynard et al., 1953). There is a high biliary concentration, 5 to 10 times that of the serum (Andriola, 1954), and there is a hepato-intestinal circulation for this drug. Maynard et al. (1953) report a fairly high excretion of the antibiotic in the stool, although as their results are simply given as milligrams per gram of wet stool, the percentage faecal excretion cannot be determined. Sweeney et al. (1960) reporting on DMTC showed that from 23 to 72% of an oral dose was excreted in the faeces and similar variable results are to be expected with the other TC antibiotics.

FLUORESCENT PROPERTIES

Udenfriend et al. (1957) demonstrated the fluorescent properties of TC and OTC which have excitation maxima at 390 mμ. and fluorescence maxima at 515 to 520 mμ. at pH 11. The fluorescent characteristics of CTC were reported as 355 mμ. excitation and 445 mμ. fluorescence, maximal at pH 11. It appears that the fluorescence reported for CTC is, in part, due

to a derived product isochlortetracycline (Fig.2.) to which the antibiotic is rapidly converted in basic solution or at neutral pH (Levine et al., 1949., Chiccarelli et al., 1956). According to Feldman et al. (1957) the derived product has an excitation or absorption maximum of 350 mμ. and a fluorescence maximum of about 425 mμ. (blue).

It has been demonstrated, by Hattner & Frost (1962) that weak alcoholic or aqueous solutions of the tetracyclines do not show strong fluorescence, but when allowed to dry on an inert material they fluoresce intensely. The suggested explanation for these phenomena is that a hydration shell surrounding the TC molecule in aqueous solutions quenches the fluorescence, as does an acid pH.

All the native tetracyclines fluoresce a distinctive golden-yellow, which is easily recognisable, and provides a ready means of identifying the presence of the drug. Since the excitation maximum of TC is at 390 mμ., whereas the major spectral line for mercury is about 360 mμ. (Fig.1.) the mercury vapour lamp is not a very efficient source of ultraviolet light for the

exhibition of TC fluorescence. Nevertheless the mercury vapour lamp provides a readily available, cheap and convenient instrument for the study of the presence and distribution of gross TC fluorescence.

Section III

TETRACYCLINE FLUORESCENCE IN BIOLOGICAL TISSUES

General Discussion

The fluorescent property of TC was first used in biological material by Bottiger (1955) to trace the absorption, distribution and excretion of the antibiotic in mice. He found that excretion was by the kidney, liver and gastro-intestinal tract with complete disappearance of the drug in 12 to 24 hours from all organs except bone, where retention occurred.

As will be described later, TC is fixed and retained by a variety of animal and human tissues, and this property is shared by its derivatives, CTC, OTC and DMTC. The physiological and diseased tissues sharing this property include various malignant tumours, bone, teeth, parasites, mitochondria, acute pancreatitis and other inflammatory or necrotic lesions, as will be discussed later.

This thesis is based on the phenomenon of TC fixation in tumours and the demonstration of this property by fluorescence induced by ultraviolet light. As the mechanisms involved may help to elucidate the mode of deposition and fixation of the drug in tumours, details will be given of the same property in relation

to non-malignant tissue. The demonstration of the phenomenon is by the fluorescent property of the drug in whatever tissue it may be laid down, so it seems necessary to discuss, as well as malignant disease, the other sites of deposition.

PART I

PERSONAL STUDIES OF TETRACYCLINE FLUORESCENCE IN TUMOURS

Introduction

The study was commenced in 1961 at the Royal Sussex County Hospital, Brighton and continued later at King's College Hospital, London. Since the first report by Rall, Loo, Lane and Kelly (1957) of the accidental discovery of persistent fluorescence attributed to TC in malignant tissue, there have been a number of reports of the exhibition of this phenomenon with varying success. The series was started with the object of verifying this virtually unique property of the drug, of attaining some skill in the recognition of the characteristic golden-yellow

fluorescence, and then of performing a study of the value of the practical applications of the phenomenon in diagnosis of malignant disease with particular reference to gastric carcinomas. Control series of benign cases with and without TC, and of malignant cases without TC are also examined.

METHODS

The principle of the exhibition of TC fluorescence in tumours is that the patient shall be prepared with TC, an interval be allowed for clearance of the drug from normal tissues, and the tumour shall then be examined in a darkened room under an ultraviolet lamp.

(a) Preparation of Patient with Tetracycline

If the tumour was on the body surface and could be illuminated with an ultraviolet lamp, careful scrutiny for fluorescence was performed before administration of TC. The dosage and route of administration of TC was varied from case to case, partly in an attempt to ascertain the factors governing the intensity of fluorescence, but mainly to fit in an adequate course of TC with an interval of at least

24 hours before the scheduled time of operation. The total oral amount of TC administered varied from 2,000 mg. to 10,000 mg. in divided doses spread over periods ranging from 12 hours to 10 days (see Table 2.) One patient received a combination of oral TC, 1000 mg., and then 8 intramuscular injections of 200 mg. each over 32 hours as he lapsed into coma. None of the patients had intolerance of the TC capsules even when receiving 500 mg. 4 hourly or as much as 10 gm. in total. There were no sensitivity reactions.

The intravenous route was chosen for most of the large bowel carcinomas as it was thought that the faeces, being a route of excretion of TC, might confuse the picture with their own fluorescence. In some instances where time did not permit an adequate oral course of the drug, the intravenous route was chosen. A standard 1000 mg. dose of TC was given in all these cases by intravenous drip of 200 to 300 ml. of normal saline. The duration of the intravenous administration varied from $\frac{1}{2}$ - 8 hours, but if given more rapidly was found to cause unpleasant flushing and a sensation of heat.

(b) Interval from Tetracycline to Examination

The interval from discontinuing TC to the time of examination of the pathological material had wide variations, especially as in some instances the neoplastic tissue was not examined until an autopsy was performed. However, the shortest period was 25 hours and the longest 48 days, although in some lesions involving skin or mucosa, serial examinations of the lesion were made during and after the administration of TC.

(c) Examination of the Lesions

A total of 40 malignant neoplasms were examined with several different ultraviolet lamps, some of which were very early models. All were mercury vapour lamps with an emission as shown in Fig. 1 principally in the 3600 Angstrom band, and were usually of the type used for wide-spread ultraviolet irradiation in physiotherapy or dermatological departments, although equally good results were obtained with the powerful beam for more localised treatment. Indeed, this latter type was tried with its curved quartz rod applicators, as supplied for therapeutic purposes, to

study tumours of the nasopharynx and larynx, but too much light in the visible part of the spectrum was produced for any useful results to be obtained. The most convenient, efficient and portable source used for the majority of the studies was the Hanau Model PL 327 with quartz burner S 81, 0.3 kw.

Tumours and other lesions were examined under three circumstances, operation specimens, autopsy material and in vivo surface lesions of the skin and mouth. The majority of the tissues were operation specimens and were examined fresh and unfixed within one hour of excision. It is important that the specimen is not fixed in formaldehyde as this results in rapid quenching of fluorescence within a few minutes, although deep frozen specimens or those preserved in absolute alcohol retain their fluorescence for several weeks.

The excised operation specimens were examined within an hour, as with the passage of time there is a gradual diminution in the intensity of fluorescence especially if exposed to sunlight. The ultraviolet lamp was directed away from the observers so that the

beam from the lamp did not directly enter their eyes, although of course there was some reflection of the rays. Special protective goggles were tried but these tended to obscure the observations and as the period of exposure to reflected rather than to direct ultraviolet rays was only of the order of 5 to 10 minutes per day no deleterious effects in the form of conjunctivitis nor visual disturbance were noted.

The examination of the specimens was made in a darkened room, but complete exclusion of light was not necessary. Rubber gloves were worn which protected the hands from ultraviolet burns.

The operation specimen was first dried with gauze swabs as it was found that blood on the surface of a tumour obscured the fluorescence, even though there is a tissue penetration of up to 1 mm. An independent observer, usually the surgeon or anaesthetist, checked my visual interpretation of the fluorescence, and a dried smear of TC was used as a standard for identification of the characteristic golden-yellow TC fluorescence.

After scrutiny of the whole specimen for fluorescence, the tumour was cut across with scalpel or scissors so that fresh cut tumour surface could be examined. Any palpable lymph nodes were cut across and the gross appearance of any fluorescence noted.

The autopsy specimens were examined in the same way as the operation specimens. Lesions of the skin and mouth were examined in vivo with the patient in a darkened room and the patient was instructed to keep the eyes closed during the procedure to avoid any ocular damage from the direct ultraviolet rays.

Colour photography of the fluorescent specimens using an ultraviolet filter over the camera lens was attempted at Brighton by the hospital photographer with unsuccessful results, although a black-and-white photograph of a typical fluorescent tumour is shown later.

RESULTS OF ULTRAVIOLET EXAMINATION

Four groups of cases were examined for fluorescence demonstrable by ultraviolet light, as follows:-

1. Benign lesions NOT prepared with TC.
2. Benign lesions prepared with TC.
3. Malignant tumours NOT prepared with TC.
4. Malignant tumours prepared with TC.

The case material and results of these groups are described separately.

1. Benign lesions NOT prepared with TC.

A representative sample of benign lesions were examined from the operating theatre and autopsy room and few benign lesions of the skin were examined in vivo. It was ascertained from the patients concerned and from the case notes, that neither TC nor any derivative had been prescribed in the previous three months.

A total of 20 such benign lesions were examined by the methods described earlier. The clinical details of these cases are not described as there was no characteristic golden-yellow fluorescence to be seen in any case. The lesions scrutinised under ultraviolet light were all proved to be non-malignant on histological examination, with the exception of the two patients with

typical varicose ulcers, and the two patients with burns. The lesions examined were as follows:-

4 Mastopathis
4 Gastric Ulcers
3 Diverticulitis of Colon
2 Varicose Ulcers
2 Burns
2 Leukoplakia of Tongue
1 Uterine Fibroid
1 Cholecystitis
1 Rectal Adenomatous Polyp

In these 20 examinations only the dull yellow autofluorescence of fat and the bluish-grey autofluorescence of other tissues could be seen except for faint lines of blue-white fluorescence along strands of connective tissue in breast lesions or on the cut bowel wall. There was no sign of characteristic yellow fluorescence of TC.

2. Benign lesions prepared with TC.

Various benign lesions were prepared with

varying doses of TC as detailed in Appendix 1. The total dose varied from 2,500 to 8,000 mg. TC spread over 2½ - 7 days. The interval from TC to examination of the lesion was from 24 hours to 3 months, although usually between 24 and 36 hours.

A total of 25 non-malignant lesions were examined as listed below:-

7 Mastopathia

5 Gastric Ulcers

3 Diverticulitis

3 Bronchitis (Bronchial biopsies)

1 Colonic Polyp

1 Crohn's Disease of Ileum

1 Gastritis

1 Varicose Ulcer (examined in vivo)

1 Ganglion of Wrist

1 Keratosis of Tongue (examined in vivo and biopsy)

1 Lipoma

The details of these cases are shown in Appendix 1. as very faint golden-yellow fluorescence was seen in 6 specimens. In 3 benign breast cysts

there was a faint powdering of yellow fluorescence on the inside wall of the cyst, perhaps to be explained by slow exchange of fluid within the cysts. In another benign tumour of the breast there were weak yellow fluorescent strands apparently in the inter-lobular connective tissue bands. A further patient had radiotherapy and simultaneous TC in error at another hospital and was found 3 months later to have chronic mastopathia with cyst formation and granulation tissue. This specimen of breast also showed extremely faint traces of yellow fluorescence. It is stressed that in all these 5 breast masses the fluorescence was extremely scanty and faint, being barely visible under the ultraviolet light. The final case with fairly strong yellow streaks was in a calcified lymph gland close to a lesion of Crohn's disease, although none of the fleshy glands nearby showed any fluorescence.

Normal tissues surrounding malignant lesions were, of course, examined in the fourth group of cases and in none was TC fluorescence to be seen.

3. Malignant Tumours NOT Prepared with TC

Tumours from patients who had not received TC or its derivatives at any time in the previous 3 months, as far as could be ascertained from the case notes or from questioning the patient or relatives, were examined with the ultraviolet lamp. In no instance was there any trace of characteristic TC fluorescence to be seen in any specimen. A total of 25 such malignant tumours were examined and as the TC fluorescent results were all negative, details of the cases will not be given. The tumours examined were frequently autopsy specimens, although some were operative specimens, and some were surface tumours examined *in vivo* and later given TC, being described in the next group in detail. The malignant tumours examined were as follows:-

5 Carcinomas of Breast

5 Gastric Carcinomas

3 Colonic Carcinomas

3 Bronchogenic Carcinomas

2 Prostatic Carcinomas

2 Rectal Carcinomas

1 Basal Cell Epithelioma of Skin

1 Chondrosarcoma of Jaw

1 Squamous Carcinoma of Prepuce

1 Hypernephroma

1 Carcinoma of Ovary

Only normal autofluorescence was seen in all cases.

4. Malignant Tumours Prepared with TC

Altogether 40 malignant tumours were prepared with TC, by the methods which have been given earlier, in oral doses ranging from 2,000 to 10,000 mg. in total or by the intravenous route in a dose of 1000 mg. The interval from administration of the drug to examination of the tumour was also widely variable from 25 hours to 48 days.

Results

Individual details of dosage of TC, interval, fluorescence and histology are given in Appendix 2. and a summary of the dosage of TC, pathology and fluorescence are shown in Table 2.

TABLE 2.

RESULTS OF FLUORESCENT STUDIES OF MALIGNANT TUMOURS

Case	Initials	Total dose TC gms.	Duration of TC	Route of TC (Oral or IV)	Interval (hours)	Pathology	Fluorescence of Tumour
1	EC	1	8 hrs.	IV	64	AdenoCa. Rectum	+++
2	JM	1	6 hrs.	IV	24	AdenoCa. Rectum	?
3	LS	1	1 hr.	IV	66	AdenoCa. Rectum	+++
4	AM	1	½ hr.	IV	48	AdenoCa. Rectum	+++
5	TB	1	2 hrs.	IV	40	AdenoCa. Rectum	+++
6	NW	1	1 hr.	IV	5 days	AdenoCa. Rectum	++
7	JA	4	56 hrs.	O	36	AdenoCa. Rectum	++
8	EF	1	1 hr.	IV	48	AdenoCa. Rectum	+
9	LL	5	5 days	O	6 days	AdenoCa. Colon	++
10	Tr.B	1	4 hrs.	IV	5 days	AdenoCa. Colon	++
11	WS	1	4 hrs.	IV	5 days	Ca. Colon	++
12	VR	1	1 hr.	IV	3 days	AdenoCa. Colon	O (blue)
13	NG	1	1 hr.	IV	40	AdenoCa. Caecum	+++
14	LC	7½	7 days	O	2 days	Ca. Breast	+++
15	DD	3	1 day	O	30	Ca. Breast	++
16	DP	5	5 days	O	7 days	Ca. Breast	++
17	AW	2½	20 hrs.	O	24	Ca. Breast	+++
18	IP	3	24 hrs.	O	28	Ca. Breast	++
19	EB	2	24 hrs.	O	24	Ca. Breast	++
20	AG	1	½ hr.	IV	48	Ca. Breast	+++
21	LH	2	16 hrs.	O	24	Ca. Breast	+++
22	JS	5	5 days	O	44	Ca. Stomach	+++
23	PM	5	5 days	O	60	Ca. Stomach	+
24	MCu	5	2½ days	O	11 days	Ca. Stomach	O
25	HF	5	2½ days	O	60	Ca. Stomach	++
26	JM	6	3 days	O	18 days	Ca. Bronchus	++
27	AP	2½	20 hrs.	O	25	Ca. Bronchus	+
28	DW	5	2½ days	O	30	Ca. Bronchus	+++
29	JW	5	2½ days	O	30	Ca. Bronchus	O
30	HC	1	8 hrs.	IV	70	Sq. Ca. Anus	++
31	WL	3	24 hrs.	O	30	Sq. Ca. Prepuce	+++
32	DT	1	1 hr.	IV	28	Sq. Ca. Skin	++
33	AP	10	10 days	O	48 days	Basal Ca. Skin	+++
34	ME	5	5 days	O	53	Basal Ca. Skin	++
35	JB	1	4 hrs.	IV	48	Malig. Melanoma Glands	O
36	PH	8	4 days	O	8 days	Ca. Renal Pelvis	+++
37	MCl	7	7 days	O	48	AdenoCa. Kidney	++
38	HW	2	16 hrs.	O	30	Salivary Ca.	++
39	EP	2.6	40 hrs.	O & IM	24	Salivary Ca.	+++
40	SC	7½	7 days	O	36	Chondro Sa.	++

Individual case details given in Appendix 2.

Intensity of Tumour Fluorescence

+++	Brilliant
++	Moderate
+	Weak
?	Dubious
O	None

In discussion the self-explanatory terms, "brilliant", "moderate" and "weak" will be applied to the overall impression of the tumour fluorescence rather than to individual areas of variable intensity. Where the term "dubious" is applied any possible fluorescence was so weak as to be difficult to identify with certainty. Such "dubious" cases of fluorescence should be included with the negative results, as there is uncertainty regarding the appearances, and they certainly cannot be regarded as exhibiting definite characteristic golden-yellow colour.

Out of the 40 malignant tumours examined, there were 4 which showed only autofluorescence and no characteristic golden-yellow colour under ultraviolet light. These 4 cases comprised an adenocarcinoma of rectum, secondary deposits in lymph nodes from a malignant melanoma, an adenocarcinoma of stomach, and a fragment from a biopsy of an oat-cell carcinoma of bronchus. In addition, there was one "dubious" fluorescent result in another adenocarcinoma of rectum, which should be grouped with the negative fluorescent results.

Of the remaining 35 tumours examined, 15 gave an overall impression of "brilliant fluorescence", 17 of "moderate fluorescence", although in some of these there were brilliant flecks, and 3 were "weak" although definite yellow fluorescence was visible.

Relation of Fluorescence to Tumour Type

A selection of malignant tumours were examined from different organs, after varying doses of TC, by different routes and with different intervals between the administration of the drug and examination of the tumours.

The largest group was of 13 adenocarcinomas of the large bowel, 8 being of the rectum and the remainder of the colon, but as there is no real histological distinction between them, they will be considered together. The range of intensity of fluorescence was mainly brilliant or moderate, although one showed "weak" fluorescence, one " dubious" and one no yellow fluorescence at all. In this last case there were some intense blue-white fluorescent flecks in the tumour. There seemed to be no relationship between the degree of differentiation and the intensity of the fluorescence,

for the "weak" fluorescence was in a well-differentiated carcinoma, the "dubious" in a poorly-differentiated carcinoma and the carcinoma showing no yellow fluorescence at all was also well differentiated. Lymph nodes were felt to be enlarged in association with 6 large bowel tumours. When sectioned these glands showed varying fluorescence and in them there was histological confirmation of the spread of the disease.

All 8 carcinomas of the breast showed moderate or brilliant characteristic yellow colour on examination with the ultraviolet light. Again the degree of histological differentiation did not seem to influence the fluorescence.

Similarly the histological differentiation did not seem to be a factor in determining the intensity of fluorescence of the 4 gastric carcinomas, two of which were "brilliant", one "moderate", one "weak" and in one no characteristic fluorescence was visible at all.

Of the 4 carcinomas of bronchus, one was squamous and 3 oat-cell in type. One of the oat-cell carcinomas did not show any fluorescence when a fragment of a bronchial biopsy was smeared on a glass slide,

but it is possible that this was due to sampling error rather than a failure of the tumour to take up TC. The remaining tumours exhibited variable but definite fluorescence.

Of the remaining 11 assorted tumours, only one failed to show any fluorescence. This was in lymph gland secondaries from a malignant melanoma. Five were squamous or basal cell carcinomas of skin, two were renal carcinomas, two salivary-gland carcinomas, and one was a chondrosarcoma of jaw. Many of these were examined in situ and all showed moderate or intense yellow fluorescence. The details of the examinations may be seen in Appendix 2.

Relation of Fluorescence to TC Dosage Regimes

The total amount of TC administered orally ranged from 2 to 10 gms. in divided doses over periods varying from 16 hours to 10 days. Neither the total dose nor the duration of the course of the drug seemed to determine the intensity of fluorescence. Both of the patients who received oral TC, but who tumours did not fluoresce, had received a total of 5 gm. TC, an amount which proved quite adequate in preparing many of

those tumours which showed good fluorescence.

The intravenous route was chosen for administration of TC in 15 patients, and in all of them a standard dose of 1.0 gm. was given. The time over which TC was given by intravenous drip varied from ½ to 8 hours. The two cases with dubious or absent fluorescence in the large bowel carcinomas received the drug over 6 hours and 1 hour respectively, and the patient with melanomatous secondary deposits over 4 hours.

There was no appreciable difference in the intensity of fluorescence induced by 1.0 gm. given by intravenous injection and by the larger doses of TC given by mouth. The lowest oral dose administered was 2 gm. over 24 hours and this gave moderately intense fluorescence. If time permits, larger oral doses might prove more efficient in inducing maximum fluorescence, but we have no evidence to support this. As the intravenous administration of 1.0 gm. can produce brilliant fluorescence it may well be that the intensity is related to the blood level achieved rather than the total amount given.

Relation of Fluorescence to the Interval from TC to Examination.

There was a wide variation in the interval between stopping the administration of TC and examination of the tumour with the ultraviolet light, The shortest interval was 24 hours and the longest 48 days. This latter patient had a rodent ulcer of the leg which was not fluorescent before TC. She was given a 10-day therapeutic course of the drug, during which time the ulcer was seen to be moderately fluorescent, and was then discharged home to attend for outpatient radiotherapy. She was admitted 48 days later for surgical treatment, and the moderate fluorescence was much the same as it had been when she was actually receiving the drug, indicating no appreciable dimming of intensity with the passage of time.

Careful scrutiny of the intensity of fluorescence in relation to the period from TC to ultraviolet examination does not reveal any significant diminution of intensity with the passage of time. The shortest interval necessary for clearance of TC from non-malignant tissues has not been determined, but it would seem likely on theoretical grounds that it should not be shorter than 24 hours.

SUMMARY OF FLUORESCENT STUDIES IN BENIGN AND MALIGNANT TISSUES.

Of 40 malignant tumours examined after TC, 35 tumours were shown to exhibit characteristic golden-yellow fluorescence when exposed to ultraviolet light. There was wide variation in the intensity and uniformity of the fluorescence.

No relationship could be discovered between the intensity of fluorescence and the site or type of malignant tumour or its degree of histological differentiation.

There was no apparent relationship between the amount of TC exhibited and the intensity of fluorescence, but the suggestion is made that it is dependent on peak blood levels achieved. With the passage of time the intensity of fluorescence in malignant tumours did not show appreciable diminution, and could be demonstrated for as long as 48 days. The maximum duration was not determined.

There was no apparent reason why 5 tumours (12.5%) should not fluoresce and it is suggested that

this lack of uptake of TC is due to individual idiosyncrasy, the cause of which was not shown.

In 20 benign lesions not prepared with TC there was no characteristic fluorescence.

In 25 benign lesions prepared with TC, faint fluorescence was seen on the inside of 3 benign cyst walls and as faint flecks in 2 benign cases of mastopathia. One calcified lymph node showed fluorescence.

In 25 malignant tumours without TC, there was no characteristic fluorescence.

PART 2

TUMOUR FLUORESCENCE

Published Observations

In 1957, the accidental discovery of TC uptake by malignant tissue was made by Rall, Loo, Lane & Kelly (1957). They were studying the pharmacological properties of a riboflavine antagonist, U6538, by examining tissues under ultraviolet light from a patient with malignant disease who had been given the antagonist. They found fluorescence in portions of the tumour, a metastatic breast carcinoma. When examining malignant tissue from other patients they found that the fluorescence was not the result of their riboflavine antagonist, and the medications received by those patients whose tumours fluoresced under ultraviolet light were reviewed. This suggested that TC, which also shows yellow fluorescence in solution, might be the cause of the yellow fluorescence in tumours. They then pursued the observation of this phenomenon by giving TC to mice or rats by injection,

intramuscularly, intraperitoneally or subcutaneously, for 2 - 5 days. They demonstrated TC fluorescence under ultraviolet light in rodents with various tumours, as follows:-

Tumours showing Fluorescence

Sarcoma	Carcinoma	Hepatoma	Lymphoma	Leukaemia
37 (mouse)	Krebs-2 (mouse)	129P (mouse)	4 (mouse)	P-288 (mouse)
180 (mouse)	Ehrlich (mouse)	134 (mouse)	2 (mouse)	P-388 (mouse)
Yoshida (rat)	Barrett (mouse)	7974 (rat)	-	-
Osteogenic (mouse)	-	-	-	-

Tumours not showing Fluorescence.

Lymphoma 1, Leukaemia 2416, Leukaemia 1210, Harding Passey Melanoma, Cloudman Melanoma S91 in mice.

Rall et al. (1957) in these studies noted that after an intra-peritoneal injection of 2mg. TC to mice, for the first 6 hours the drug appeared in most areas except the brain. Tumour tissue, the liver, parts of the intestines, and bony tissue retained the

fluorescence for 24 hours, but thereafter the characteristic golden-yellow TC fluorescence was seen only in tumour, bone and teeth where it persisted, essentially undiminished until death of the animal in 10 to 20 days. The fluorescence in the tumours was distributed diffusely in the more peripheral areas and was not apparent in the grossly haemorrhagic or necrotic zones.

The same workers demonstrated that 1 mg. TC per mouse, in single or individual doses, produced fluorescence in half the animals with sarcoma 37, up to 4 mg. in most of their animals, and 8 mg. or over in all these mice. Taking the mouse weight as 20 gm. and a dose of 2 mg., with extrapolation to a 70 Kg. man, this would be equivalent to a total dose of 7,000 mg., the usual therapeutic course of TC given over 1 week.

They also demonstrated that all three of the TC antibiotics then in wide usage - TC, CTC and OTC - were qualitatively similar in causing the fluorescence in mouse sarcomas.

Since these initial observations a number of reports (Table 3) confirm the phenomenon of TC uptake

TABLE 3.

GROSS FLUORESCENCE OF TUMOURS AFTER TETRACYCLINE DRUGS - SURVEY OF LITERATURE

Authors	Year	Tumours showing Fluorescence	Tumours etc. NOT showing yellow fluorescence	Notes
Rall et al	1957	2 Carcinoma, Breast	2 Leukaemias	14/19 experimental animal tumours showed fluorescence
McLeay et al	1958) 1960) 1962)	14 Adenocarcinoma, Colon 4 Adenocarcinoma, Breast 3 Adenocarcinoma, Stomach 3 Carcinoma, Bronchus 4 Carcinoma, Oropharynx 2 Squamous Carcinoma, Cervix 1 Adenocarcinoma, Kidney 1 Thymoma, Thymus 1 Squamous Carcinoma, Larynx 1 Adenocarcinoma, Pancreas 2 Metastatic Carcinoma, Bone 1 Adenocarcinoma, Thyroid 3 Osteogenic Sarcoma, Bone 1 Paget's Disease, Bone 1 Ewing's Tumour, Bone	Nil	More than 1000 normal tissues showed blue autofluorescence. More than 200 tumours without tetracycline showed only blue autofluorescence.
Vassar et al	1960	3 Squamous Carcinoma, Cervix 1 Squamous Carcinoma, Skin - for 3 weeks 1 Squamous Carcinoma, Penis 1 Chorionepithelioma Metastasis, Testis to Skin - for 3 weeks 1 Adenocarcinoma, Pancreas - for 4 weeks 2 Undifferentiated Carcinoma, Bronchus - for 8 and 14 weeks respectively	Nil	
Philips et al	1960	8 Adenocarcinoma, Colon 2 Adenocarcinoma, Rectum 3 Adenocarcinoma, Breast 2 Adenocarcinoma, Stomach 2 Reticulum cell Sarcoma, Stomach 1 Hepatoma, Liver 1 Squamous Carcinoma, Lip 1 Squamous Carcinoma, Tonsil 1 Squamous Carcinoma, Mouth (metastatic) 1 Epidermoid Carcinoma, Parotid 1 Squamous Carcinoma, Tongue	2 Adenocarcinoma, Colon) Blue 2 Adenocarcinoma, Rectum) fluor- 1 Adenocarcinoma, Breast) escence 1 Osteogenic sarcoma - Metastatic to chest wall) 1 Squamous Carcinoma Metastasis, site unknown) Dull grey 2 Transitional Carcinoma Kidney) auto-fluor- 3 Adenocarcinoma, Stomach) escence	Fibroadenomas, cystic disease and ductal hyperplasia of breast, thyroid adenomas, mixed parotid tumour, duodenal and gastric ulcers, benign rectal polyps, diverticulitis, villous adenoma of rectum, haemorrhoids, numerous fresh normal tissues from tetracycline-prepared patients, and carcinomas from patients who had not had TC ALL showed only autofluorescence
Bailey & Levin	1961	1 Osteogenic Sarcoma secondary to Paget's Disease 3 Osteogenic Sarcoma	1 Fibrosarcoma 1 Anaplastic Sarcoma (without calcification)	One of the Osteogenic Sarcomas had much calcification and fluorescence, but the other 2 of the fibrous type had only little fluorescence
Prout et al	1962	3 Prostatic Carcinoma bone metastases		Although primary tumour & soft tissue metastases showed no fluorescence
Carter et al	1962	7 Carcinoma, Stomach 12 Carcinoma, Colon 5 Carcinoma, Breast 2 Benign Tumours, Bone	3 Carcinoma, Colon 4 Carcinoma, Breast 6 Benign Tumours, not bone	
Milch et al	1961	1 Reticulum-cell Sarcoma) 1 Osteogenic Sarcoma) 1 Malignant Giant Cell Tumour) - of bone 5 Metastatic Tumours)	4 Osteochondromas 2 Chondrosarcomas	-fluorescent only at cartilaginous base. -one no fluorescence and in the other Fluorescence wherever evidence of matrix calcification with Alizarin red S positive areas \ only close to the invading margin
Takayama	1964	8 Carcinoma, Breast 2 Carcinoma, Stomach	3 Mastopathy 1 Chronic Interstitial Mastitis 4 Benign Tumours	Extremely vivid yellow of "panniculus adiposus" of breast (different from TC fluorescence)

and retention by malignant tumours as demonstrated by characteristic fluorescence under ultraviolet light.

McLeay (1958) was the first to make clinical observations of this fluorescence. He examined surgical specimens of normal tissues from over 1,000 patients without cancer and from 42 patients with cancer, none of whom had received TC, and was able only to demonstrate autofluorescence on examination of the tissue with an ultraviolet light source of 3660 Angstrom units. An additional control group was of 11 patients who had received TC but had benign lesions, and they similarly showed only autofluorescence. However 11 patients with malignant disease prepared with TC, allowed an interval of 12 hours in which to clear TC from normal tissues, and from whom the surgical specimen was then examined, showed in all cases a characteristic yellow fluorescence in the tumours.

By 1962, McLeay and Walske had extended this original series of observations to include a control group of more than 200 carcinomatous cases who had not received TC and showed only blue autofluorescence. He had increased his group of carcinomas to 37, all of which

exhibited golden-yellow fluorescence under ultraviolet light. Using biological assay they found a TC concentration of 10 µg. per gm. in good fluorescing tumours ranging down to 1.0 µg. per gm. in poorer fluorescing tumours. On preliminary ultraviolet microscopy they thought that the fluorescence was situated within the tumour cell.

Vassar et al. (1960) challenged the specificity of the fluorescence. Whilst in all 9 cases of carcinoma pre-treated with TC they were able to demonstrate typical bright yellow fluorescence, they comment on the difficulties of the inexperienced observer with the auto-fluorescence of adipose tissue (pale yellow), and the microscopic autofluorescence of lipo-fuscin (brilliant yellow orange), porphyrins (red), and histocytes containing granules of lipoproteins. Their microscopic studies showed fluorescence invariably confined to macrophages and tissue debris in the tumour stroma, and stress that in no case was a malignant cell seen to fluoresce. They point out that in patients with non-specific skin ulcers fluorescence may persist up to 72 hours, compared with periods of up to 14 weeks fluorescence after TC in malignant tissue.

In the same year, Phillips et al. (1960) also found only autofluorescence in benign tumours or inflammatory diseases, but in 12 out of 35 malignant tumours the characteristic yellow fluorescence was not seen as it was in the other 23 malignant cases. In half of these apparently negative cases a bluish fluorescence was seen which the authors think might be a very weak positive result as it was so distinct from surrounding autofluorescence. They thought that their negative or equivocal results might be due to an inadequate dose of TC being administered in those cases, and certainly their results tend to indicate that in part the dose of TC administered influences the intensity of yellow fluorescence. Extraction of TC from tumour tissue was performed with a fair co-relation between the intensity of fluorescence and the amount of drug obtained - up to 7.6 mg./gm. tumour tissue, a much higher concentration than that found by McLeay & Walske (1962). Their impression was that the highly anaplastic tumours had a greater avidity for TC than the well-differentiated.

Carter et al. (1962) found TC fluorescence in 24 out of 31 malignant tumours after intravenous TC

preparation of the patients, and the histological studies suggested the fluorescence was not within the tumour cells. Only 2 benign bone tumours showed fluorescence.

The preceding studies have included mainly alimentary and breast cancers, but bone tumours have been shown by McLeay et al. (1960) and Bailey & Levin (1961, 1963) to exhibit the same phenomenon (see Table 3.) and it is of interest to note that the second authors felt that the fluorescence was related to the presence of active calcification in the tumours or at sites of reactive bone formation. Their two negative results occurred in a fibro-sarcoma and a highly anaplastic fibrous tissue sarcoma, neither of which showed any evidence of calcification.

Milch et al. (1961) studied 10 malignant bone tumours of which 5 were metastatic carcinomas and demonstrated TC-induced fluorescence in them all. It is of importance however that in addition he demonstrated fluorescence in 4 benign osteochondromas. His explanation, supported by ultraviolet microscopy, was that the localisation of fluorescence occurred only in

those areas in which matrix calcification was observed, the same areas where Alizarin red-S staining occurred and where there were distinctive morphological features in the matrix on phase, dark field and polarized light studies.

However, Ackerman and McFee (1963) reporting their studies on an unspecified number of fresh autopsy specimens where the patients had received therapeutic courses of a TC compound at varying intervals before death, stated that the deposition of TC in malignant tissue was "erratic and undependable". The total dose of the drug was not stated but they could find no obvious pattern for predicting the presence or lack of fluorescence in relation to histology, dose or route of administration. Unlike previous workers, they found that fluorescence was often associated with necrotic elements of malignant tissue. In addition they demonstrated bright areas of yellow fluorescence in non-cancerous tissue. Apart from the well-recognised retention by bone, fluorescence was shown in 3 specimens of pancreatic fat necrosis, a necrotic area of diverticulitis, pyonephrosis, a gall stone and

"several other necrotic areas" in autopsy tissues. It should be noted however that in their studies the interval from discontinuing the drug to death was "at least 16 hours" so it is possible that in sites of poor excretion and poor viability a delayed clearing of TC was being observed. Again they note the presence of TC deposits on ultraviolet microscopy in necrotic tissue and stroma rather than within malignant cells. In studies on rats and hamsters using transplanted Novikoff hepatomas and amelanotic melanomas, no fluorescence was demonstrated, perhaps due to tumour type differences.

Discussion

Since the original observations of Rall et al. (1957), it has been shown by McLeay (1958), Vassar et al. (1960), McLeay & Walske (1962) and Takayama et al. (1964) that in their experience all malignant tumours except leukaemias fluoresce a golden-yellow colour after preparation of the patient with TC (see Table 3.) The total number of soft tissue malignant tumours examined by these authors is 56. Large numbers of normal tissue and benign lesions with and without TC preparation, and malignant tumours without TC, showed only autofluorescence. Vassar et al. (1960) commented on the difficulties of interpretation of autofluorescence by the inexperienced observer.

However, Phillips et al. (1960) found only two-thirds of their malignant tumours fluoresced bright yellow. Of their remaining 12 malignant cases, half showed a definite blue fluorescence which they thought was a weak TC result as it was so distinct from grey or dull yellow autofluorescence and as they tended to use rather low dosage regimes. Autofluorescence was seen in 21% of their malignant tumours, in all of their

benign cases prepared with TC, and in all malignant tumours not prepared with TC. It is of interest to note that in my own series, one rectal carcinoma showed this same blue fluorescence.

Similar experience was obtained by Carter et al. (1962) with 77% of their malignant tumours showing yellow fluorescence after intravenous TC preparation. This percentage compares with 87% yellow fluorescence in my own series of soft tissue malignancy.

Results with malignant bone tumours show a rather different pattern. It would appear from the results of Bailey & Levin (1961), and Milch et al. (1961) that in bone sarcomas fluorescence is only to be found if there is evidence of matrix calcification. Secondary carcinomatous deposits in bone show fluorescence, but Prout et al. (1962) observed that in the case of prostatic carcinomas the primary tumour and soft tissue metastases did not fluoresce. It would also appear that benign bone tumours may fluoresce, especially at the bases where calcification may be occurring. In my own series only one malignant bone tumour, a chondrosarcoma of jaw was seen and showed persistent fluorescence.

The major attack on the specificity of TC fluorescence was levelled by Ackerman & McFee (1963), but as their article was unsupported by specific numbers and the interval from TC to examination in some cases was as short as 16 hours, it is difficult to assess the importance of their criticisms. Certainly there may be persistence of TC fluorescence in necrotic and calcifying tissues, as will be discussed later in the sections on the nature of the fluorophore in sites other than malignant tissue, but this does not invalidate the tumour results. Indeed, in benign lesions in the present series, fluorescence could be found in minute amounts in cyst walls and tissue of some benign breast tumours, possibly due to the calcium content of milk, but it is emphasised that there was no comparison with the amount seen in malignant tumours. Only one calcified gland showed a moderate amount of yellow fluorescence.

The relationship between degree of differentiation and intensity of fluorescence has been discussed by several authors. McLeay et al. (1958) suggests that carcinomas with the most rapid growth showed most

fluorescence, and Phillips et al. (1960) had the same impression. In my own series the differentiation of a tumour did not seem to determine its fluorescence.

The limit of duration of TC fluorescence has not been analysed but in my own series it persisted for 7 weeks in one patient and Vassar et al. (1960) observed fluorescence 14 weeks after administration of TC.

With low dosage schedules of TC there may be an increasing deposition of TC in malignant tumours with increasing dose (Phillips et al., 1960). It is suggested that, provided adequate blood levels are achieved, either by 5 gm. orally in divided doses or by 1.0 gm. intravenously, there is no direct correlation of fluorescent intensity with total dose of TC. No cause has been demonstrated by any workers as to why some malignant tumours do not fluoresce or fluoresce less intensely than others.

The Fluorophore in Malignant Tissue

Whereas fluorescein was observed by Moore (1947, 1948.b) to have a tendency to localise temporarily in tumour tissue when administered parentally,

TC compounds appear not only to localise but also to concentrate and remain bound to malignant tissue for prolonged periods. The mode of deposition of the TC and the nature of the fluorophore in tumours has stimulated much research for this remarkable property of prolonged sequestration of drug by tumour tissue has obvious therapeutic implications as yet unrealised.

The major problem to be discussed in this section is the reason for localisation of TC in tumours as opposed to most other normal tissues (with the exception of bone and teeth) for there is no doubt that preferential concentration of TC within the malignant tumour occurs whilst TC is being administered. Although it has been shown that normal soft tissues will fluoresce whilst therapeutic blood levels obtain, the tumour, even at that time, shows more intense fluorescence than surrounding normal tissue. Secondly, the reason for the prolonged retention of TC fluorescence in the tumour is of importance particularly as it may shed some light on the differences in metabolism of malignant disease as compared with physiological tissue.

Investigations into the nature of the fluorophore were started by the original observers of the phenomenon using CAF_1 mice with Sarcoma S-37 (Loo, et al. 1957). After preparation with intraperitoneal TC, they obtained tumour tissue which they homogenized in water. The homogenate was dialyzed against dilute hydrochloric acid (0.1 N), as TC remains stable for at least 4 hours in this concentration of acid. When the pH of the filtered dialyzate was adjusted to above 7 the fluorophore came down as a flocculent precipitate which flurosced bright greenish yellow under ultraviolet light. With a dialyzate similarly prepared from tumour tissue af control mice which had received no TC, a similar flocculent precipitate was obtained. This was however not fluroscent. In both cases the precipitates gave positive ninhydrin and biuret tests, thus suggesting their peptide nature. A more concentrated solution of fluorophore was prepared by these workers by centrifugation of the dialyzate. After saturation of alkaline dialyzate with salt the fluorophore could be partially extracted into butanol. Examination of the

ultraviolet absorption spectrum of this extract compared with the spectrum of TC itself in butanol revealed that the excitation peak at 367 mμ. of the parent compound had undergone a shift of 15 mμ. to 382 mμ., the blank itself obtained from control rats without TC showing no absorption at this range. Such a bathochromic shift could be ascribed to the formation of a complex between TC and peptide in tumour tissue.

Paper electrophoresis showed the migration rate of the fluorophore at pH 8.6 differed quite noticeably from that of TC and similarly, paper chromatography showed quite different values. Moreover in weak acid neither the fluorophore nor the control non-fluorescent precipitate displayed any new absorption bands or any shift of existing bands. Loo and his associates therefore concluded that the complex formed between TC and the peptide must be a loose one with ready dissociation in acid. Assuming that the peptide of the control and that of the TC-treated tumour exhibit identical absorption patterns, they were able to calculate the absorbance owing to the prosthetic group and this was found to be exactly identical with

the spectrum of TC at that concentration and pH.

The identification of this prosthetic group of the fluorophore as being TC is supported by the evidence that after exposing to normal hydrochloric acid for 1 hour, the spectrum of the prosthetic group can be shown to be the same as that of anhydrotetracycline, as would be expected, for the effect of a strong acid is to dehydrate the TC molecule to this compound with rapidity. Thus these authors, without actually isolating the prosthetic group, had demonstrated that the fluorophore in the tumour from a TC-treated mouse was most likely to be a complex formed of the parent compound and a peptide.

Kohn (1961b) also of the National Cancer Institute, Bethesda, quotes unpublished experiments of Lane, Titus & Loo indicating that the TC complex in tumours is dissociated by EDTA. His experiments were designed to test the hypothesis that metal ions may act in the systems of TC fixation in tumours and bone by simultaneously binding TC and suitable macromolecular sites. Higuchi & Bolton (1959) found that deoxyribonudeic acid (DNA) formed a very firm complex

with TC. Kohn therefore studied the interaction of TC with DNA and with serum albumin in the presence and absence of divalent cations, using the technique of equilibrium dialysis. He found that little or no TC became bound to DNA in the absence of divalent metal ions (zinc, calcium, manganese, magnesium) but that binding did occur in their presence. In the case of serum albumin, TC binding occurred in the presence of zinc but not calcium. TC chelates of calcium and zinc showed fluorescence which was enhanced markedly by the presence of DNA and albumin. As a result of his fluorescent experiments he felt he had shown support for the picture of a bond between DNA (or albumin) and the metal component of the TC chelate.

That there is a protein-binding of the drug was confirmed by Wozniak (1960) who studied the binding of TC drugs with dog and human plasma by dialysis equilibrium technique under physiological conditions of temperature and pH. The percentage bound was about 32% for TC, 51% for DMTC and 64% for CTC. A study with TC gave no difference in binding at 37 or 4°C. but binding increased with increasing alkalinity from pH 6.7,

when 30% was bound, to pH 8.1 when nearly 60% was bound.

Machado et al. (1964) studied the localisation of TC in transplantable sarcoma-37 tumours in mice utilising the radioactivity and fluorescence of tritiated TC as measures of concentration. The tritiated TC used in this study differed from the parent molecule only by the substitution of tritium for hydrogen in the 7 - position. This study on large groups of animals showed three different patterns of organ fluorescence. Organs such as the liver and heart presented a diffuse homogeneous yellow fluorescence on the cut surface appearing within the first 24 hours of injection of the drug and not present to significant degree after 48 hours. Bone fluorescence was also homogeneous, appeared within 24 hours and persisted for the duration of the experiment (8 days). Tumour fluorescence was not homogeneous, appeared within 24 hours and was distributed in well-demarcated areas differing in shape and size, and located both on the surface and within the tumour. Fluorescence was related to tumour age, i.e. the duration from

implantation to sacrifice. Forty seven per cent of the 10-day old tumours and 100% of the 13-day or older tumours were fluorescent. None of the tumours of less than 10 days of age showed the characteristic localised fluorescence. Similar results were obtained with the more slowly growing Ehrlich's solid tumour in C57 mice.

By fluorescent microscopy, against the faint greenish-blue autofluorescence contrasted the spotty bright yellow to gold fluorescence throughout the tumour cell cytoplasm, a finding in agreement with the earlier observations of McLeay & Walske (1962) in human tumours. No fluorescence was seen in the intersticial spaces and the nuclei produced a negative image. The fluorescent spots all corresponded to groups of tumour cells in more or less advanced states of degeneration. The cytoplasm of these cells was always eosinophilic and most of the nuclei were small and hyperchromatic. No macrophages were seen in the fluorescent areas in contradistinction to the report of Vassar et al. (1960) who reported the localisation of TC in tumours to be in the associated macrophages. From the fluorescent studies

it seems that TC is retained in greater quantities in those areas of the tumour that are dead or dying. The studies using tritium-labelled TC confirm this finding, by showing that the apparent concentration is not an artifact due to selective quenching of the fluorescence.

The greatest intensity of fluorescence was seen in the transition zone between the obviously viable and the obviously necrotic fragmented cells. In the centre of a frankly necrotic area the absence of fluorescence, as noted by Rall et al. (1957) and Phillips et al. (1960), could be explained by the lack of blood supply to provide a route of entry for the TC. One of the earliest signs of cell injury is the loss of selective membrane permeability which might lead to a passive assimilation of free or protein-bound TC.

Majno et al. (1960) in a critical series of experiments on the time intervals of cellular death and necrosis comments on the denaturing of proteins giving rise to increased autofluorescence of liver cells developing between 15 and 30 minutes after sudden complete ischaemia. It may be that the TC uptake by

necrosing tumour cells is related to this protein denaturation in the cytoplasm.

Shear (1933) reviewing the earlier literature of tumours concluded that the non-necrotic areas of degenerating tumours contained relatively little calcium whereas the necrotic areas contained extensive amounts of calcium. A later study by Suntzeff & Carruthers (1944) revealed a linear relationship between increasing necrosis and increasing calcium content up to 0.077 mg. per 100 mg. in epidermal tumours of mice, whereas the calcium content of the completely viable tumour, 0.009 mg. per 100 mg., was quite depressed relative to the tissue of origin, 0.044 mg. per 100 mg. epidermis. Thus a body of circumstantial evidence lends some credence to the hypothesis that the predilection of TC for devitalised tumour tissue exists by virtue of its ability to chelate calcium.

When Machado et al. (1964) administered Alizarin red-S, an acid dye which chelates calcium, systemically to mice bearing S-37 tumours, the dye was visible grossly after 36 hours only in the necrotic

areas of the tumours and in the bones. However, since closely related dyes may localise either in the necrotic (trypan red) or in the viable (trypan blue) areas of tumours as shown by Duran-Reynals (1939), this localisation of Alizarin red-S cannot necessarily be attributed to its known calcium-chelating propensity, although it seems likely.

The concept of calcium-chelation being a major mechanism for sequestration in tumours has biochemical support in the work of Albert (1953), Albert & Rees (1956) who demonstrated in vitro a high avidity of the TC's for calcium. In histological studies by Malek & Kolc (1960) of spontaneous mammary tumours in bitches, differing degrees of TC-induced fluorescent intensity seemed to be related to the degree of calcification, although the exact site in relation to the malignant cell was not described.

Moreover, a detailed study by Riley (1963) of the fluorophore in a transplanted human tumour in the cheek pouch of cortisone-treated Syrian hamsters showed TC-induced fluorescent islands which tended to enlarge, never decreased and were not islands of

necrosis, inflammation or infection. Ultraviolet microscopy revealed the golden yellow fluorescence of TC to be present within tumour cells. The presence of calcium, as demonstrated by the von Kossa stain and by microradiography could be well correlated with the presence of fluorescence. Calcium was always present in those areas of the tumour which fluoresced. When sections were treated with EDTA to remove calcium, fluorescence could not be demonstrated. It is emphasised that gross calcification of the tumour was not present. There was not calcification of the stromal connective tissue elements of the tumour but rather microcalcification within tumour cells.

In contrast Yabe et al. (1964) did not find selective, persistent TC-induced fluorescence in gastric adenocarcinomas transplanted into the subcutaneous tissues of C57BL mice. The tumour was descended from a gastric adenocarcinoma induced in mice by injections of 20-methylcholanthrene into the stomach wall. The reason for failure of selective localisation in this tumour is not clear, but it could be simply an idiosyncracy of that particular tumour, or it might conceivably be

related to "tumour age", as the authors simply say that they exhibited TC when the tumour was "two or three weeks old".

However there is evidence for other tissue components as well as calcium, peptides and proteins having a capacity for binding TC. There is an affinity for serum beta-lipoproteins which is so pronounced in the presence of calcium ions that the reaction has been proposed by Lacko et al. (1959) as method for the quantitative precipitation of this fraction. This particular affinity of TC exists also for the beta-lipoproteins of liver mitochondria (Pamukco et al. (1963), although Du Buy & Showacre (1961) feel that this localisation plays an insignificant role in the phenomenon of macroscopic tissue fluorescence. Similarly, although it has been shown in the beautiful autoradiographic studies by Andre (1956) using tritium-labelled TC that there is a significant amount of TC in the cell nucleus, there is no evidence for tumour-cell nuclear fluorescence.

Since in addition to TC several chemical species of grossly unrelated structure, such as haematoporphyrins (Rassmussen-Taxdal et al. 1955) radioiodine

(Clode et al., 1961) and dyes such as trypan red (Duran-Reynals, 1939) and fluoroscein (Moore, 1947), appear to localise preferentially in tumour tissue, a single basic physiological defect or physio-chemical interaction might underlie the behaviour of all of them. There is probably an element of increased capillary permeability involved in the less viable areas of tumours (Duran-Reynals, 1939) predisposing to leakage of various native and foreign materials into these areas. However, there undoubtedly exists some sort of mechanism for binding or sequestering these substances from the general circulation. In the case of TC it appears most likely that chelation with calcium is the most important factor in the remarkably prolonged retention of this fluorescent drug by malignant tissue.

PART 3

BONE FLUORESCENCE

The localisation of TC drugs in bone is of relevance to their localisation in tumours in so far as the mechanism for deposition may be similar and the exhibition of the phenomenon is by the same observation of a characteristic golden-yellow fluorescence when the material is exposed to ultra-violet light. It therefore seems desirable that an outline should be given of work in the field of TC-induced bone fluorescence.

Normal Bone

Following the initial observation of tumour fluorescence by Rall et al. (1957) the same group of workers, Milch, Rall and Tobie (1957, 1958) report their studies on bone from various experimental animals. After parenteral TC 5 to 200 mg./Kilo, they demonstrated fluorescence under ultraviolet light lasting in soft tissues for 30 minutes to 6 hours. By 12 hours the fluorescence was confined to bone and teeth. Brain at no stage showed fluorescence and

after 12 hours it could be demonstrated in an occasional specimen of liver. When once the fluorophore was deposited in bone it was not displaced over a period of 10 weeks observation of the experimental animals. It was observed that young bone showed the most intense fluorescence, whereas old bone tended to give a less intense creamy colour. Articular and epiphyseal cartilage showed under ultraviolet light, both grossly and with ultraviolet microscopy, a deep blue or bluish-grey autofluorescence which contrasted vividly with the golden-yellow fluorescence of new bone proliferation. Periosteal and endosteal surfaces were more brilliant in young animals than in old. They also showed that fluorescence in bone could be demonstrated within 30 seconds of exhibition of the drug, even when doses as low as 0.3 mg./Kilo were used.

It was noted that TC was deposited in the same sites as Alizarin as reported by Cameron (1930), and in the same sites as Radio-calcium ($Calcium^{45}$) as reported by Arnold, Jee & Johnson (1956). There is a structural similarity of TC with its four 6-membered rings and Alizarin with its three similar rings. It would seem

possible that both may bind to calcium by the same, or similar, mechanisms.

Thus the distribution of tetracycline in bone is not homogeneous and is determined by the rebuilding process and free surface chemistry of the bone mineral. Sites of new bone formation show the highest concentrations but old bone is also able to bind TC in low concentrations owing to calcium ions on the surface of the apatite. The amount of TC deposited per gramme of bone depends on the number of reactive cylinders or osteons in the cortex and on the proportion of cortical to cancellous bone. Turnover, or remodelling, usually occurs at a rather higher rate in cancellous bone than in cortical bone, and thus more calcium ions are available for chelation with TC.

The duration of binding of TC in bone has been shown by Frost (1961) to be for at least 9 years and the drug can be detected with ease in bone sections by the characteristic fluorescence when exposed to ultraviolet light. These properties can be utilized to study skeletal metabolism, particularly as there is preferential incorporation into new bone deposits.

It appears from the work of Harris, Jackson & Jowsey (1962), comparing microradiography, Ca^{45} autoradiography and fluorescent microscopy after TC, that all actively growing surfaces are intensely labelled by TC. There are, however, other low-intensity sites of incorporation of TC, namely diffuse distribution, "edge sclerosis" (a thin rim of increased mineral density lining the inner edge of some non-growing osteons, trabeculae and Volkmann's canals), and finally surrounding many lacunae. A point which must be stressed is that actively growing bone shows a higher concentration of the fluorophore and is therefore distinguishable from the low-intensity sites detailed above. However new bone formation is often so focal that interpretation of metabolic activity based on observations of a limited sample is hazardous. Nevertheless, as Frost et al. (1960a) have shown, TC is most valuable as a safe, convenient and important tool in the study of bone formation in vivo. As TC appears to be fixed in bone, giving staining of Haversian canals, osteocyte lacunae in Haversian systems and calcified cartilage whilst they are actively

mineralising, retrospective studies for many years after TC administration may be performed.

Pathological States of Bone

A. Teratogenic and Bone Growth Effects

TC has been shown by Bevelander and his co-workers (1960ab) to cause skeletal mal-development when given to the larval sand dollar and to the chick embryo. This work was extended by Urist & Ibsen (1963) who found that saturation of the rapidly growing skeleton of a chick with OTC caused the development of rachitic deformities and interference with calcification. Tubaro (1964) continued this work on chick embryos and found that DMTC produced many more foetal abnormalities than did TC or CTC, a feature which he related to its greater stability.

In humans, apart from a warning of the theoretical danger with one case report from Carter & Wilson (1962) in relation to foetal danger, no further cases appear to have been reported. Nevertheless the premature infant was shown by Cohlan et al. (1963) to have a temporary 40% depression of normal skeletal growth if given TC, and in the rat a 28% depression of

foetal growth could be caused by transplacental passage of the drug, so there is good reason for caution in the prescribing of this drug in pregnant women or infants.

B. Pyogenic Osteomyelitis.

This disease even in this day of effective antibiotics still poses a therapeutic problem. Frost et al. (1960b) point out, using TC as a marker in their experiments, that bone is highly impermeable when dead even though those channels containing a patent blood supply in life showed surface staining whilst TC was being administered. If small portions of dead bone are present slow diffusion of antibiotic to the bacteria in the many spaces of bone might be effective in sterilizing the bone, provided the drug be given for long periods of time. However, large pieces of dead bone will never be permeated and thus the disease runs a relapsing course from a bacterial reservoir of the dead bone of very high diffusion impedence.

The antibacterial activity of TC after its deposition in bone has been studied by Anderson et al. (1959) using rabbits' bone; the animals having been

prepared previously with TC. Those animals which had received parenteral TC within 96 hours of sacrifice showed an antibacterial activity against test organisms, but if more than 96 hours had elapsed there was no inhibition of organisms even though the bone showed fluorescence. The explanation may be either that of slow diffusion of the drug in bone or the formation of a firm inactivating bond to bone at about 96 hours in the process of mineralisation. In contrast Boyko (1959) reports the retention of antibacterial activity by bone fluorophore for up to 5 weeks in mice and 2½ months in rabbits (the limits of his observations) after administration of TC. Plaza-Roca (1959) suggests that possibly liberation of active antibiotic might occur during resorption of bone, such as bone grafts if obtained from the iliac crest, the last region in man to ossify completely.

C. Fractures

Malek & Kolc (1960) studied the penetration of CTC into animal tissues affected by different pathological processes. In fractures intensive absorption by the entire bone from joint to joint was shown by highly

intense fluorescence, while the two broken ends and adjacent area of the fracture did not fluoresce. The fractured bone seemed to absorb the antibiotic to the detriment of other bones. In later stages of healing, fluroescence moved increasingly into the area of the callus. Experimental fractures have been studied by Urist & Ibsen (1963) who found that during the first week of healing the bone formed in the callus was highly reactive, binding OTC in high concentrations with progressive reduction thereafter.

D. Osteoporosis

The phenomenon of TC fluorescence has been used to study this condition (Urist et al. 1962b) but results are difficult to interpret because fluorescence can be seen only in that 1% of the skeleton which is exchangeable, labile or reactive, whereas the 99% remaining is not labelled. Patients with osteoporosis may have high or normal accretion rates, or even, in severe senile osteoporosis the accretion rate may be low (Urist & McLean, 1963).

E. Arthritis.

Studies have been made on arthritic femoral

heads by various workers including Milch (1963) with the observation that shortly after an injection of TC these femoral heads do not exhibit fluorescence as would be expected if the blood supply were intact. This failure of blood supply was found in all forms of hip arthritis, including rheumatoid and post-traumatic forms, but whether this is a primary or secondary phenomenon is uncertain.

F. Paget's Disease of Bone

McLeay et al. (1960) and Urist & Ibsen (1963) have noted vivid TC fluorescence in the calcified mosaic of Paget's Disease with the suggestion of high calcium turnover in the affected sites.

The Nature of the Fluorophore in Bone

Titus et al. (1958) investigated the fluorophore found in bone in TC-treated rabbits. By similar methods to those given on tumour fluorophore (see earlier) they demonstrated that the fluorescent material in bones after TC was most likely to be the unaltered TC as it was possible to obtain the absorption spectrum of TC from bone-fluorophore solution. Paper chromato-

graphy and the use of strong acids supported this conclusion as with tumours. They found that the fluorescent material could be removed by ethylenediaminetetra-acetic acid (EDTA) at pH 7.3 from the fluorescent bone and since this compound is known to chelate metallic cations, it was suggested that such metallic cations were involved in the binding of TC to the bone structure. This suggested that TC is either primarily bound to the inorganic structure of bone or is bound to the organic matrix through metallic cations. This was felt to be consistent with previous observations by Regna et al. (1951) that these drugs form chelate compounds with metallic cations, such as calcium, at physiological pH.

Finerman & Milch (1963) reported additional in vitro evidence for the binding of TC to calcium. Using ground fat-free, cortical animal bone powder treated with buffered TC solution, they were able with a spectrophotometer to calculate the percentage TC absorbed. Significant binding occurred to the fat-free bone powder. No binding was observed however with bone salt which had been pretreated with EDTA and which

contained no detectable calcium. Ethylenediamine (ED) was used to treat the bone powder to remove the organic phase and this preparation avidly bound TC to the same extent as did a preparation of calcium phosphate, but to a greater extent than calcium carbonate. It therefore appeared that ED treatment, by removing the organic phase, appeared to remove either a diffusion barrier or an otherwise hindering bond between crystal and matrix phases. An apparently greater total calcium crystal surface, approximating to that of inorganic calcium phosphate, was thus made available to the TC. Conversely, treatment with EDTA in effect removed the crystal phase so that no TC binding occurred. These workers therefore concluded that persistence of TC in biological tissues is probably directly related to the calcium content of that tissue. They felt that it seemed reasonable to conclude that TC probably reacted primarily with calcium ions in hydroxyapatite-seeded nucleation sites on collagen fibrils. This would certainly explain the localisation and persistence of TC *in vivo* in newly proliferated bone and in certain areas of calcified cartilage as described by Milch *et al.* (1958, 1961).

Deleu & Bohr (1964), having shown a diffuse uptake of TC by bone grafts, investigated further this phenomenon in bone devitalised by freezing, boiling, storing in alcohol or by simply keeping it as fresh bone. For 24 hours the bone was soaked in a solution of TC in citrated plasma and then washed for 48 hours. They found that mineralized dead bone took up TC on every surface. As in living bone there was a greater uptake in the growing part, in the zone of demarcation, within the osteoid seam. In non-growing surfaces, like osteons which show edge-sclerosis or in resorption cavities, there was limited uptake. Besides this surface incorporation, there was also a diffuse uptake which was more prominent in cancellous than in cortical bone. Thus the uptake of TC is not dependent on the activity of living bone cells or on the physical properties of the intercellular components, which are greatly modified by boiling or alcohol. The binding cannot be directly related to calcium uptake because there were no free calcium ions in the citrated plasma.

Additional support for calcium being an important factor in the binding of the TC group of drugs

in biological material was offered by Ibsen & Urist (1962) in detailed studies of reactions of OTC and varying levels of calcium at physiological ranges of pH, ionic strength and drug concentration by spectrophotometric methods. This revealed a stepwise addition of metal to OTC. When 1:1 or higher calcium to OTC complexes are formed it seems unlikely that all of the co-ordinating positions of calcium are satisfied. This apatite-surface calcium could react with OTC and permit accumulation of this fluorophore in bone. Possibly spacial configuration of apatite-surface calcium allows two or more calcium atoms to bind simultaneously one molecule of TC, enhancing the force of attraction.

In an accompanying article Urist et al. (1962a), using biological effects of altered concentrations of free ionised calcium and OTC on isolated frog's heart, were able to obtain remarkable agreement with the concept of a 1:1 complex of metal to OTC found by spectrophotometry. They point out that the usual human dosage of this drug (1 gm. per day) produces a serum concentration of approximately 1.0 µg. per ml., of

which 75% is protein-bound and only 25% free in solution to form complexes with metal ions. Under these conditions calcium and OTC formed complexes chiefly in the 1:1 ratio, but in bone matrix where calcium ion concentration may be higher, the proportions might increase to 2:1 or even 3:1. Because of the high concentration of calcium ions, in relation to other polyvalent ions in blood, the Mass Law would operate in favour of calcium complex formation and this effect would be localised and enhanced in calcifying tissues.

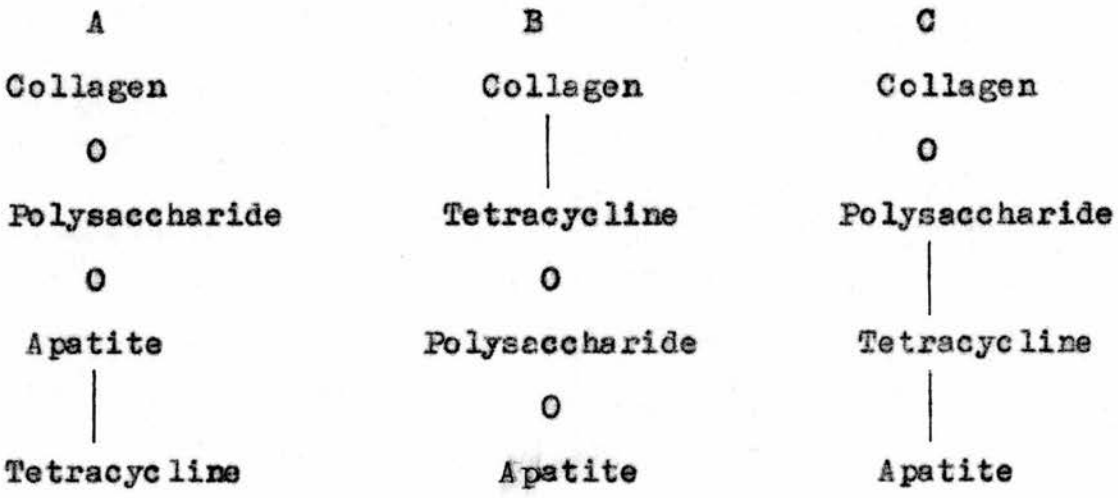
These authors point out that although the inorganic calcium phase of the binding in bone is emphasised, it is also possible that TC forms complexes with an organic constituent during synthesis of calcifiable new matrix. Non-calcifying tissues such as tumours, healing wounds, sclera, tendons, scales, hair and feathers fluoresce, particularly after prolonged administration of TC and this could be construed as evidence that the fluorophore in bone is bound to polysaccharide (Plaza-Roca, 1959), polypeptide (Loo et al. 1957), or collagen (Milch et al., 1961).

It is suggested by Urist & McLean (1963) that

the three possible sites of binding of the fluorophore in bone are on the crystal surfaces as complexes with calcium, as complexes with collagen or in complexes that share calcium ions with polysaccharide in newly mineralized tissues (Fig. 3.).

Figure 3

POSSIBLE SITES OF BINDING OF TETRACYCLINE IN MINERALIZED TISSUE



Site A (Fig. 3.) is well supported by the previously quoted evidence of binding of OTC by apatite crystals in aqueous solutions and by anorganic bone. Site B is difficult to prove since collagen binds only relatively small quantities *in vitro* and it cannot be divested of all mucopolysaccharide and metal ions that form complexes with TC. Site C is considered a possibility but direct chemical evidence is lacking.

Although the walls of gastric ulcers have a tendency to bind TC in much smaller quantities and for shorter periods than tumours (Hakkinen & Hartiala, 1959) and contain much larger concentrations of sulphate than does gastric mucosa (Hakkinen, 1959), this does not prove that TC binding in bone or elsewhere depends on sulphated mucopolysaccharides, as has been suggested by the above authors.

Whereas collagen binds TC *in vitro* in small amounts, the complex does not fluoresce and probably does not exist in bone matrix. Osteoid is non-fluorescent in rachitic rats treated with TC.

It therefore seems likely in bone that the large TC molecule is bound to the bone mineral

microcrystallites in the crystal surfaces during deposition (Urist & Ibsen, 1963).

Thus the relatively large amount of TC bound in newly deposited mineral can be explained by smaller crystallites, with a relatively large surface area suspended in tissue with maximum content of water, and higher chemical reactivity of hydrogen-bonded, calcium-deficient apatite. The high reactivity of the bone mineral with TC declines in less than 24 hours after deposition possibly in part from transformation of calcium-deficient apatite to hydroxyapatite. In addition the crystals grow larger, thereby decreasing the surface area, while the matrix becomes denser, thus enclosing the inorganic phase in organic matrix that is relatively impermeable to large molecules such as TC.

PART 4

FLUORESCENCE IN OTHER TISSUES

Calcified Tissues

A. Teeth. There is a voluminous dental literature on the staining of teeth with the TC group of drugs. The brown pigmentation seen in childrens' teeth, particularly after prolonged treatment with TC was described by Shwachman et al. (1958). The teeth are fluorescent under ultraviolet light but the disfiguring brown pigment is a degradation product of TC. Wallman & Hilton (1962) proved the presence of TC in such teeth by ultraviolet spectroscopy, but of much greater import would seem to be the enamel hypoplasia which may be associated, especially in neonatal patients. Indeed transplacental passage has resulted in stained teeth in the foetus when the drug was administered to the mother (Kutscher et al., 1963).

B. Calcareous Tendinitis deposits removed after exhibition of OTC by Urist & Ibsen (1963) gave

strong homogeneous fluorescence with the suggestion that the looser packing of the apatite crystals favours the binding of the drug.

C. Metastatic Calcification. Using dihydrotachysterol (A.T.10), Hakkinen (1958) induced metastatic calcification in rats particularly on the surface of the kidneys, but also in the stomach, lungs and heart, which was readily demonstrable as intense fluorescence as the animals had received TC as well.

D. Calculi. The property of fluorescent marking of calcified calculi both renal (Mulvaney et al., 1964) and biliary (Ackerman & McFee, 1963) may perhaps be of use in the future in studies on the life history of calculi. It may be mentioned that no demonstrable inhibition of bacterial growth occurred from particles of fluorescent urinary calculi.

Non-Calcified Tissues

A. Gastric Ulcers. Hakkinen (1959) studied TC fluorescence in experimental gastric ulcers in animals and noted persistence of fluorescence for

a few days, but from my own and other authors' observations it would seem that in benign gastric ulcer in man any fluorescence is minimal and short-lived and not to be confused with the gross persistent fluorescence seen in malignant tissue.

B. Tissue Injury Again Hakkinen (1959) reports fluorescence persistent for 3 days in areas of tissue injury as does Mustakallio (1962) in relation to traumatic injuries, but it seems likely that in these situations one is simply seeing the results of absent blood supply in sloughs and necrotic tissue from whence TC once deposited cannot be cleared.

C. Ischaemic Tissues Malek et al., (1963a) have studied the distribution and temporary retention of TC drugs in experimental animal kidneys and have shown far less localisation in experimental pyelonephritis than in renal ischaemia, where calcification is more prominent. These findings are in accord with the TC fluorescence persistent

in areas of heart muscle which have been the site of myocardial infarction (Malek, 1963b).

D. Micro-organisms and parasites. In studies of protozoal and bacterial phagocytosis it was found by Du Buy et al. (1964) that TC was taken up, as shown by fluorescent microscopy, by extracellular organisms, but that until the host cell died the intracellular parasites were not visible.

Saxen & Vainio (1964) found that the fluorescence of TC, which was incorporated into degenerating cells, proved to be a sensitive marker of early viral cytopathic effects.

At the other end of the scale of micro-organisms, it has been mentioned by Tobie & Beye (1960) that loa-loa, the eye-worm, fluoresces through the skin after the sufferer has been given TC, and that this may be of value in diagnosis.

E. Pancreatitis. The affinity for calcium of the diseased tissue of acute pancreatitis is

presumably the explanation of TC-induced fluorescence in this condition as reported by Ackerman & McFee (1963) and in experimental acute pancreatic necrosis (Malek & Kolc, 1960).

SECTION IV

DIAGNOSTIC APPLICATIONS OF TETRACYCLINE FLUORESCENCE IN TUMOURS

Introduction

The property of TC fluorescence of malignant tumours may be utilised as a diagnostic aid. This is not to say that the role of radiology, endoscopy and the usual methods of exfoliative cytology have been superceded, but rather to assess the value of TC fluorescence as an additional method. At present the only endoscope capable of transmitting ultraviolet light has been developed by Whitmore (1964, 1965) when he used a special quartz fibre-optic cystoscope in the early diagnosis of bladder tumours. The two problems in the design of such an instrument are the heat produced by the ultraviolet light source and the transmission of the ultraviolet light, which is absorbed by glass so that quartz transmission of the rays has to be used. So far an appropriate gastroscope has not been developed. Apart from direct examination of malignant lesions of skin, the oropharynx and

operative specimens, the main field of interest has been in exfoliation of TC fluorescent material from malignant tumours. The major part of the work on exfoliated material both by myself and, since embarking on this project, by a large number of other authors, has been on gastric lavage material. Other exfoliative sites have included the bronchus, ascitic and pleural fluids and, in a few instances colonic and duodenal lavage specimens.

The advantage of TC fluorescence is that it does not require a highly trained cytologist for its recognition, a point of some importance in places without the services of such a specialist. There is an increasing shortage of trained cytologists and technicians because of the expanding programme of routine cytological surveys, particularly in relation to cancer of the cervix uteri.

It seems important to assess the reliability of the TC fluorescent test in exfoliated tumour material, as compared with routine cytology, for the former method is so much quicker and easier than the

complex staining and scrutiny of standard cytological slides. In addition it may be that the naked-eye gross assessment of TC fluorescence, whilst crude, nevertheless is assessing degenerate tumour material which the exfoliative cytologist would be unable to identify.

PART 1

PERSONAL SERIES OF GASTRIC LAVAGE STUDIES

Studies of patients with both benign and malignant gastric lesions have been performed personally since 1962 at the following hospitals:- Royal Sussex County Hospital, Brighton, Brighton General Hospital, King's College Hospital, Dulwich Hospital, Brook Hospital and Woolwich Memorial Hospital. All aspects of the procedures have been performed by myself alone and, so as to attain uniformity of technique, in no instance was the gastric lavage delegated to anyone else. The examination of lavage material has also been performed by myself, although in addition a second medical observer was asked to make an independent assessment of fluorescence compared with a dried smear

of known TC on filter paper. These independent observers' assessments of fluorescence in every case agreed with my own. At times it was obviously impossible for me to be completely unbiased in assessing fluorescence where the patient was in my clinical care, but in those cases examined from other units, care was taken not to make a clinical diagnostic assessment before examining for fluorescence.

METHODS

The details of TC dosage are given later, together with the intervals from treatment to lavage, when reporting the results of the lavages of the various groups of patients.

If aluminium hydroxide gel, which has been shown to reduce absorption of TC (Waisbren & Hueckel, 1950), was being given it was stopped and an alternative antacid used. No alteration was made to the diet, although theoretically milk products should have been avoided to improve absorption of TC (Scheiner & Altemeir, 1962).

The main principles of the lavage technique are those of Raskin et al. (1958), although with some modifications detailed below. At first the overnight residue, preliminary wash with normal saline, and buffer lavage specimens were scrutinised separately. However, it was felt that this imposed too great a number of slide examinations for the cytologist and that if the first two specimens were not included desquamated material might be lost. After the first 6 cases a pooled specimen of overnight residue and normal saline lavage was examined, care being taken to neutralise the lavage fluid immediately the tube was withdrawn.

The patient was fasted overnight for 12 hours and the lavage performed between 8 and 10 a.m. Sips of water were allowed to aid the swallowing of the stomach tube. A plastic No. 18 Levin stomach tube was passed either by the nursing staff or myself, preferably pernasally, but occasionally the tube had to be passed by mouth if there was nasal obstruction. Confirmation of its position was obtained by aspirating

residual gastric juice, at first this was kept separate from the gastric washings but later it was pooled as frequently it was of small volume. The solutions used for gastric lavage were at first buffered isotonic acetate solution to a pH of 5.5, but usually sterile normal saline was used as being more readily available. The acetate buffer was prepared as follows:- 1.3 parts solution A (0.2NAcetic Acid, 11.6 ml. to 1 litre) with 8.7 parts solution B (0.2MSodium Acetate Trihydrate 27 gm. to 1 litre). The amount of solution used varied. If the resting juice obtained was clear 1 pint (540 ml.) was used for lavage. If however the gastric juice contained any food residue, a cleansing lavage was first performed with as much saline as was necessary to obtain a clear return. The diagnostic lavage was then completed in the following way.

A vigorous lavage was always performed using either a 50 or 100 ml. glass syringe or using a 200 ml. metal, or metal and glass, Le Riche bladder syringe. A more powerful jet of fluid can be delivered by the larger syringes and it is thought that this is

important in obtaining exfoliation.

Half of the total amount of solution was forcibly injected down the tube with the patient lying supine, the head being supported by two pillows. For 5 - 10 minutes repeated withdrawal and reinjection of fluid was performed. The patient then turned to lie on his right side and part of the remaining fluid was injected, withdrawn and reinjected. This procedure was repeated with the patient prone and then lying on his left side. In each of these four positions the epigastrium was vigorously massaged unless there was too much tenderness, and the position of the tube was frequently raised or lowered.

Finally with the patient lying supine again the stomach was emptied and the tube removed. The whole procedure usually took about 30 minutes.

Preparation of the Smears

Immediately gastric lavage was completed the contents were brought to neutrality, if necessary, by the addition of sodium bocarbonate solution, using

litmus paper as indicator.

As soon as practicable, and always within 45 minutes, part of the lavage fluid was put into four universal containers and centrifuged at 3000 r.p.m. for 15 minutes. The remainder of the fluid was sent to the laboratory for routine exfoliative cytology after staining either with Papanicolaou stains or haematoxylin and eosin.

The supernatant fluid was decanted from the universal containers and the sediment smeared on to Whatman No. 3 filter paper and in some instances on to glass microscopic slides. The smears were allowed to dry in a warm room, not exposed to daylight which enhances the gradual decay of the fluorescent property of TC.

Examination of the Smears

As soon as practicable, but never more than 6 hours later, the smears were examined in a darkened room with a portable ultraviolet lamp described earlier. The smears were viewed by holding the filter paper (or

slide) over the top of the box containing the lamp so that the beam of ultraviolet light was directed away from the operator to prevent direct ultraviolet light entering the eyes. The smears were held within a few inches of the light and compared with a standard obtained by allowing TC solution to dry on a filter paper. This comparison with known TC fluorescence was of importance in differentiating the golden-yellow fluorescence of TC from other fluorescence, particularly white fluorescence or dull yellow colour which may be seen in normal gastric juice. Photographs are shown on page 226, but do not accurately reproduce the true fluorescent colours.

RESULTS OF GASTRIC FLUORESCENT TEST

Four groups of patients have had their gastric contents examined for fluorescence:-

1. Juice from patients with Peptic Ulcers - NOT prepared with TC
2. Lavage from patients without Gastric Carcinomas - prepared with TC
3. Lavage from patients with Gastric Carcinomas - NOT prepared with TC
4. Lavage from patients with Gastric Carcinomas - prepared with TC.

The case material and results of these groups are described separately.

1. Juice from Patients with Peptic Ulcers - NOT Prepared with Tetracycline

The juice was a portion of the resting juice obtained from 12 gastric ulcer and 12 duodenal ulcer patients, who had not received TC, in the course of a routine assessment of gastric secretory function.

NO FLUORESCENCE of the characteristic golden-yellow was seen on exposing the neutralized dried smears to ultraviolet light. In many instances although there were no traces of typical yellow fluorescence, there was to be seen either a white fluorescence, particularly on the filter-paper smears, or a dull yellow staining (not fluorescence) of bile. No difficulty was encountered in differentiating this from the true TC fluorescence as shown by a dried smear of TC solution on filter paper.

2. Lavage from Patients without Gastric Carcinomas - Prepared with Tetracycline

Case Material

These 45 patients comprised:-

19 patients with Benign Gastric Ulcers

3 patients with Duodenal Ulcers

13 patients with dyspepsia in whom no definite evidence of ulcer could be found, although sometimes Ba meal and Gastroscopy were suspicious

3 patients with Pernicious Anaemia

1 patient with nutritional iron deficiency anaemia

1 patient with hypertrophic gastritis

2 patients with carcinoma pancreas } not invading the stomach

1 patient with carcinoma gall bladder }

1 patient with previous gastrectomy

1 patient with previous gastro-enterostomy

All these patients had at least one barium meal examination, and certainly all the patients with a gastric ulcer had at least two such radiological examinations. Gastroscopy was performed in 16 patients. Abdominal operation was performed on 15 patients. In those seven who had a partial gastrectomy for gastric ulcer, histology showed only chronic benign gastric

ulceration with no suspicion of malignancy. In the two patients with carcinoma of pancreas and the one with carcinoma of the gall-bladder, provided histologically either at laparotomy or autopsy, there was no invasion of the stomach.

It is thought that in all cases, the possibility of gastric carcinoma has been virtually excluded by laparotomy, autopsy, repeated barium meal examinations, gastroscopy or clinical follow-up over a period of at least 6 months. The possibility that one of these patients will develop an overt carcinoma of the stomach in the future cannot be denied, but seems unlikely. Those patients with gastric ulcer who have not had gastrectomy all showed radiological or gastroscopic evidence of complete healing.

Tetracycline Preparation

In 24 of the 45 patients, TC preparation was with 5 gm. TC orally in divided doses over 5 days, and in a further 6 patients with the same amount over 2½ days. In the remainder given oral TC the total dosage

ranged from 6 to 8 gm. over a period of 3 to 10 days. The intravenous route of administration was chosen for 3 patients because of vomiting and a single intravenous dose of 1.0 gm. given over one or two hours.

Interval

The interval between completing administration of TC and gastric lavage, was from 30 to 48 hours. The interval was 36 hours in 26 patients, 30 hours in 14 patients, and 48 hours in 5 patients.

Results

In 43 of the 45 patients, no characteristic golden-yellow fluorescence could be seen, although frequently there were white fluorescent particles in the smears. On two examinations a dull yellow bile staining was associated with the white fluorescence but the colour was not at all like that of TC.

In 2 patients' smears there was a trace of golden-yellow fluorescence indistinguishable from that of TC. Each of these patients had had a standard 5-day course of 5 gm. of TC and an interval of 36 to 48 hours respectively.

The first of these false-positive results was from a man of 60 years who suffers from systemic lupus erythematosus taking prednisone, whose blood urea was normal. He had dyspepsia for 6 months, and barium meal showed a lesser curve gastric ulcer, as did gastroscopy, although there was no suspicion of malignancy on these examinations. A few weeks later laparotomy was performed with a view to gastrectomy, but on opening the stomach the ulcer was seen to be soundly healed, so excision was not performed. Two years later he is well with no abdominal complaints whatsoever.

The second of these apparently fallacious results was in a lady of 57 years with a history of one year typical ulcer dyspepsia and occasional vomiting. On two barium meals there was a persistent small lesser curve ulcer crater which had disappeared on later barium meal and no abnormality could be seen on gastroscopy. Six months later she was symptom-free and gaining weight.

It is possible that in the future these two

p atients may develop a gastric carcinoma and they are being followed carefully at clinic with this in mind. Emphasis is laid on the fact that in both these patients the yellow fluorescent result was extremely weak and in each was present in only one of the 4 smears.

Gastric cytology was performed by the pathologists of the hospitals concerned on part of the gastric lavage in all but two of the patients, and in none were malignant cells seen.

3. Lavage from Patients with Gastric Carcinomas - NOT Prepared with Tetracycline

Only 5 of these patients have had a gastric lavage performed before the exhibition of TC and in none of these has there been any trace of golden-yellow fluorescence. It would probably have been wise to have performed such a pre-TC test on all patients but this has been undesirable in the ill patients, and often difficult to arrange where surgical intervention was pressing.

4. Lavage from Patients with Gastric Carcinomas - Prepared with Tetracycline

Case Material

There were 30 patients with malignancy studied by gastric lavage after TC preparation. Of these, 15 had gastrectomy or biopsy proof of gastric carcinoma, a further 8 patients had laparotomy proof of gastric malignancy although no biopsy was taken, and in the remaining 7 patients the clinical course and radiological appearances virtually proved the diagnosis of gastric malignancy.

Of the 15 histological specimens examined 4 were well-differentiated, 6 were poorly differentiated (3 of the mucous-secreting or "signet-ring" type), 1 very poorly differentiated and 1 anaplastic type of adenocarcinoma of the stomach. There were 2 cases with linitis plastica without microscopic invasion of the mucosa (see Appendix 3).

Tetracycline Dosage

Varying methods of preparation of the patients with TC were employed, partly to suit the

convenience of the surgeon, and partly to assess the various possible dosage regimes in relation to lavage fluorescence. In those patients who were vomiting, the intravenous route was employed to ensure no confusion of results due to impaired gastric emptying and absorption of the drug.

The numbers of patients receiving the various dosage regimes are as follows:-

500 mg. qds for 2½ days - 5000 mg. - 11 patients
250 mg. qds for 5 days - 5000 mg. - 11 patients
500 mg. qds for 4 days - 8000 mg. - 2 patients
1000 mg. intravenous over
½ - 4 hours - 1000 mg. - 6 patients

Intervals from Tetracycline to Lavage

The intervals from stopping the TC to the time of lavage varied from 30 to 48 hours, with the most usual interval being about 36 hours. It was thought that any shorter interval might produce false-positive results through failure of TC clearance from normal body fluids.

Routine Exfoliative Cytology was performed by the Pathology Departments of the various hospitals. Regrettably cytology was only performed in 18 instances of the 30 specimens of lavage material. The shortage of trained staff in some of the hospitals and the need on occasion to perform the lavage at weekends rendered it impossible to obtain cytological examination of the lavage material in 12 cases.

Results of Fluorescent Test in Gastric Malignancy

The individual details of the various cases are given in Appendix 3, and in Table 4. Of the 30 patients with gastric malignancy pretreated with TC, 23 (77%) showed a positive gastric fluorescent test. Eight patients showed brilliant fluorescence, 10 patients showed moderate fluorescence and 5 weak, but definite, fluorescence. There was great variation between the amount and intensity of the TC fluorescence on the smears. In some the whole of the dried smear showed small flecks and large spots of golden-yellow fluorescence whereas in others the positive result could consist of a few minute flecks of characteristic fluorescence of the size of grains of salt. The

TABLE 4.

RESULTS OF GASTRIC LAVAGE STUDIES IN MALIGNANT CASES

Case	Initials	Total dose gms. TC	Duration of TC	Route of TC (Oral or IV)	Interval (hours)	Fluorescence	Cytology
1	WF	5	5 days	O	36	+++	?+
2	RB	5	2½ days	O	36	++	?+
3	WW	5	2½ days	O	30	O	O
4	HF	5	2½ days	O	36	++	O
5	FO	5	2½ days	O	36	+	O
6	CW	5	5 days	O	36	+	O
7	GS	5	2½ days	O	32	+	N.D.
8	AA	1	4 hrs.	IV	48	+++	+
9	AK	1	½ hr.	IV	40	++	N.D.
10	FW	8	4 days	O	36	++	N.D.
11	PM	5	5 days	O	36	O	N.D.
12	MC	5	2½ days	O	36	O	O
13	TS	1	4 hrs.	IV	36	++	O
14	EB	5	5 days	O	36	O	O
15	FW	8	4 days	O	36	++	O
16	FE	5	5 days	O	36	+++	N.D.
17	JS	5	5 days	O	30	O	N.D.
18	LJ	5	2½ days	O	30	+	O
19	DH	5	2½ days	O	30	++	O
20	SM	5	5 days	O	32	+++	+
21	SL	5	5 days	O	30	O	N.D.
22	HF	5	2½ days	O	30	++	+
23	JO	1	1 hr.	IV	30	+	O
24	SA	5	5 days	O	30	++	O
25	LD	1	1 hr.	IV	32	+++	+
26	FW	1	1 hr.	IV	30	++	N.D.
27	AC	5	5 days	O	30	+++	N.D.
28	AL	5	5 days	O	32	+++	N.D.
29	MW	5	2½ days	O	30	+++	N.D.
30	JJ	5	5 days	O	30	O	N.D.

Individual case details in Appendix 3.

Intensity of Lavage Fluorescence

+++	Brilliant
++	Moderate
+	Weak
O	None

Cytological results

+	Malignant cells seen
?+	Suspicious of malignant cells
O	No malignant cells seen
N.D.	Cytological examination not done.

ease of detection was the guide to the assessments of "brilliant", "moderate", and "weak" which were dependent on the amount of fluorescence on the overall appearance of the smears, rather than the actual intensity of any particular fleck or spot of fluorescence.

In 7 cases (23%) of gastric malignancy, there was no characteristic golden-yellow fluorescence visible on the dried smears. None were classified as dubious or equivocal as it was felt that such results are not of value to the clinician, and a definite statement as to the presence or absence of fluorescence was essential. The spreading of half the smears on filter paper and half on glass slides was found to be of value in assessment of the results, particularly when the wet smear on the filter paper on drying produced a white ring of fluorescence around the deposit. Especially in these instances, the examination of smears on the slides when reviewed obliquely under the ultra-violet lamp was helpful in deciding on the question of true TC fluorescence.

It was with increasing facility that the

scrutiny of the smears could be assessed and, particularly in the early cases, it was found invaluable to compare the smears with a known TC smear. The presence of white fluorescence, presumably from traces of food debris, and of yellow non-fluorescent bile staining could be a source of confusion unless reference was made to the standard TC smear, where the true distinctive golden-yellow fluorescence of TC could be seen under the ultraviolet lamp.

In a few instances dried smears on glass slides were examined under an ultraviolet microscope, but the intensity of fluorescence of a non-specific type so confused the appearance of the smears that it was felt that no more useful information could be gained from this technique than from the naked-eye scrutiny of the smears under an ultraviolet lamp.

Relationship of TC Fluorescence to Tumour Types

An attempt has been made to correlate the positive results obtained with the histological types of the tumours. The 15 gastric carcinomas examined histologically as to tumour differentiation in

relation to the results of the fluorescent test are given in Table 5.

Tumour Types	Fluorescent Test			
	Brilliant	Moderate	Weak	Nil
Well-differentiated	1	-	1	2
Poorly differentiated	-	3	2	3
Anaplastic	-	1	-	-
Linitis Plastica	-	-	-	2

Table 5. Relation of Fluorescent Test Results to Tumour Differentiation

It can be seen that from the small number of cases available no particular pattern of histological differentiation and fluorescent result can be demonstrated.

The gross appearance of the tumour was classified from an assessment of the pathological appearances of the stomach, the operative findings if gastrectomy was not performed, or the radiological or gastroscopic appearances. It is realised that this is a crude assessment for what may be, for example, a plaque of cancer at one time may through necrosis appear like a malignant ulcer later. Similarly on

radiology, a filling defect may be thought to be of a "cauliflower" tumour whereas more accurately it should be regarded as a large plaque. Nevertheless, the assessment into broad macroscopic types is given below with the results of the fluorescent test in Table 6.

Type of Tumour	Fluorescent Test			
	Brilliant	Moderate	Weak	Nil
"Cauliflower"	5	6	2	2
"Plaque"	3	2	1	3
Ulcer	-	1	1	2
Linitis Plastica	-	-	-	2

Table 6. Relation of Fluorescent Test to Macroscopic Tumour Type

In the 7 cases (23%) where a negative fluorescent result was obtained, there seems to be a ready explanation in two instances (cases 3 and 17, Appendix 3, Table 4), where at operation was found linitis plastica without apparent histological invasion of the mucosa, so that malignant tissue would not be available for exfoliation. In case 17 (Appendix 3) the gastrectomy specimen was examined with the ultraviolet lamp shortly

after the lavage. Although there was vivid fluorescence of the cut edge of the stomach, no appreciable fluorescence could be seen on the mucosal surface and it is not surprising that in the lavage material no exfoliated fluorescence could be seen. (In one of the other negative fluorescent results (case 12, Appendix 3.) vigorous lavage was not performed because of the presence of much blood, which may explain the failure of the test in this case. In the remaining 4 negative results, there is no ready explanation for the failure of fluorescence. It may be that lavage was not sufficiently vigorous to cause shedding of tumour material into the stomach, or it may be that one is encountering the individual variation of tumours in their TC uptake, as suggested by Case 11, where examination of the excised tumour revealed only very weak fluorescence.

From these results it would appear that the tumours presenting as cauliflower-like filling defects or plaques of malignant tissue with rigidity and irregularity of the stomach on barium meal are the

most likely to be diagnosed by the TC fluorescent test. It would seem likely that these two gross types of tumour have greater exfoliation of tumour cells, by virtue of their greater surface area, or perhaps the weakness of their stroma, than does the malignant ulcer. There are however too few malignant ulcers in the series for this point to be definitive.

The fact that most of the carcinomas were advanced cases was unavoidable in view of the unselected nature of this series. It is most regrettable that by the time the majority of patients with gastric carcinoma present at hospital their disease is so widespread as not to be curable by surgical intervention.

Relation of TC Fluorescent Results to TC Dosage Regimes

As previously detailed, there were 4 different regimes of TC administration and it is important to analyse the fluorescent results in relation to each regime (See Table 7.)

TC Regime	Fluorescence Test			
	Brilliant	Moderate	Weak	Nil
5000 mg./2½ days	1	5	3	2 (including 1 linitis plastica)
5000 mg./5 days	5	-	1	5 (including 1 linitis plastica)
8000 mg./4 days	-	2	-	-
1000 mg. intravenous	2	3	1	-

Table 7. Relation of Fluorescent Test to TC Regime

From Table 7. it will be seen that each of the 4 regimes of preparing the patient for the test with TC was capable of inducing typical fluorescence in the gastric lavage material.

Even though the intravenous method utilises a much smaller total dose it would appear that the high blood levels achieved compensate for the much smaller amount of TC. In any case parenteral administration of TC is essential for some cases where gastric stasis may lead to fluorescent particles from the tablets remaining

in the stomach for more than the minimum 30-hour interval from stopping the drug to performing the test. Gastric stasis or vomiting may also lead to impaired absorption and the possibility of a "false-negative" result. The minimum intravenous dose has not been ascertained but no difficulties were encountered with 1000 mg. TC being given over a period of more than ½ hour, although if given more quickly unpleasant flushing may trouble the patient.

The large total oral dose of 8000 mg. was effective but, as it was tried in only 2 patients, evidence is insufficient to say whether increasing the TC load achieved more fluorescence in the tumour and in the resultant lavage material.

A total oral dose of 5000 mg. was given to 2 groups of 11 patients, the first receiving 500 mg. q.d.s. for 5 days. No unpleasant side effects were encountered with the more concentrated regime. The numbers do not permit any deduction as to which regime is the more efficient in preparing the patient. There

were 5 negative tests with the 5-day regime as compared with 2 negative tests with the 2½ day regime, but on the other hand there were more brilliant fluorescence results with the longer course.

A dosage of 250 mg. q.d.s. takes 5 days to achieve a blood TC level of 3 to 4 ug./ml. whereas the 500 mg. q.d.s. achieves 4 ug./ml. "within 3 or 4 days" (Dowling, 1955). If indeed the blood level rather than the duration of exposure of the carcinoma to TC is the important factor in determining the intensity of fluorescence, it would seem logical to use the higher dosage of 500 mg. q.d.s. In addition the delay of a week in being able to report the fluorescent results is often undesirable, so the compressed regime of 2½ days TC administration would appear to be perfectly adequate for routine use.

Relation of TC Fluorescent Results to the Interval from TC to Lavage

The interval from discontinuing TC to time of lavage varied from 30 to 48 hours. In 2 patients

with intervals of 48 hours and 40 hours, a fluorescent result of brilliant and moderate intensity respectively was obtained. The remainder all had lavage between 30 and 36 hours. Within the limits given above it does not seem that the exact interval is critical, but to avoid "false-positive" results it would seem unwise to curtail the interval much below 30 hours, although no direct evidence has been ascertained on this point.

Relation of Fluorescence Test to Cytological Examination

In those 18 cases where routine cytological examination after staining with Papanicolaou or haematoxylin and eosin was performed, only on 4 occasions were the cytologists able to report malignant cells definitely present in the gastric lavage specimen. In 2 other cases they were suspicious of atypical cells being malignant, and in 12 cases they reported no malignant cells to be seen. The relationship of the fluorescent test results to those of routine cytology is shown in Table 8.

Fluorescence		Cytology			
		Positive	?+	Negative	Not Performed
Brilliant	8	3	1	0	4
Moderate	10	1	1	5	3
Weak	5	0	0	4	1
None	7	0	0	3	4
Total		4	2	12	12

Table 8. Relation of Fluorescent Test to Cytology

From this analysis there is the suggestion that the more material being exfoliated from a tumour the greater is the likelihood of routine cytology being diagnostic and the more brilliant is the TC fluorescent test. Nevertheless the results of cytology with only 6 (33%) positive or suggestive results compares badly with the 77% success rate of the TC fluorescence test in this series.

SUMMARY OF RESULTS OF TC FLUORESCENT TEST

No characteristic fluorescence was found in the resting juice of 24 patients with peptic ulcers, nor in 5 patients with gastric malignancy who had not received TC.

In 45 patients without gastric malignant disease, who had received TC, there were only 2 weak golden-yellow smears, both in apparently benign gastric ulcers. Standard exfoliative cytological studies showed no malignant cells.

Of 30 patients with gastric malignancy prepared with TC, 23 (77%) gave a positive fluorescent test for malignancy and 7 (23%) gave a negative test. The results are analysed in relation to tumour differentiation, gross type, mode of administration of TC, and interval from discontinuing the drug to gastric lavage. Standard exfoliative cytological examination for malignant cells was performed in 18 cases from the gastric carcinoma group and in only 6 instances (33%) were suspicious or definite malignant cells seen.

PART 2.

THE VALUE OF THE TETRACYCLINE FLUORESCENCE TEST FOR GASTRIC CARCINOMA

Published Experience

Klinger & Katz (1960, 1961) administered TC 250 mg. t.d.s. for 5 days to 59 patients and performed gastric lavage before and 24 hours after stopping TC. The centrifuged deposit was dried and examined under an ultraviolet lamp for the characteristic yellow fluorescence of TC. In all patients before TC, the deposit showed no fluorescence, whereas in 17 out of 18 patients with gastric carcinoma a positive test was obtained with the demonstration of variable but definite naked-eye yellow fluorescence. A negative test was also obtained before and after TC in 41 controls (see Table 9.) Any free acid was neutralised to pH6 with sodium bicarbonate solution, as they found that acid quenches TC fluorescence.

A similar technique was employed by Kantor (1961) and Berk & Kantor (1962) using DMTC as they

TABLE 9.

EXFOLIATIVE FLUORESCENT STUDIES IN GASTRIC CARCINOMA - SURVEY OF THE LITERATURE

Authors	Year	Drug	Daily Dose mg.	Route	Duration Days	Total dose mg.	Interval hours	Gastric Malignancy Positive	Gastric Malignancy Negative	Non Malignant Controls Positive	Non Malignant Controls Negative	Notes
Klinger, J.	1960		750	Oral	5	3,750	36	46	27	2	202	59 cases had lavage before TC - no fluorescence.
Katz, R.	1961	TC	2,000	Oral	2	4,000	36	3	10	0	20	
Arteaga, I)	1964		300	IM	5	1,500	12	5	3	0	9	OTC used in 8 Cancer and 21 Controls with poorer results.
Berk, J.E. Kantor, S.M.)	1961 1962 1963	DCTC	600	Oral	5	3,000	30	34	5	4[a]	134	[a]2 showed "mucosal atypism" in benign gastric ulcer.
Sherman, H.H. et al.	1963	TC	1,000 &/or 300 - 600	Oral IM	4 1-2	4,000 300 - 1,200	24+ 36	16	3[b]	2[c]	89	[b]2 lymphomas and 1 Carcinoma with no gross fluorescence but positive on fluorescent microscopy. [c]One cirrhosis + one adenomatous polyp with "focal atypism".
Aberle, S.	1963	TC	750	Oral	5	3,750	24+	1	1	7[d]	12	[d]All benign gastric ulcers. Positive fluorescence in basal gastric secretions without TC in 13 out of 53 samples.
Hahn-Pederson, A. et al.	1963	TC	1,000	Oral	5	5,000	30	2	4	7[e]	11	[e]6/12 DU and 1/6 GU false positive
Klass, A.	1963	DCTC or TC	600 2,000	Oral	5 2	3,000 4,000	30	2	3	5	3	Comparing routine cytology - all malignant cases positive and all non-malignant cases negative.
Segura, J. Martinez, A.	1963	DCTC	600	Oral	5	3,000	24	14	14	3[f]	22	[f]including 2 benign gastric ulcers. Invalid results because in earlier cases used poor technique
Slatopolsky, E.	1963	DCTC	600	Oral	5	3,000	48	17	3	1	59	-
Forni, E.	1963	DCTC	600	Oral	5	3,000	30	9	6	8	26	15 examinations before TC - negative fluorescence.
Oria, M Ferraris, C.M.	1964	DCTC	600	Oral	5	3,000	30	17[g]	1[h]	1	27	[g]inc. 1 gastric sarcoma [h]peptic ulcer with areas of neo-plastic change.
Torino, L.	1964	TC	2,000 or 500	Oral IM	2 2	4,000 1,000	36 36	13[i]	2[j]	3	27	[i]includes 3 sarcomas. [j]1 carcinoma and 1 lymphoma
Tomenius, J Lindkvist, P quoting Hanngren Blomqvist	1964	DCTC	600	Oral	5	3,000	30+	6	1	0	26	-
	1964	DCTC	600	Oral	5	3,000	30+	6	2	1	22	-
Rugtveit, A. Hope, L.	1964	TC	3,000 or 750	IV Oral	3+hrs 4	3,000 3,000	24-96	17	1	9	9	Controls were all benign GU except for 1 normal stomach + 1 Haemangio-endothelioma (positive fluorescence).
Worsley, G.H. et al	1963	TC or DCTC	750 600	Oral	5	3,750 3,000	30	2	3	1	20	Comparing routine cytology 3/5 positive carcinomas but all 21 control peptic ulcers negative.
Kravchik, I.	1964	TC	1,000	Oral	5	5,000	30	16	0	2	37	Before TC all showed negative fluorescence.
Cummins, A.J. et al	1964	DCTC	600	Oral	5	3,000	32	11	12	5	98	Omitting 1 questionable result from each group. 61 before DCTC - no fluorescence. Routine cytology 16/23 positive in gastric carcinoma & 87/103 negative in controls with 16 "unsatisfactory".
Sandlow, L.J. Mecheles, H.	1963 1964	TC	2,000 or 500	Oral IM	2 2	4,000 1,000	36	53[k]	2[l]	7	143	[k]including 3 lympho & 1 leiomyosarcoma. [l]One failure to neutralise acid & one linitis plastica without mucosal involvement.
Echeverria, E. et al.	1963	DCTC	600	Oral	5	3,000	24-30	18	2[m]	5[n]	34	4 excluded because fluorescence before TC. [m]2 malignant lymphomas. [n]3 with "mucosal atypism" in benign GU

found the fluorescence of 150 mg. of this drug was greater than that of 250 mg. of TC. All 10 cases of gastric cancer showed a positive fluorescent test, but there were 3 "false positives". In two of these however it was suggested that the gastric ulcer was "premalignant" because of mucosal atypism, and the remaining "false positive" case was of a patient with an unproven benign gastric ulcer, still under observation, whose gastric washings showed cells suspicious of malignancy. These workers also stress the importance of adjusting the pH to between 6 and 7, and the early examination of the smears from the gastric lavage as soon as the specimen was dry.

It was following these two reports that my own series was started, but since then there have been many publications giving varying experience, so the present series would still seem to have some relevance.

Sherman et al. (1963) next reported a series of cases of gastric lavage for carcinoma again showing a high (16/17) positive fluorescence,

but they showed that the remaining "false-negative" case on gross examination showed positive fluorescence when the smear was examined with an ultraviolet microscope. In their series they reported 64 controls without gastric disease and 27 non-carcinomatous gastric cases with negative fluorescence. There were, however, 2 "false-positive" results, one from a patient with hepatic cirrhosis and one from a patient with a gastric adenomatous polyp which on microscopy although benign, had areas of "cellular atypism" corresponding to areas of fluorescence under the ultraviolet microscope.

These studies seemed promising to these first three groups of authors who felt that their results indicated a useful method for the differentiation of carcinomatous and non-carcinomatous gastric disease, whilst accepting some fallibility.

However, Aberle (1963ab) threw doubts on the validity of the procedure. He examined 53 specimens of basal gastric secretions from 48 patients

without prior administration of TC and without gastric carcinoma or gastric ulcer, prepared the sediment in the usual way by neutralisation, centrifuging and allowing to dry and examined the smears, comparing them with a freshly prepared known positive standard under ultraviolet light. Positive fluorescence was noted in 13 out of 53 specimens, and 6 of these positive results were obtained from 13 specimens from patients who had undergone gastric surgery. Aberle (1963) also reported "false-positive" fluorescence in 7 of 19 tests in patients prepared with TC who had benign gastric lesions, a "false-negative" result in one or two cases of gastric carcinoma.

Berk (1963) rapidly answered these criticisms of Aberle (1963ab), emphasising that the latter had only studied a few patients after TC administration and that of these only 2 had gastric carcinomas. He quoted his current (1963) total of 38 patients with gastric carcinoma with a positive fluorescence test of 87% and 140 subjects with normal stomachs or benign lesions of the stomach in whom only

4% "false-positive" results were obtained.

On the basis of his figures he felt that the degree of accuracy was "of an acceptably high order". Emphasis is placed by this author on the difficulty of identifying the characteristic fluorescence of TC and implies that inexperience in interpreting the presence of characteristic fluorescence might account for the unduly high proportion of false positive results in benign gastric lesions and in basal secretions reported by Aberle.

However, Hahn-Pederson et al. (1963) reported very poor results with this test in a small number of cases. Only 2 out of 6 cases of gastric carcinoma showed fluorescence of dried deposit after TC, whereas there were 7 false-positive results in 18 cases of peptic ulcer. They concluded that the TC fluorescent test was not a satisfactory method for differentiating between benign and malignant gastric ulcers.

A small series of 5 cases of carcinoma and

8 proved benign lesions was reported by Klass (1963) from Detroit and compared with his results of exfoliative cytology on the same specimens. Whereas his cytological results were always correct in these small number of cases there were 3 "false-negative" and 5 "false-positive" TC fluorescent results in these two groups of malignant and benign cases respectively. This author felt that the discrepancy in his results as compared with other series was probably accounted for by difficulties in examining the dried smear under ultraviolet light. He points out that bile appears yellow under ultraviolet light but does not fluoresce, whereas impurities in the filter paper fluoresce a brilliant whitish colour. He concluded that the test was of no value as long as there was no more objective, quantitative method for assessing the result of the test.

In Columbia, Segura & Martinez (1963) using the method of Berk (1961) with DMTC found that only half of their 28 cases of carcinoma showed fluorescent washings, but their results are invalid for in the

earlier cases of their study their technique was faulty. From Mexico, Echeverria et al. (1963) report successful experience with the test in all of 18 cases of gastric carcinoma, but 2 malignant lymphomas gave negative results. There were, however, in the control group of 39 patients, 5 "false-positive" results of which 3 were in gastric ulcers where atypical mucosal changes were suggestive of pre-malignancy. Four cases were excluded because gastric lavage before DMTC showed fluorescence, a finding similar to that of Aberle.

Slatopololsky (1963) from Cleveland, Ohio, again using DMTC gave a good report with 3 out of 20 gastric carcinomas failing to give fluorescence and only one control out of 60 giving "false-positive" fluorescence. He found that three days treatment of the patient with DMTC was sufficient to prepare some of his patients and that a 24 hour gap was all that was necessary to allow clearance of the drug from normal body fluids.

In Italy experience seems to differ. Forni

(1963) reports poor results with only 9 out of 15 carcinomas and 8 out of 34 controls showing fluorescence. However, Oria & Ferraris' (1964) experience was good with 17 gastric tumours giving fluorescence and only one negative result in a peptic ulcer with areas of neoplastic change. They had one "false-positive" result in 28 controls. Torino (1964) also found encouraging results with only 1 carcinoma in 14 malignant cases not producing a fluorescent deposit. Of the 30 controls, 3 gave fluorescence. It is of interest that a lymphoma gave a negative test, as did two of these tumours in Echeverria's (1963) series. In contrast 4 sarcomas were found in these last two Italian series to produce a fluorescent sediment.

From Sweden, Tomenius & Lindkvist (1964) using DMTC after the method of Berk & Kantor found reasonably satisfactory results as did Hanngven & Blomqvist whom they quote. These workers record 12 out of 15 positive tests in gastric malignancy, and only one false positive result in 49 examinations of benign gastric lesions.

From Norway however Rugtveit & Hope (1964), whilst obtaining comparable positive results in gastric malignancy (17/18), found in their control group of 18 cases, mainly gastric ulcers, equal numbers of negative and false-positive results. For this reason they conclude that the test is of little value in differentiating between cancer and gastric ulcer. They also showed fluorescence in the cut surface of gastric ulcers and cancers, surprisingly enough not related necessarily to the results of fluorescence of lavage specimens.

Worsley et al. (1963) in Montreal reported a small series of 5 gastric carcinomas in which 3 were positive on exfoliative cytology and 2 positive on fluorescent testing. They felt that the test was "inferior to good cytological techniques and had not proved of great value", but it must be stressed that their series was small.

From Russia, Kravchick (1964) supports the value of the TC test, finding all 16 gastric carcinomas produced TC fluorescence in the gastric lavage smears, although there were 2 positive results out of 39 non-

malignant cases. In addition a preliminary test before TC never gave the characteristic fluorescence.

In 1964, Cummins et al. reported their experience with the fluorescent test using the method of Berk & Kantor and comparing their results with those obtained with exfoliative cytology using conventional staining methods. From their series of 25 cases of gastric malignant disease (including 1 leiomyosarcoma) they had only 44% positive result with fluorescence but 64% with gastric cytology. In 104 patients with benign gastric lesions, there were 94% negative fluorescent tests for malignancy, but only 84% were definitely negative on cytological grounds. They suggest, as a possible explanation for false results, retention of TC particles in the stomach by pyloric obstruction, necrotic debris in benign ulcers, ingestion of other fluorescent material, and malignant lesions proximal to the stomach. Unlike Aberle (1963b) they did not find fluorescence of the gastric sediment before TC treatment. Their conclusions were that the fluorescence test was "neither simple nor reliable".

Sandlow et al. (1963) and Sandlow & Necheles (1964ab) have published a large series of cases including 55 gastric malignancy in whom they found that the TC fluorescent test of gastric washings was positive with an accuracy of 96%. In the 75 non-ulcer controls, there were 7% "false-positive" and in 75 benign gastric ulcers 4% "false-positive". By repeating the gastric washings 60 hours post-TC in all cases with positive results they have recently reduced the number of "false-positives".

The latest cumulative results by the two original advocates of the TC fluorescent test, together with the results given above from Sandlow & Necheles (1964ab) constitute the three largest series and all find the test of value with a fairly high rate of diagnosis of gastric cancers and an acceptably low rate of "false-positive" results in controls. Thus Klinger, Arteaga & Katz (1964) from Chile with a standard 5-day course of TC, 250 mg. t.d.s., obtained fluorescence in the gastric deposit of 46 out of 73 carcinomas. Even though this detection rate of 63%

is not very impressive they had only 1% false results in their non-malignant cases. In cases of pyloric stenosis where gastric retention of the oral drug could give fallacious results they use intramuscular TC. Their experience of an abbreviated more intensive course of TC (500 mg. q.d.s. for 2 days) and of OTC would suggest that their original method and drug are preferable.

Berk & Kantor (1963) from Detroit who have been the advocates of DMTC in a standard regime of 150 mg. q.d.s. for 5 days, were equally impressed with the test, obtaining positive fluorescence in all but 5 of 39 gastric carcinomas. A "false-positive" rate of 3% seems acceptable.

The discrepancies between the published series is disturbing and perhaps may be attributed to a failure of technique as stressed by Berk & Kantor (1963) who emphasise strict attention to detail if erroneous results are to be minimised. It is of some interest that they eluted material from the filter paper and were able to demonstrate by chromatographic means that the

fluorophore has the same motility as unmodified TC.

Technique of the Tetracycline Fluorescent Test

Strict attention to detail in the performance of the test is essential in order to minimise erroneous results, and attention to the following points is emphasised:-

1. Preparation of the Patient

As it seems probable that the intensity of fluorescence is in part related to the individual nature of the tumour and in part to the blood level of TC achieved, it is essential that adequate amounts of a TC drug be given. Berk & Kantor (1963) advocate not less than 3.0 gm. of DMTC be given in divided doses by mouth. Although they have shown that this is an adequate preparation, on theoretical grounds there is the possible danger of increasing the number of false positive results because of the delayed renal excretion and persistence of higher tissue levels, especially if there is any degree of renal insufficiency.

For this reason many workers, notably Klinger, Arteaga & Katz (1964), have continued to use TC as originally advocated by them in 1960. The dosage originally used by these workers was 250 mg. TC orally t.d.s. for 5 days, but it may be significant that this total dose of 3.75 gm. gave a rather low diagnostic rate of 63% of 73 tumours. When they compressed their TC into a total dosage of 4.0 gm. over 2 days their diagnosis rate fell to an unacceptably low figure of 23% of 12 tumours. Sandlow & Necheles (1964ab), however, with the same concentrated regime obtained 53 positive diagnoses and their 2 negative results included a linitis plastica without mucosal involvement and a case examined with a faulty technique. My own results, coupled with those of Kravchik (1964) and Sherman et al. (1963) would suggest that the slightly higher dosage of 250 mg. q.d.s. for 5 days (total 5.0 gm.) gives a reasonably high diagnosis rate. On some occasions it may be necessary to compress the TC regime so as to obtain quicker results. In so far as a dosage of 500 mg. q.d.s. produces blood levels of 4 ug./ml. in 3 or 4 days, whereas 250 mg.

q.d.s. takes 5 days to achieve that level, in theory the higher dosage regime would seem to be satisfactory. Certainly Sandlow & Necheles (1964ab) found that a total dose of 4.0 gm. over 2 days was highly satisfactory, and in my own 10 cases prepared with 5.0 gm. over 2½ days the results were also satisfactory.

The derivative OTC was tried by Klinger et al. (1964) with poorer results, but the drug, DMTC, has been tried by many workers following the example of Berk & Kantor (1961). It appears that 150 mg. DMTC gives equivalent blood levels to 250 mg. TC and these workers advocated a dosage of 150 mg. DMTC q.d.s. orally for 5 days with positive results in 87% of 39 tumours, and only 3% "false-positive" results in benign diseases. Comparing all published reports TC produced 76% positive tumour results compared with 74% for DMTC so there is no significant difference between the two drugs in this respect. It might be expected that DMTC would produce more false positive results than TC by virtue of its higher plasma protein binding and slower excretion. In fact comparison from

the collected literature gives 7% "false-positive" results with TC and 4% with DMTC, although these figures may be fallacious because of differing techniques. If there is renal insufficiency, DMTC will persist longer and might give more false positive results than TC.

For very rapid preparation of the patient or when there is any suggestion of failure of absorption due to vomiting or gastric stasis, the drug must be given by a parenteral route. Sandlow & Necheles (1963) recommend that where more than a small amount of clear secretion is found on initial aspiration, the lavage should be cancelled or parenteral administration of the drug be given before proceeding a few days later. Intramuscular administration of TC has been found by Klinger et al. (1964), Sherman et al. (1963), Sandlow & Necheles (1964) and Torino (1964) to be a perfectly adequate method. The dosage regimes varied from 600 to 1500 mg. spread over 1 to 5 days, but insufficient details are given by the authors for the optimum intramuscular regime

to be assessed.

In my own series I did not use intramuscular administration but, where parenteral administration was essential, preferred a single intravenous drip over ½ to 4 hours of 1000 mg. of TC as being quick and achieving very high blood levels. In all 6 cases prepared by the intravenous route the fluorescence was brilliant or moderate so it would seem that this method gives good results. In the literature the only authors to use intravenous TC were Rugtveit & Hope (1964) in a dose of 3000 mg. and, although their tumour diagnosis rate was satisfactory in a small series, they were amongst those who found a high percentage of "false-positive" results.

In summary therefore the dosage schedules which seem to be adequate for preparing the patient are:-

TC 250 mg. t.d.s. or q.d.s. orally for 5 days

TC 500 mg. q.d.s. orally for 2½ days

DMTC 150 mg. q.d.s. orally for 5 days

TC 250 mg. b.d. by intramuscular injection for 2 or more days.

TC 1000 mg. by intravenous drip over ½ - 4 hours.

2. Absorption of the Drug

The mechanical problems of absorption of TC where there is pyloric stenosis or vomiting have already been dealt with in the preceding section. More subtle influences may lead to poor absorption of the drug. Milk, milk products and aluminium hydroxide are frequently being given to patients with dyspeptic symptoms and these factors can impair absorption of the TC drugs (Scheiner & Altemeir, 1962). After a single oral dose of 300 mg. DMTC an average peak concentration of 2.08 ugm. per ml. is reached in 3 to 6 hours, whereas if milk or cheese are eaten at the same time, the peak level is about 0.6 ugm. per ml. Similar effects with the other tetracyclines have been shown by Martin & Harper (1959) who demonstrated that the mechanism was probably the formation of a calcium caseinate complex with TC drugs. With aluminium hydroxide gel even more marked reduction in blood levels of TC has been observed by Waisbren & Hueckel (1950), perhaps due to absorption of the antibiotic on to the gel.

Whilst the patient is being prepared with

oral TC or DMTC for the gastric lavage, no aluminium hydroxide should be given and diet should not be supplemented with milk or milk products.

3. Interval between stopping the drug and lavage

The importance of allowing an adequate period for clearance of the drug from normal tissues so as to avoid false positive results in benign conditions must be stressed. Although some workers have obtained perfectly good results with a 24 hours interval, as there is still a trace of TC or DMTC circulating at this time it would seem wise to allow a minimum period of 30 hours before lavage.

4. The necessity for vigorous lavage

Fluorescence of the gastric sediment depends on the exfoliation of cells from malignant tissue. Vigorous lavage of the stomach, using a large syringe with the patient placed in several positions and simultaneous massage of the stomach through the abdominal wall are important in achieving adequate material for examination. Whereas for exfoliative cytology degenerate cells are unsuitable for

examination, in the TC fluorescence test the overnight gastric residue should not be discarded as it may contain fluorescent material. It has been found that about half-an-hour needs to be spent in performing an adequate lavage.

5. Neutralisation of the lavage fluid

TC fluorescence is quenched by a high concentration of hydrogen ions (Berk & Kantor, 1962). The lavage fluid must be neutralised, if necessary, to a pH of about 7 using sodium bicarbonate, or if preferred a buffered acetate lavage fluid may be used. As fluorescence *in vitro* of TC is even greater at a pH of 9, Kohn (1961) was trying this degree of alkalinisation but has not reported his results.

6. The time of examination under ultraviolet light

The sediment must be allowed to dry before scrutiny as the hydration shell of TC quenches fluorescence. As fluorescence progressively decreases with time the ultraviolet examination must be performed as soon as possible and not longer than 6 hours after lavage.

7. Misleading results from tumours elsewhere

The possibility of fluorescent material deriving from malignant disease in the lungs, mouth, pharynx or oesophagus being swallowed by the patient and giving a positive result on gastric lavage must be considered. It is indeed possible that similar fluorescent exfoliation from tumours of the pancreas or bile passages could regurgitate into the stomach giving confusion to the site of the primary tumour. Cummins et al. (1964) report a patient with questionable fluorescence on gastric lavage who had a carcinoma of trachea eroding into the oesophagus and malignant cells were to be found in the gastric washings. Bernhardt et al. (1963) have demonstrated malignant cells from bronchogenic carcinomas in 6 out of 10 patients. Colmore (1964) prepared 15 patients with bronchogenic carcinoma with 5.0 gm. TC and after a 48-hour interval performed gastric lavage. He obtained 12 fluorescent results as compared with 1 such result in 15 controls.

8. Identification of TC fluorescence

To be interpreted as a positive test, the dried sediment should be of a bright golden-yellow colour which is quite distinctive and, with experience, easily identified. A dried smear of dilute drug should be used as a standard for comparison to avoid confusion with the commonly seen white fluorescence or dull yellow bile colour.

The major criticism levelled at the TC fluorescence test is its lack of objectivity in identifying TC fluorescence and it is probably this fact which has led to some poor results, especially in inexperienced hands. In an effort to give an objective interpretation of the test Socci et al. (1964) have used a photographic method to compare the colour of the fluorescence of a pipette of gastric sediment with that of a standard tube of TC. They claim that their colour photography gives absolutely true colours allowing objective interpretation and reporting, but of course the processing of colour film takes time and once again the comparison is subjective.

A more scientific approach has been reported in the last few months by Berk et al. (1965). They devised two new extraction techniques using paper chromatography for specific identification of DMTC and column chromatography with fluorometric analysis to measure the quantity present. In this preliminary communication they report that although DMTC was not evident by paper chromatography in the gastric sediment of normal persons pre-treated with DMTC, a minute amount could be found in 5 out of 6 normals by the second technique. The quantity of DMTC was considerably less than in his two cases of gastric cancer and they suggest that simple paper chromatography may serve as an objective screening test. If this is positive then the more complex procedure of column chromatography and fluorometric analysis may be used as a second stage to determine the actual quantity of DMTC present. A large number of tumours and benign cases will have to be examined to determine the limits of the test, but this objective approach would seem to offer great possibilities for the future.

Discussion

In the preceding description of the reported series, many conflicting opinions are given as to the value of the TC fluorescent test in the diagnosis of gastric malignancy. With such dissension an explanation must be sought for the differing evaluations in the literature.

The criticisms of Aberle (1963ab) were the most vigorous as he found that out of 53 samples of gastric juice examined without the exhibition of TC, as many as 13 specimens showed yellow fluorescence which he could not differentiate from that of TC. If indeed this reported false fluorescence were correct, the TC fluorescent test would have no validity whatsoever. From the 18 published series however he receives support for this finding only from Echeverria et al. (1963) who found apparently typical fluorescence in 4 cases out of 63 examined before TC.

In contrast Klinger, Arteaga & Katz (1964) with 59 cases, Forni (1963) with 15 cases, Cummins

et al. (1964) with 61 cases, Kravchick (1964) with 55 cases, and the present series with 17 cases, from which gastric juice was examined before TC, showed no typical golden-yellow fluorescence at all. Thus in the 207 cases examined by these workers, no false fluorescence could be found before TC, whereas the two other groups found 17 in 116 examinations. One suspects that the difference lies in the criteria for visual appraisal of the smears.

If one analyses the overall results from the 18 published series and my own there were 75 "false-positive" flucrescent results (6.5%) in non-malignant controls after TC or DMTC, compared with a total of 1,073 negative results. It is of some interest that in the 75 "false-positive" results are included 6 cases where there was "mucosal atypism" either in gastric ulcers or in a polyp suggesting a possibly early or pre-malignant state. If the results of Aberle (1963a) and Echeverria et al. (1963) are excluded on the grounds that their ability to recognise true TC fluorescence is in doubt, the number of "false-

positive" results falls to 63 out of 1,090 examinations, i.e., 5.8%.

The TC fluorescent test was positive in 331 cases from the collected series of gastric malignancy, including 8 cases of gastric sarcoma, with negative fluorescence in 113 cases of which 5 were gastric lymphomas. Thus the overall diagnosis rate was 74.5% with a failure of diagnosis in 25.5%. Again excluding the two dissident authors who found fluorescence in basal secretions, the diagnosis rate for gastric malignancy becomes 73.9%.

It is of interest to analyse the results of the larger series of cases where experience in the technique would be greater and when the recognition of TC fluorescence would presumably be more accurate. Thus taking all groups' experience where the numbers tested exceeded 50 patients, and excluding the results of Segura & Martinez (1963) who in their early cases used a poor technique, we find that 76.8% of malignant cases were diagnosed correctly and, perhaps more significantly, the "false-positive" results in benign

patients fall to 2.9% of 887 cases.

Thus it would appear that the diagnosis can be made in about three-quarters of patients with gastric malignant disease and that there is a fallacious positive fluorescent result in 2.9 to 5.8% of benign cases, the better results being obtained by the more experienced workers. This compares with 77% positive results and 4% "false-positive" results in my own series.

These results would seem to be extremely encouraging, although one does not know what proportion of the tumours tested were at an advanced stage where diagnosis by other means, such as barium studies, gastroscopy or exfoliative cytology would give comparable or better results. The two main problems in the diagnosis of gastric carcinoma are the early diagnosis and the differentiation of benign from malignant lesions without resort to laparotomy. No evidence can be quoted for the value of the TC fluorescent test in <u>early</u> diagnosis. It would seem, however, that at least in the later stages the test is of value.

A comparison of the diagnostic accuracy of cytology, radiology and endoscopy in particular relation to that of the TC fluorescent test can only be made from a study of different series in the literature. There is considerable variation in the skill, care and experience of the cytologist, radiologist and endoscopist and there is a tendency for only those workers with vast experience and enthusiasm to report their work, which in general will give a much better diagnosis rate than is in fact obtained in routine work in hospitals throughout the world.

A useful comparison is made by Henning, Witte and Bressel (1964) who obtained a diagnostic accuracy of 73.5% with cytology, 89.4% with radiology and 72.7% with endoscopy in a large series of oesophageal and gastric malignant tumours, the best figures being obtained from the oesophagus and cardia. Reviewing the literature of many authors with differing methods of inducing gastric exfoliation, rinsing, dabbing, balloon, nylon knots, etc., no major

difference in cytological results can be shown, and the diagnostic accuracy in 3480 cases was 69.8%. It must be stressed that these exfoliative cytology results tend to come from special units with highly trained experienced cytologists and that there is a variation in successful diagnosis from about 50% to over 90% (Raskin et al. 1959). Doubtless in less expert hands the diagnosis rate for gastric cytology will fall below 50%.

In a survey of the literature for comparison of cytology and radiology by Henning et al. (1964), of 858 oesophageal and gastric malignant tumours, 72.3% were correctly diagnosed by X-ray examination compared with 80.8% cytological diagnosis. In a similar collection of 67 cases, 65.7% were diagnosed on endoscopy.

It will be seen therefore that the overall diagnosis of malignancy of the stomach by the TC fluorescent test, from the collected series, is 74.5% which compares well with that of cytology (69.8%), radiology (72.3%) and endoscopy (65.7%) in the

collected series of malignant tumours of stomach and oesophagus.

However, in the assessment of any technique for diagnosis of malignancy, the number of positive diagnoses must be assessed in relation to the number of fallacious or "false-positive" results with benign conditions. Again Henning et al. (1964) have produced most valuable collected figures from the literature. The comparison of false positive results in 113 cases of benign disease was 1.5% for exfoliative cytology and 4.4% for radiology. In 178 cases a false diagnosis of malignant disease was made in 2.8% by gastroscopy and oesophagoscopy. One of the difficulties in assessing collected series, particularly in cytology, is that in the literature it is not always clear what was a questionable and what was a probably positive misinterpretation, so these results may be slightly over-optimistic. In Henning's own series, doubtful reports were excluded and only the highly probable and definite false positive cases included. In 153 patients with benign disease, a fallacious diagnosis was reached

in between 3 and 5 per cent for each of the three diagnostic procedures. With reference only to cytological "false-positive" results in the literature from 1947 - 1962 of 7893 benign cases examined, an incorrect malignant diagnosis was made in 1.9%, although the variation of different authors was from 4.7% to zero.

It will be seen therefore that in this respect the TC fluorescent test is not so accurate, with a false positive result of 5.8% from the collected series although only 2.9% from the more experienced workers and 4% in my own series.

One may assume that only rarely will the detection of a malignant tumour of the stomach be missed provided all three diagnostic procedures are performed regularly and simultaneously in every case by highly skilled experts. Unfortunately such highly-trained specialists are not always to be found especially in the smaller hospitals and herein may well lie at least part of the value of the TC

fluorescent test. It would also seem reasonable to advocate its use with exfoliative cytology, as the gastric lavage conveniently provides material for examination under the ultraviolet lamp and after staining for microscopic examination for malignant cells.

The value of cytology and perhaps of the TC fluorescence test lies mainly in the clarification of radiologically or gastroscopically questionable findings. In rare cases cytology has been responsible for tumour diagnosis in the absence of other evidence. Schade (1964), particularly, has contributed to the early diagnosis of in situ carcinomas by cytology. As he says, "only the diagnosis of a carcinoma in situ counts as a success for cytology", for the radiologically or endoscopically visible gastric carcinoma is unlikely to have the prospect of permanent cure by surgical excision. The screening of selected populations by gastric lavage is a possible approach to the problem of early diagnosis of carcinoma of the stomach. The

association of gastric cancer with pernicious anaemia is generally accepted, the incidence being estimated to be 3 to 20 times that in the general population (Hitchcock *et al*. 1957, Mosbech & Videbaek, 1950). The frequency of gastric cancer in achlorhydric or hypochlorhydric patients is 3 to 4 times that of patients with normal acid secretions (Hitchcock *et al*. 1957). Any screening programmes therefore would logically involve patients with pernicious anaemia and elderly persons, especially men, who are achlorhydric or hypochlorhydric. Although the experience of MacDonald *et al*. (1964) in such a selected group, was disappointing in that the carcinomas discovered were also demonstrable by barium studies, future experience may show such an approach is worthwhile in discovering early *in situ* carcinoma.

The evidence for possible early detection of cancer by the TC fluorescence test is extremely scanty and certainly in my own series no such evidence can be deduced. We are certainly watching the two cases with negative cytology, apparently benign gastric ulcers

but weak positive fluorescence, most carefully to see if there is any suggestion of overt carcinoma. In the literature the extent of the radiological changes in the carcinomatous group is seldom mentioned, and certainly in my own cases practically all were obvious on barium meal. Nevertheless Berk & Kantor (1963) report "mucosal atypism" in gastric ulcers in 2 of their 4 apparently "false-positive" results, and Sherman et al. (1963) report one of their 2 "false-positive" results had "focal atypism" in an adenomatous polyp. Similarly Echeverria et al. (1963) found in 3 of their 5 "false-positive" cases, gastric ulcers "with atypical mucosal changes suggestive of pre-malignancy". On the other hand Oria & Ferraris (1964) had their only false negative result in a peptic ulcer with areas of neoplastic change. It may be that the combination of exfoliative cytology and the TC fluorescence test will prove useful in any screening programme.

PART 3.

OTHER DIAGNOSTIC APPLICATIONS OF TETRACYCLINE FLUORESCENCE IN TUMOURS

The bulk of the literature on the diagnostic applications of tumour fluorescence with TC deals with gastric malignancy, but the same principles may be utilised for other malignant disease. My own experience in other situations is very limited and will be mentioned in the relevant sections.

A. Sputum. The possibility of fluorescent malignant material from the lungs, pharynx or oesophagus being found in the gastric lavage material has already been mentioned as a source of possible confusion in determining the site of malignancy.

The work of Colmore (1964) using a standard method of TC preparation and obtaining 12 fluorescent gastric lavage results in 15 patients with bronchogenic carcinoma, suggests a diagnostic method for patients unable to produce specimens of sputum for examination.

Slatopololsky (1963) examined the sputum of 5 patients with bronchogenic carcinoma after a 5-day

course of DMTC and an interval of 48 hours. He found fluorescence in the sputum smears in 4 of these patients. Similarly, Sandlow & Necheles (1964) mention 2 positive sputum smears in bronchogenic carcinoma.

The only detailed study has been by Hiduchenko (1965) who prepared his patients with 4.0 gm. TC over 4 days and allowed a 30 to 36 hour interval before obtaining and preparing the sputum by adjusting the pH to between 7 and 8. The sputum was then smeared and allowed to dry overnight but a defect of the technique was that the smears were not examined for fluorescence until 24 hours had elapsed. Smears were examined before the course of TC and 5 patients were excluded because of the presence of yellow fluorescence but 3 of these had had TC earlier. There was also one patient with carcinoma and one with tuberculosis. Of 19 bronchogenic carcinomas examined with this technique 16 were positive, 3 equivocal, but none negative. Of 22 other pulmonary diseases, 17 were negative, 2 equivocal and 3 showed definite yellow fluorescence, of which one was in

renal failure and would thus probably have persistent high circulating TC blood levels. It appears therefore that although a high diagnosis rate of bronchogenic carcinoma can be obtained from fluorescent sputum smears, there are apparent "false positive" results.

My own experience with the technique is with only 6 cases, each of whom had a bronchogenic carcinoma, prepared with 5.0 gm. TC and examined after an interval of 36 hours. The sputum was simply smeared on glass slides and there was no adjustment of pH. In 4 of the 6 cases, there were flecks of characteristic fluorescence on the smears. In 6 patients with non-malignant pulmonary disease, there was no golden-yellow fluorescence, after a similar preparation with TC. Exfoliative cytology was positive in 3 of the 6 carcinomas and there was one carcinoma which showed fluorescence but a negative cytology on 3 occasions.

Bronchial aspirate at bronchoscopy was examined in only 1 patient with bronchogenic carcinoma who had been prepared with TC and the smear showed an occasional fleck of fluorescence and was

cytologically positive.

Smears were taken on to glass slides from 4 bronchial biopsies of carcinoma and in 3 instances a positive fluorescent result was obtained.

The total number of cases of carcinoma of lung so far reported are insufficient for a true evaluation of the TC technique to be made. Many varying reports on the value of exfoliative cytology have been published mainly using the Papanicolaou method of staining, and a differing number of sputum examinations. In the best hands where scrupulous attention to detail is carried out and multiple sputum examinations are made, results may be as high as 79% positive diagnosis with only occasional erroneous results (Koss et al. 1964). It is emphasised however that in less skilled hands and with fewer specimens examined, results will be much poorer. As with gastric carcinoma, the shortage of specially trained pathologists and technicians indicates the need for a simpler test. Perhaps the TC fluorescent test on sputum will prove of some value in conjunction with cytology or where such facilities are lacking.

B. Duodenal Drainage. Cytological diagnostic procedures of malignant tumours in the region of the duodenum have not been widely practiced in this country and I have no experience of the TC fluorescence test on duodenal drainage material. This technique has been used by Sandlow et al. (1964ab) who prepared their cases with TC, allowed a 36 hour interval and then performed duodenal intubation with secretin stimulation. In 7 cases of pancreatic carcinoma they obtained typical TC fluorescence, but none in 6 cases of chronic pancreatitis and 12 normal controls. Slatopololsky (1963) mentioned that with a similar technique but using "Urecholine" (bethanecol chloride) as pancreatic stimulus, he obtained 2 fluorescent results in 3 carcinomas of the pancreas, and in a patient with a hepatoma as well.

An analysis of the literature by Henning et al. (1964) revealed a cytological diagnosis rate of 63.5% of 181 cases with 2.8% false positive results. However few centres in this country practice this technique of diagnosis in this difficult area for

tumour detection. The TC fluorescent test has only been tried in the few cases reported above, but where duodenal intubation and pancreatic stimulation is being performed cytological examination could well be supplemented by the TC fluorescent test.

C. Serous Effusions. Effusions in the 3 principal serous cavities, the pleura, the pericardium and the peritoneum, due to malignant diseases have been examined by the technique of the TC fluorescent test in a few cases.

Of the 4 pleural effusions due to carcinoma of the bronchus, prepared with a 5-day course of TC 250 m.g. q.d.s. and allowed an interval of 36 hours before aspiration, 2 cases demonstrated bright golden-yellow fluorescent particles on the dried smear. In both cases exfoliative cytological examination gave suspicious results for neoplastic cells. On the other hand there were no fluorescent particles in the fluid from 2 cases of heart failure, 2 cases of pneumonia, and 1 case of pulmonary embolism. There was, however, in 2 of these non-malignant cases a very faint rim of

powdery golden-yellow fluorescence at the edge of the dried smear of the centrifuged fluid, although no fluorescent particles in the cellular debris, which would suggest that TC may be retained in serous effusions longer than in the blood stream.

In 3 patients with ascites due to portal cirrhosis and one due to heart failure, the same faint powdery ring could be seen at the edge of the smear in one cirrhotic case, although the debris showed no fluorescence. Two patients with ovarian carcinomatous ascites were examined by the same technique and in one of these there were distinct particles of moderate fluorescence in the deposit.

Sandlow & Necheles (1964ab) report 12 malignant pleural effusions all showing fluorescence after preparation with TC, but 12 benign effusions, due to infection and heart failure, showed none. The same authors report all 12 malignant ascites positive, and 12 benign ascites due to cirrhosis and heart failure as showing no fluorescence. Cummins et al. (1964) mention a false positive result in cirrhosis but

3 negative results in other cirrhotics, and a negative test in a patient with hepatic metastases.

These results of the TC test need verification, but as there is some difficulty in the cytological evaluation of effusions because of misinterpretation of mesothelial cells, there would seem to be an indication for a combination of the two techniques. In comparison with the fluorescent results given above we may note a diagnostic accuracy for cytology of 87% in pleural and pericardial fluids quoted by Grunze (1964) with 2% false positive results, although Naylor & Schmidt (1964) claim only 54% decisively positive malignant diagnoses from serous effusions.

D. Urine and Bladder. Only one case of urinary tract malignancy, a hypernephroma, has been reported by Slatopololsky as showing TC fluorescence in a dried smear of urinary sediment. Whitmore (1965) at a lecture to the Royal College of Surgeons in London stated that he had tried, in 42 patients with urinary tract malignancy, a modified TC fluorescence using alkalinisation and millipore filtration but had

abandoned the procedure "as not being a useful screening test".

Direct scrutiny of the bladder using a fibre-optic ultraviolet cystoscope was described at the same lecture (1965) with an increase in the number of cases previously published (Whitmore et al. 1964). They used oral TC 1.0 gm. daily for 2 to 6 days, an interval of 12 to 72 hours being allowed for the urine and normal tissues to be free of the drug. Out of a total of 38 bladder carcinomas, 31 showed fluorescence on ultraviolet cystoscopy and 2 were equivocal. The most striking feature of their report was that 8 out of 10 tumours were carcinomas in situ which were not obvious with ordinary light cystoscopy. This of course opens up an important aspect of TC fluorescence in the search for the early bladder carcinoma in populations at high risk, for example workers in the rubber industry, as a further step in exfoliative cytology screening programmes. In addition the technique may be used to identify the extent of spread and the presence of other early neoplastic

lesions in patients with known bladder tumours. A point, however, which must be borne in mind is that calcareous deposits in the bladder also fluoresce (Barlow, 1965).

E. Colonic Washings. The only work reported on TC fluorescence in colonic washings is by Carter et al. (1962) who prepared their patients with 1500 mg. of TC by intravenous drip on 3 successive days. After an interval of 24 hours colonic lavage was performed until the return was free from particulate matter. A one-litre normal saline enema was given and when returned the fluid was prepared as for gastric smears. Of 16 colonic washings, 7 fluorescent results were subsequently shown to be from colonic carcinomas, one non-fluorescent result from a carcinoma caecum, and one fluorescent result from a patient who died shortly after of acute pancreatitis. The other non-malignant colonic lesions showed no fluorescence.

Lavage and cytology can account for a larger percentage of early diagnoses and five-year survivals in cancer of the colon than in tumours of

the upper alimentary tract (Raskin & Pleticka, 1964) It is therefore paradoxical that colonic cytology has attracted much less support as a diagnostic procedure than gastric cytology. The above authors' diagnostic accuracy was extremely high with only 3.7% of carcinomas undetected, 2.8% "false- positive" results and 8% unsatisfactory examinations in a total of 569 patients including 77 carcinomas beyond the reach of the sigmoidoscope. No other laboratory in the world can rival these results, but the figures show the degree of accuracy of colonic cytology which can be achieved in the best hands. The reason for the infrequency of utilisation of the technique is probably the difficulty of performing the test well. Nevertheless, to supplement exfoliative cytology with a TC fluorescent test would seem reasonable in order to obtain the maximum information from this tedious and rather unpleasant diagnostic procedure.

F. Skin. Lipnik (1963) outlines a screening test for malignant conditions of the skin by applying a solution of 1% DMTC with 0.1% cyanocobalamine directly to the

lesion. A challenge solution of 4.9% trichloracetic acid is then applied to the tumour and if the test is positive, the yellow-green fluorescence must remain within the crusts of the tumour tissue for 10 seconds. He noted a bright yellowish green fluorescence in 68 of 75 malignant and premalignant lesions including basal cell epitheliomas, squamous cell carcinomas and certain senile keratoses. Of 191 benign tumours tested, 186 gave a negative result.

However, Burrows (1964) was unable to confirm these good figures, for he obtained 43% false negative results in 42 malignant lesions, and 54% false positive results in 37 benign lesions.

The principle of the test seems somewhat dubious in so far as the speed of disappearance of the fluorescence with the acid must depend on the rate and depth of penetration of the lesion, both by the TC and by the acid.

That malignant lesions of the skin do show persistent fluorescence when TC is administered

systemically has certainly been shown by myself to occur (see Table 2., Appendix 2.), but no topical applications were made. It seems doubtful if there is any important place in skin tumour diagnosis for TC, as any crusts or sloughs, because of their absent blood supply, may show fluorescence until separation occurs, and indeed Mustakallio (1962) suggested that TC fluorescence might delineate the extent of necrotic superficial injuries.

G. Biopsy Material. The suggestion has been made by Takayama et al. (1964) that the phenomenon of TC localisation in tumours should be utilised as a substitute for frozen-section examination in the operating theatre as the gross fluorescent examination takes 1 or 2 minutes as opposed to 15 minutes for frozen section. They administered 500 mg. of TC by intravenous drip twice daily for 4 days with an interval of only 12 hours before operation. The intravenous route was chosen to achieve a high blood level of 15 ugm. per ml. All 8 of their breast cancers showed typical fluorescence as did 2 gastric cancers

and 2 renal cancers. They contrast the characteristic light yellow fluorescence of tumours with the extremely vivid yellow fluorescence of "panniculus adiposus" and the autofluorescence of 4 benign tumours, 3 cases of mastopathia, and one of chronic interstitial mastitis. The number of cases is certainly not large enough for them to advocate the widespread substitution of ultra-violet examination for frozen-section examination, unless such facilities are unobtainable. Even under those conditions one would hesitate to adopt their idea as in breast lesions, where frozen-section is most often required, weak fluorescence was demonstrated in my cases on the lining of cysts and in one case where radiotherapy had ill-advisably been given for inflammatory disease.

One might, perhaps, utilise TC fluorescence in the darkened operating theatre to delineate the extent of a tumour, but within the abdominal cavity or where there is fat the dull-yellow autofluorescence of fat might well be confusing. In any case as ultraviolet light only penetrates for 1 mm. into

tissues, the tumour would need to be cut across before its extent could be seen. In bone tumours the fluorescent aspect might be utilised as mentioned by Bailey & Levin (1961) who found it of help in one excision of osteogenic sarcoma but the autofluorescence of fat in the marrow may be a source of error and indeed led these authors to amputate higher than was necessary in one patient.

SECTION V.

POSSIBLE THERAPEUTIC IMPLICATIONS OF TETRACYCLINE LOCALISATION IN TUMOURS

INFLUENCE OF ANTIBIOTICS ON TUMOUR GROWTH

The possible relationship of tumour growth and the uptake of antibiotics has been the subject of sporadic reports in the literature, with differing conclusions. Sokoloff & Eddy (1951ab) showed that CTC promoted the growth of certain experimental tumours but could also induce regression of others. Gummel & Luhrs (1953) noticed an increase in experimental tumour growth during administration of chloramphenicol, penicillin and TC. On the other hand, several groups working with a variety of animal tumours could discern no appreciable effects with CTC, TC, or OTC. (Goldman, 1951; Ambrus *et al.*, 1953; Koga, 1954; Sugiura, 1959).

In human malignant disease, Ayre (1951) reported a regression in carcinoma *in situ* of the

cervix in 6 patients being given CTC. A good effect was reported by Bateman et al. (1952, 1953, 1955) in several carcinomas, but these workers changed their minds as their observations progressed. Goldman (1951) reported no noticeable alteration in the clinical course of patients with Hodgkin's disease as a result of administration of TC.

As a result of his clinical observations and of extensive animal experiments, Uemura (1964) concludes that although some antibiotics may occasionally produce either enhanced or inhibited growth in a malignant tumour, most of the factors regulating their growth are probably beyond the reach of bacteriostatics, so that their influence is extremely weak and of no clinical importance.

RADIOACTIVE ISOTOPE-LABELLED TETRACYCLINE

The diagnostic and therapeutic possibilities of isotope-labelled TC have stimulated much interest throughout the world in relation to tumour uptake of the drug. Unfortunately neither the Radiochemical Centre

at Amersham nor the major drug manufacturers of TC have been able to provide me with an isotope-labelled TC for use in animal experiments on uptake and therapy of tumours, so no personal work has been possible in this field.

Various radioactive isotopes can be incorporated into the TC drugs. Snell et al. (1955) labelled OTC with radioactive carbon, C^{14}, using biosynthetic methods with a substrate containing C^{14}-labelled sodium acetate. Using similar methods Miller et al. (1956) were able to incorporate C^{14} into CTC.

The first practical application of radioactive TC was by Andre (1956) who prepared tritium-labelled TC by substitution of tritium at the 10-position using platinum oxide as a catalyst. With his stable compound he studied the distribution of the drug, in experimental animals using autoradiography. Takesue (1960) used the same compound to assay serum levels and found a good correlation with microbiological methods. Tritiated TC was also used by Bakay (1962) to study the dynamics of the drug in relation to meningitis and confirmed that

although the blood-brain barrier is almost impermeable to TC at the normal therapeutic levels, the barrier readily breaks down in meningitis or brain abcess.

The first report in relation to tumours was by Hlavka & Buyske (1960), using the isotope I^{131} substitution into 7-iodo-6-deoxytetracycline prepared by their co-workers (Boothe et al., 1960). Unfortunately, Hlavka & Buyske found a lack of selectivity of the drug in a dose of 0.015 mg. per gm. for tumour tissue in dogs with spontaneous mammary tumours, with maximum concentration at first in the liver which contained twice as much as the tumour and the kidney, and very much more than muscle or bone. After 24 hours the differences were much less pronounced. Similar lack of selectivity for tumours was found in mice with spontaneous mammary carcinomas.

However McCleay et al. (1960) describe briefly their studies with I^{131}-labelled TC in a larger dose of 0.25 mg. per gm. on mice with C3H spontaneous mammary tumours. Scintillometer studies showed localisation of radioactivity in the tumour. In a

higher dosage of 3 mg. per gm., I^{131}-TC produced a definite necrosis of the tumour with survival for the duration of the study of 40 days, whereas tumour-bearing mice not given the radioactive drug survived for no longer than 20 days. No radiation effects were observed in any other organ, but McLeay (1962) in a personal communication comments that the concentration of the drug in the liver is a complicating factor in pursuing this line of research towards the problem of human cancer. McLeay's initial report was followed by one from himself and co-workers (Dunn et al. 1960). Sodium iodide was used to block thyroid uptake in rodents before the I^{131} derivative was administered. In this study in normal rodents, bone appeared to have the highest uptake but the liver had a very variable specific activity. In mice with spontaneous mammary tumours, tumour tissue usually had the highest activity, but there was variation of uptake compared with bone and liver.

The same group of workers (Eskelson et al., 1963) synthesised N-(4 radioiodopyrazole-methyl)-tetracycline and studied its distribution in mice with

tumours. The compound was found to accumulate in the liver of mice and dogs, but not in the mouse tumours to anything like the same degree. They then compared the distribution with that of 9-radioiodo-6-deoxy-6-demethyl-tetracycline. Because of large variations in tumour uptake they were unable to draw any significant conclusions beyond the suggestion that mouse tumours have a preferential uptake for the non-amide substituted compound, although still small in relation to that of the liver over a 96-hour period. One wonders about the stability of the compounds for the mice, which had not been pretreated with iodide, showed a high uptake in the thyroid gland.

This seems to be all the work which has been done on isotope-labelled TC in relation to tumours and it seems a great pity that the initial report with the doubling of life-span and necrosis of tumours in mice has not been investigated further with other substituted compounds and with other tumours. An alternative approach was mentioned by Phillips et al. (1960) who had succeeded in conjugating a boronated TC compound

(tetracycline boronic acid) which was being studied in animal tumours from the aspect of ultimate neutron-capture therapy. Their preliminary observations indicated that the administration of this compound permitted deposition of fissionable material (boron) in cancer tissue, which could then be exposed to a neutron beam. No further reports appear to have been published on this subject.

CYTOTOXIC AGENT COMBINATION WITH TETRACYCLINE

An alternative approach to cancer therapy in this context is by preparing a stable combination of TC with a cytotoxic agent. A TC mustard compound has been prepared by Kaplan et al. (1965) by conjugating $\beta-\beta^1$-dichlorodiethylamine hydrochloride (nor HN_2) with TC. They tested the compound on mice with spontaneous tumours and showed some antitumour activity, although whether the mustard compound was released mostly in the injection solution, in the animals' bloodstream, or at the neoplastic cell was not shown.

SUMMARY

The historical aspects and current theory of fluorescence have been described with reference to this property of the TC drugs demonstrable by ultra-violet light.

The entire literature, relating to the characteristic golden-yellow fluorescence induced in malignant tumours by the TC's, has been reviewed. The nature of the fluorophore and its fixation in tumours, bone and other tissues has been discussed with the role of calcium chelation being emphasised.

To verify and examine this property of tumours, a study of 40 cases has been made with the demonstration of typical fluorescence in 90% of malignant neoplasms after the prior administration of TC. Tumour-type and histological differentiation does not determine the fluorescent intensity, which is in part related to the TC blood levels achieved. Occasional benign lesions showed faint fluorescence after TC.

Exfoliation of fluorescent material has been studied with especial reference to the diagnosis of gastric carcinoma. Smears prepared from gastric washings showed characteristic fluorescence in 77% from gastric carcinomas, and 4% from control cases prepared with TC, but none could be seen in either group if TC had not been given. Standard cytological examination showed malignant cells in only one-third of carcinomas studied and none in the controls. Analysis of results has been made with regard to tumour type and differentiation and the TC regime employed. Scrupulous attention to detail must be paid if fallacious results are to be avoided.

The published reports on the gastric fluorescent test have been analysed and in general the results obtained in the larger series were similar to my own. The critics of the test have reported small numbers and it is thought that their inexperience in recognition of the typical fluorescence may have led to their poor results.

The value of the fluorescent test has been assessed in relation to large collected analyses of other diagnostic methods. Cytology, radiology and gastroscopy may each lead to a correct diagnosis in two-thirds to three-quarters of gastric carcinomas, as can the TC fluorescent test on gastric washings. The proportion of false malignant diagnoses in benign diseases for each of these techniques is similar to that of the fluorescent test.

The principle of the test has been extended to include the examination of sputum, effusions, colonic washings and duodenal juices. My own limited experience with sputum, pleural effusions and ascites has been presented and the published series reported, with the suggestion that TC fluorescence may have wider applications in tumour diagnosis.

The TC fluorescent test has been shown to have a place in the diagnosis of malignant disease, particularly of the stomach and perhaps of other

organs. It may be combined with cytology to obtain maximum information from exfoliated material and has the advantage in not needing a skilled cytologist for its interpretation.

Finally, the possible future therapeutic implications of TC binding by tumours have been discussed with reference to the published reports of radio-isotope labelling and combination with cytotoxic drugs.

Appendix 1.

CONTROL GROUP OF NON-MALIGNANT DISEASE

- PREPARED WITH TETRACYCLINE (see Page 31.)

1. Mr. S.A. Age 66.

   Presented with features of colonic obstruction.

   TC 1000 mg. intravenous over 30 minutes.

   Interval 2 days

   Fluorescence of opened excised pelvic colon - NIL

   Histology - Acute Diverticulitis.

2. Mr. B.M. Age 23

   Presented with symptoms of chronic small bowel obstruction and a mass in R.I.F.

   TC 1000 mg. intravenous over 30 minutes.

   Interval 4 days

   Fluorescence of excised ileal mucosa and thickened ileal wall - NIL.

   No fluorescence of several fleshy lymph nodes but one gritty hard obviously calcified lymph node showed moderate yellow fluorescent streaks.

   Histology - Crohn's Disease of Ileum

3. Mr, F.J. Age 55

   Presented with haematemesis and melaena. Ba meal suggested antral ulceration.

   TC 500 mg. q.d.s. for 4 days. Total 8000 mg.

3. cont.

Interval 24 hours

Fluorescence of partial gastrectomy specimen - NIL.

Histology - Marked Antral Gastritis and
Intestinal Metaplasia.

4. Mrs. F.P. Age 51.

Had radiotherapy at same time as course of TC 3 months earlier for presumptive carcinoma breast at another hospital.

TC 250 mg. q.d.s. for 5 days. Total 5000 mg.

Interval 3 months

Fluorescence - extremely weak traces of yellow in sectioned specimen.

Histology - Chronic Mastopathia with cyst formation and granulation tissue.
No evidence of malignancy.

5. Mrs. D.W. Age 43

Presented with lump in breast.

TC 500 mg. q.d.s. x 5 doses. Total 2500 mg.

Interval 26 hours

Fluorescence of excised mass - weak powdering of yellow fluorescence on lining of cyst.

Histology - Mastopathia with dense connective tissue with distorted ducts and intraluminal proliferation.

6. Mrs. P.H. Age 50.

Presented with lump in breast.

TC 500 mg. 4 hourly x 6 doses. Total 3000 mg.

Interval 26 hours

Fluorescence of excised mass - weak strands of yellow fluorescence.

Histology - Mastopathia - condensation of intralobular connective tissue and extensive duct proliferation.

7. Mr. J.S. Age 42

Presented with lump in breast.

TC 500 q.d.s. for 4 days. Total 8000 mg.

Interval 28 hours

Fluorescence of opened cyst - weak powdering of yellow fluorescence on inside of cyst wall.

Otherwise NIL.

Histology - Retention Cyst covered with dense fibrous tissue and infiltration of inflammatory cells, some ducts with stasis and round-cell infiltration. Mastopathia

8. Mrs. S.T. Age 50.

Presented with lump in breast.

TC 500 mg. q.d.s. for 3 days. Total 6000 mg.

Interval 30 hours

Fluorescence of incised mass - NIL.

Histology - Mastopathia with duct proliferation.

9. Mr. T.F. Age 69.

Presented with cough and chest X-ray showing patchy atalectasis.

TC 500 mg. 4 hourly x 6. Total 3000 mg.

Interval 24 hours

Fluorescence of bronchial biopsy fragments - NIL.

Histology - Unstable Metaplasia
but no malignancy.

10. Mrs. E.W. Age 73

Presented with warty area on tongue.

Pre-TC - NIL fluorescence in vivo.

TC 1000 mg. intravenous over 1 hour.

Interval 24 hours

Fluorescence in vivo and biopsy - NIL.

Histology - Simple Keratosis.

11. Mrs. L.M. Age 79

Presented with lump of thigh for 1 year.

TC 500 mg. 4 hourly x 6 doses. Total 3000 mg.

Interval 30 hours

Fluorescence of lump - NIL.

Histology - Lipoma.

12. Mr. G.A. Age 65

Presented with dyspepsia for 4 years with lesser curve GU on Ba meal.

TC 250 mg. q.d.s. for 5 days. Total 5000 mg.

Interval 36 hours

Fluorescence of gastrectomy specimen - NIL of ulcer crater nor of cut edge.

Histology - Benign Chronic Gastric Ulcer.

13. Mrs. A.B. Age 56

Presented with vomiting and dyspepsia for 9 months with large ulcer on posterial wall of stomach on Ba meal.

TC 500 mg. q.d.s. for 2½ days. Total 5000 mg.

Interval 30 hours

Fluorescence of gastrectomy specimen - NIL of ulcer crater nor of cut edge.

Histology - Benign Chronic Gastric Ulcer.

14. Mrs. J.K. Age 53.

Presented with 3/52 pain, vomiting and anorexia. Ba meal showed fairly large GU on high posterior wall of stomach with possibility of malignancy.
TC 500 mg. q.d.s. for 2½ days. Total 5000 mg.
Interval 36 hours
Gastric lavage - no fluorescence.
Further interval of 25 hours
Fluorescence of Gastrectomy specimen - NIL of ulcer crater nor of cut edge.
Histology - Benign Chronic Gastric Ulcer.

15. Mrs. R.S. Age 72.

Presented with 5 year history of dyspepsia and proven gastric ulcers.
TC 500 mg. q.d.s. for 3 days. Total 6000 mg.
Interval 36 hours
Gastric lavage - no fluorescence. No malignant cells seen.
Further interval of 28 hours
Fluorescence of gastrectomy specimen - NIL of ulcer crater nor of cut edge.
Histology - Benign Gastric Ulcer with Atrophic Gastritis.

16. Mr. F.L. Age 54

Presented with 2 year dyspepsia and weight loss.

Ba meal - large lesser curve ulcer with raised edge.

Gastroscopy appearances highly suspicious of malignancy.

TC 500 mg. q.d.s. for 3 days. Total 6000 mg.

Interval 36 hours

Gastric lavage - no fluorescence. No malignant cells seen.

Further interval of 24 hours.

Fluorescence of gastrectomy specimen - NIL of surface nor of cut edge.

Histology - Benign Gastric Ulcer with extensive Chronic Gastritis and Intestinal Metaplasia.

17. Mr. S.C. Age 48

Presented with rectal bleeding.

Ba enema filling defect in descending colon.

TC 250 mg. q.d.s. for 5 days. Total 5000 mg.

Interval 30 hours

Fluorescence of excised polyp - NIL on surface nor at excised base nor on section.

Histology - Benign Colonic Polyp.

18. Miss E.S. Age 51.

Presented with lump in breast.

TC 500 mg. q.d.s. for 2½ days. Total 5000 mg.

Interval 32 hours

Fluorescence of excised mass - NIL.

Histology - Mastopathia with extensive duct proliferation.

19. Mrs. Y.S. Age 58.

Presented with lump in breast.

TC 250 mg. q.d.s. for 5 days. Total 5000 mg.

Interval 34 hours

Fluorescence of excised mass - NIL except for faint dusting of yellow fluorescence on inside of cyst wall.

Histology - Mastopathia with duct proliferation and some duct stasis, with a single Retention Cyst.

20. Mr. S.L. Age 50.

Presented with haemoptysis and fever. Chest X-ray - NAD.

TC 250 mg. q.d.s. for 7 days. Total 7000 mg.

Interval 48 hours

Fluorescence of bronchoscopic biopsy - NIL.

Histology - Chronic Bronchitis.

21. Mr. W.H. Age 48.

Presented with bronchitis and haemoptysis.

TC 250 mg. q.d.s. for 7 days. Total 7000 mg.

Interval 24 hours

Fluorescence of bronchoscopic biopsy - NIL.

Histology - Chronic Bronchitis with some metaplasia.

22. Mrs. P.H. Age 29.

Presented with lump on wrist

TC 500 mg. 4 hourly x 6. Total 3000 mg.

Interval 24 hours

Fluorescence of excised cystic mass - NIL.

Histology - Simple Ganglion.

23. Mrs. J.T. Age 69.

Presented with constipation and dubious barium enema.

TC 250 mg. q.d.s. for 5 days. Total 5000 mg.

Interval 4 days

Fluorescence of opened colon - NIL.

Histology - Diverticulitis.

24. Mrs. J.P. Age 72.

Presented with rectal bleeding and dubious barium enema.

TC 1000 mg. IV over 2 hours.

Interval 34 hours

Fluorescence of opened colon and mass - NIL.

Histology - Diverticulitis with pericolic extension.

25. Mrs. S.H. Age 52.

Presented with ulcer of leg for 2 years.

Fluorescence - NIL before TC.

TC 250 mg. q.d.s. for 5 days. Total 5000 mg.

Fluorescence during course of TC of ulcer in vivo - showed weak but definite yellow fluorescence.

Interval 24 hours - weak fluorescence of scab.

Interval 48 hours - fluorescence of ulcer (scab having separated) - NIL.

No histology as clinically Varicose Ulceration which healed completely with bed rest.

Appendix 2.

DETAILS OF MALIGNANT TUMOURS EXAMINED FOR TETRACYCLINE FLUORESCENCE - SUMMARIZED IN TABLE 2. (page 35)

1. Mr. E.C. Age 74.

Presented with diarrhoea and rectal mass.

TC 1000 mg. intravenous over 8 hours.

Interval 64 hours

Fluorescence of excised opened rectum - weak yellow fluorescence of floor, but cut edge of ulcer showed brilliant yellow flecks.

Adjacent lymph gland cut surface - weak yellow fluorescence.

Histology - Moderately well differentiated Adenocarcinoma Rectum

Broder's Grade II. Duke's Class B.

2. Mr. J.M. Age 66.

Presented with rectal bleeding and abdominal distension.

TC 1000 mg. intravenous over 6 hours.

Interval 24 hours

Fluorescence of opened excised rectum - nil of ulcer surface and dubious of cut edge. Note - enormous hepatomegaly due to secondary deposits.

Histology - Poorly differentiated Adenocarcinoma Rectum

Broder's Grade III.

3. Mr. L.S. Age 46

Presented with rectal bleeding.

TC 1000 mg. intravenous over 1 hour.

Interval 66 hours

Fluorescence of opened excised rectum - moderate yellow on surface of ulcer; brilliant yellow of cut surface; brilliant in lymph node cut across.

Histology - Moderately well differentiated Adenocarcinoma Rectum with hyperchromatic cells lining well-formed acini and tubules. Carcinoma in adjacent lymph nodes.

Broder's Grade II. Duke's Class C.

4. Mrs. A.M. Age 66.

Presented with diarrhoea and rectal bleeding.

TC 1000 mg. intravenous over 30 mins.

Interval 48 hours

Fluorescence of opened excised rectum - weak yellow on surface, brilliant yellow on cut edge of ulcer. Brilliant yellow in lymph node cut across.

Histology - Adenocarcinoma Rectum with well-formed acini penetrating deeply into muscle layers. Carcinoma in adjacent lymph node.

Broder's Grade II. Duke's Class C.

5. Mr. T.B. Age 84.

Presented with recurrence of rectal tumour after previous excision 2 years earlier.

TC 1000 mg. intravenous over 2 hours.

Interval 40 hours

Fluorescence of opened resected rectum, moderate yellow on surface, brilliant on cut edge.

Histology - Well differentiated
Adenocarcinoma Rectum
Broder's Grade II.

6. Mr. N.W. Age 44.

Presented with rectal bleeding.

TC 1000 mg. intravenous over 1 hour.

Interval 5 days

Fluorescence of opened resected rectum - nil on surface of ulcer, moderate yellow of cut edge.

Histology - Well differentiated
Adenocarcinoma Rectum
Broder's Grade II. Duke's Class A.

7. Mr. J.A. Age 27

Presented with rectal bleeding.

TC 500 mg. t.d.s. over 56 hours. Total 4000 mg.

Interval 36 hours

7. cont.

Fluorescence of opened excised rectum - weak yellow on ulcer surface, moderate yellow with brilliant flecks of cut edge.

Histology - Moderately well differentiated

Adenocarcinoma Rectum

Broder's Grade II. Duke's Class C.

8. Mr. E.F. Age 87

Presented with rectal bleeding.

TC 1000 mg. intravenous over 1 hour

Interval 48 hours

Fluorescence of opened excised rectum - nil on surface of tumour, weak yellow on cut edge of ulcer.

Histology - Well differentiated

Adenocarcinoma Rectum.

9. Miss L.L. Age 80.

Presented with abdominal mass and bowel irregularity.

TC 250 mg. q.d.s. for 5 days orally. Total 5000 mg.

Interval 6 days

Fluorescence of excised mass of tissue, moderate overall fluorescence with brilliant flecks and spots.

Histology - Well differentiated

Adenocarcinoma Colon

Broder's Grade II. Duke's Class B.

10. Mr. Tr.B. Age 66.

Presented with constipation and rectal bleeding.

TC 1000 mg. intravenous over 4 hours.

Interval 5 days

Fluorescence of opened excised colon, nil of efflorescent branches of tumour but moderate yellow with intense flecks in base and in adjacent lymph node.

Histology - Well differentiated

Papillomatous Adenocarcinoma Colon

Broder's Grade II. Duke's Class B.

11. Mr. W.S. Age 59.

Presented with constipation.

TC 1000 mg. intravenous over 4 hours.

Interval 5 days

Fluorescence of opened resected colon - weak yellow on surface of ulcer, moderate yellow with brilliant flecks in cut mass and lymph node.

Histology - Poorly differentiated

Carcinoma Colon

Broder's Grade III. Duke's Class C.

Lymph nodes invaded.

12. Mr. V.R. Age 73.

Presented with diarrhoea.

TC 1000 mg. intravenous over 1 hour.

Interval 3 days

Fluorescence of opened excised colon - NO yellow fluorescence but bright flecks of blue/white fluorescence in areas of the cut tumour.

Histology - Well differentiated

Adenocarcinoma Colon

Broder's Grade II. Duke's Class B.

13. Mrs. N.G. Age 74.

Presented with anaemia, pain and mass in R.I.F.

TC 1000 mg. intravenous over 1 hour.

Interval 40 hours

Fluorescence of large mass of tumour, areas of negative, moderate and brilliant yellow. Lymph glands showed similar variation.

Histology - Well differentiated

Adenocarcinoma Caecum

with extensive spread.

Broder's Grade II. Duke's Class B.

14. Mrs. L.C. Age 65.

Presented with gross furgating carcinoma of breast.
Being treated with radiotherapy.
Before TC, no fluorescence in vivo. TC 500 mg. stat. then 250 mg. q.d.s. orally for 7 days.
Total 7500 mg.
Interval (i) 48 hours - Fluorescence brilliant generalised yellow in vivo of ulcerated breast
(ii) 8 days - Moderate in vivo fluorescence
Histology - Carcinoma Breast.

15. Mrs. D.D. Age 71.

Presented with lump in breast.
TC 500 mg. 4 hourly x 6 orally. Total 3000 mg.
Interval 30 hours
Fluorescence of simple mastectomy specimen - nil of surrounding breast tissue cutting into specimen circumscribed moderate yellow fluorescence with brilliant flecks.
Histology - Irregular islands of cancer cells in dense connective tissue, the cells having vesicular nuclei and small amounts of cytoplasm.
Carcinoma Breast.

16. Miss D.P. Age 64.

Presented having had mastectomy 2 years earlier, recent ulcerating lumps in axilla and neck.
Before TC, no fluorescence on examination of areas with ultraviolet
TC 250 mg. q.d.s. for 5 days orally. Total 5000 mg.
Fluorescence at end of course, moderate yellow neck ulcer and brilliant fleck on small axillary ulcer in vivo.
Interval 7 days
Fluorescence unchanged.
Histology - Heavily Keratinized
Squamous Cell Carcinoma Breast
Broder's Grade II.

17. Mrs. A.W. Age 60.

Presented with lump in breast.
TC 500 mg., 4 hourly x 5 orally. Total 2500 mg.
Interval 24 hours
Fluorescence of radical mastectomy specimen - brilliant fluorescence of tumour tissue. Deep to pectoralis major, strands of moderate fluorescence, apparently lymph vessels with carcinoma.
Histology - Spheroidal Cell Carcinoma Breast with marked scirrhous reaction. Lymph nodes invaded. Patey-Scarff Grade II.

18. Mrs. I.P. Age 51.

Presented with lump in breast.

TC 500 mg. 4 hourly x 6 orally. Total 3000 mg.

Interval 28 hours

Fluorescence of radical mastectomy specimen - moderate yellow of tumour mass with brilliant flecks.

Histology - Carcinoma Breast

Clumps of irregular vacuolated cells in dense fibrous tissue.

19. Miss E.B. Age 72

Presented with lump in breast.

TC 500 mg. q.d.s. for 1 day. Total 2000 mg.

Interval 24 hours

Fluorescence - moderate diffuse yellow of cut tumour

Histology - Spheroidal-cell Carcinoma Breast

Patey-Scarff Grade II.

20. Mr. A.G. Age 66.

Presented with lump of breast.

TC 1000 mg. intravenous over ½ hour.

Interval 48 hours

Fluorescence of cut tumour - patchy brilliant yellow

Histology - Carcinoma Breast (Male)

Little tendency to form tubules, abundant fibrous reaction.

21. Mrs. L.H. Age 83

Presented with lump in breast.

TC 500 mg. 4 hourly x 4. Total 2000 mg.

Interval 24 hours

Fluorescence of simple mastectomy specimen - moderate diffuse yellow with brilliant streaks of cut tumour (SEE PHOTOGRAPH A).

Histology - Adenocarcinoma Breast with good deal of intraluminal proliferation, but also scirrhous reaction and much proliferation by single cells and small groups. Patey-Scarff Grade II.

22. Mr. J.S. Age 73

Presented with loss of weight and dyspepsia.

TC 250 mg. q.d.s. for 5 days orally. Total 5000 mg.

Interval 30 hours - gastric lavage - no fluorescence of deposit.

Interval to operation 44 hours.

Fluorescence of excised stomach - cut surface brilliant yellow, but only faint blotch of reddish yellow on mucosal surface.

Histology - Adenocarcinoma of Stomach - Linitis Plastica.

23. Mr. P.M. Age 52

Presented with dyspepsia.

TC 250 mg. q.d.s. for 5 days. Total 5000 mg.

Interval of 36 hours to gastric lavage - no fluorescence of deposit.

Interval to gastrectomy 60 hours

Fluorescence - Dubious traces of yellow on surface and cut edge of tumour.

Histology - Carcinoma Stomach

Mass of oedematous connective tissue with numerous signet-ring type cancer cells.

24. Mrs. M.CU. Age 67

Presented with anaemia.

TC 500 mg. q.d.s. orally for 2½ days. Total 5000 mg.

Interval 36 hours to gastric lavage - very gentle because much altered blood - no fluorescence.

Interval 11 days to gastrectomy.

Fluorescence of tumour - NIL.

Histology - Well differentiated ulcerated Adenocarcinoma Stomach

25. Mr. H.F. Age 64.

Presented with loss of weight and abdominal pain.
TC 500 q.d.s. orally for 2½ days. Total 5000 mg.
Interval 36 hours
Gastric lavage - moderate spots of yellow fluorescence.
Interval before gastrectomy - 60 hours
Fluorescence of excised stomach - moderate of ulcerated surface of tumour and moderate of cut edge
Histology - Flat, thick, ulcerated anaplastic Carcinoma of Stomach.

26. Mr. J.M. Age 60

Presented with headache, vomiting and signs of intracranial tumour with collapsed lobe on chest X-ray. Bronchoscopy - negative.
TC 500 mg. q.d.s. orally for 3 days. Total 6000 mg.
Interval 18 days
Fluorescence brilliant yellow of autopsy material from lung (brain not examined).
Histology - Oat-cell Carcinoma Bronchus

27. Mr. A.P. Age 62

Presented for investigation of collapsed right upper lobe of lung.
TC 500 mg. 4 hourly x 5 orally. Total 2500 mg.
Interval - 25 hours

27. cont.

Fluorescence - weak yellow of bronchial biopsy fragment smeared on slide.

Histology - Oat-cell Carcinoma Bronchus

28. Mrs. D.W. Age 64

Presented with confusion and hilar mass on chest X-ray.

TC 500 mg. q.d.s. for 2½ days. Total 5000 mg.

Interval 30 hours

Fluorescence of smear of fragment of bronchoscopic biopsy - brilliant yellow.

Histology - Squamous Cell Carcinoma Bronchus

29. Mr. J.W. Age 60

Presented with haemoptysis.

TC 500 mg. q.d.s. orally for 2½ days. Total 5000 mg.

Interval 30 hours

Fluorescence of smear of fragment of bronchial biopsy - NIL.

Histology - Oat-cell Carcinoma Bronchus

30. Mr. H.C. Age 61

Presented with discharging anal mass.

TC 1000 mg. intravenous over 8 hours.

Interval 70 hours

Fluorescence of opened excised rectum and anus - nil of ulcer surface but moderate diffuse yellow with bright flecks on cut edge.

Histology - Squamous Carcinoma of Anal Canal
Broder's Grade II.

31. Mr. W.L. Age 58.

Presented with painful ulcerating prepuce.

Before TC no fluorescence on examination of penis with ultraviolet lamp.

TC 500 mg. 4 hourly x 6 orally. Total 3000 mg.

Fluorescence in vivo at end of course of TC - brilliant yellow on all raw surfaces.

Interval 30 hours

Fluorescence of excised prepuce - spots of brilliant yellow on some ulcerated and cut surfaces, otherwise moderate intensity of ulcerated areas.

Histology - Squamous Carcinoma of Prepuce
Broder's Grade II.

32. Mr. D.T. Age 65

Presented with ulcer on amputation stump.

TC 1000 mg. intravenous in 1 hour.

Interval 28 hours

Fluorescence of surface of ulcer - weak yellow, moderate yellow of cut tumour. Scraping from subjacent marrow cavity negative.

Histology - Well differentiated

Squamous Cell Carcinoma Skin

Marrow scraping showed no malignancy.

33. Mrs. A.P. Age 75

Presented with enlarging ulcer of leg for years.

Received radiotherapy prior to TC.

Before TC no fluorescence on examination with ultraviolet lamp.

TC 250 mg. q.d.s. for 10 days orally. Total 10,000 mg.

Interval 48 days

Fluorescence - brilliant yellow patches over ulcerated area in vivo.

Histology - Basal Cell Carcinoma of Skin

34. Miss M.E. Age 87

Presented with enlarging ulcer of cheek.

Being treated with radiotherapy.

Before TC no fluorescence in vivo.

TC 250 mg. q.d.s. for 5 days orally. Total 5000 mg.

34. cont.

Interval 53 hours

Fluorescence - diffuse moderate of ulcer in vivo

Histology - Basal Cell Epithelioma of Cheek.

35. Mr. J.B. Age 39

Malignant melanoma of leg removed 1½ years earlier.

Presented with swelling in groin.

TC 1000 mg. intravenous over 4 hours.

Interval 48 hours

Fluorescence of cut glands - nil.

Histology - Replacement of glands by
Malignant Melanoma.

36. Mrs. P.H. Age 69.

Presented with loin mass and pain.

TC 500 mg. q.d.s. for 4 days orally. Total 8000 mg.

Interval 8 days

Fluorescence - variable intensity from moderate to brilliant through whole mass of tissue.

Histology - Transitional Cell Carcinoma of Renal Pelvis
with papillary overgrowth of pelvic epithelium and extensive invasion of kidney and surrounding tissues.

37. Mrs. M.Cl. Age 60

Presented with pain, mass in loin and cachexia.

TC 250 q.d.s. for 7 days orally. Total 7000 mg.

Interval 48 hours - then died.

Fluorescence of large mass of tissue at autopsy - patchy varying from nil to brilliant.

Histology - Fairly well differentiated Papillary Adenocarcinoma of Kidney with fairly extensive necrosis.

38. Mr. H.W. Age 59.

Presented with parotid swelling.

TC 500 mg. 4 hourly x 4 orally. Total 2000 mg.

Interval 30 hours

Fluorescence of tumour fragments - moderate yellow.

Histology - Salivary Carcinoma with low-grade sialadenitis and tumour tissue in the form of cords and islands of rather dark cells widely spaced in heavy fibrous stroma.

Broder's Grade II.

39. Mr. E.P. Age 79.

Presented with lump in cheek ulcerating into mouth
TC 500 mg. x 2 orally, then patient had stroke, then
200 mg. by intramuscular injection x 8. Total 2600 mg.
Interval 24 hours then death.
Fluorescence of autopsy fragment - brilliant yellow.
Histology - Carcinoma of Salivary Gland
Infiltration of subepithelial tissues
by irregular masses of cells resembling
epidermal basal cells.

40. Mr. S.C. Age 68

Presented with tumour of jaw ulcerating into the
mouth. Being treated with radiotherapy.
Before TC no fluorescence on examination of mouth
with ultraviolet lamp.
TC 500 mg. stat., then 250 mg. q.d.s. orally for
7 days. Total 7,500 mg.
Interval (i) 36 hours - Fluorescence moderate
diffuse yellow in vivo
(ii) 8 days - Fluorescence moderate
diffuse yellow post mortem.
Histology - Chondrosarcoma of Mandible

Appendix 3.

DETAILS OF TETRACYCLINE FLUORESCENT TEST IN GASTRIC LAVAGE OF MALIGNANT TUMOURS - SUMMARIZED IN TABLE 4 (page 110.)

1. Mr. W.F. Age 78

Presented with 5 months' dyspepsia, anorexia and loss of weight.

Ba. meal - large tumour in upper half of stomach.

TC 250 mg. q.d.s. for 5 days. Total 5000 mg.

Interval 36 hours

Lavage fluorescence - brilliant smears.

Cytology - "some suspicious atypical cells" suggestive of squamous cell carcinoma.

Gastrectomy - Ulcerated, well differentiated, papilliferous Adenocarcinoma

2. Mrs. R.B. Age 61.

Presented with 2½ years' dyspepsia.

Ba. meals - had two 1½ years earlier - NAD.

- third showed tumour mass in mid-gastric region.

TC 500 mg. q.d.s. for 2½ days. Total 5000 mg.

Interval 36 hours

Lavage fluorescence - moderate streaks and flecks.

Cytology - "suspicious atypical cells"

Laparotomy - inoperable tumour. Gland biopsy - very poorly differentiated Adenocarcinoma

3. Mrs. W.W. Age 65.

Presented with 2 months' anorexia, epigastric pain and weight loss.

Ba. meal - large mid-gastric tumour.

TC 500 mg. q.d.s. for 2½ days. Total 5000 mg.

Interval 30 hours

Lavage fluorescence - NIL.

Cytology - no malignant cells.

Gastrectomy - Anaplastic carcinoma of the linitis plastica type with no mucosal invasion.

4. Mr. H.F. Age 64. (= Case 25. Appendix 2)

Presented with 8 months' weight loss, epigastric pain and epigastric mass.

Ba. meal - pyloric filling defect.

Lavage fluorescence before TC - NIL.

TC 500 q.d.s. for 2½ days. Total 5000 mg.

Interval 36 hours

Lavage fluorescence - moderately intense spots.

Cytology - no malignant cells.

Gastrectomy - flat thick antral anaplastic carcinoma.
Fluorescence of tumour - moderate intensity.

5. Mr. F.O. Age 55.

Presented with 6 months' weight loss and vomiting.

Lavage fluorescence before TC - NIL.

Ba. meal - pyloric filling defect.

TC 500 mg. q.d.s. for 2½ days. Total 5000 mg.

Interval 36 hours

Lavage fluorescence - weak flecks.

Cytology - no malignant cells.

Gastrectomy - large ulcerated poorly differentiated Adenocarcinoma

6. Miss C.W. Age 54.

Presented with 8 months' weight loss and anaemia.

Ba. meal - rigid lesser curve.

TC 250 q.d.s. for 5 days. Total 5000 mg.

Interval 36 hours

Lavage fluorescence - weak flecks.

Cytology - no malignant cells.

Gastrectomy - well differentiated papilliferous Carcinoma.

7. Mr. G.S. Age 74.

Presented with 2 years' weight loss and anorexia.

Ba. meal - irregular filling defect in upper ½ of stomach.

TC 500 mg. q.d.s. for 2½ days. Total 5000 mg.

Interval 32 hours.

7. cont.

Lavage fluorescence - few weak flecks.

Cytology - not performed.

Laparotomy - obvious inoperable gastric malignancy.

No biopsy.

8. Mr. A.A. Age 60.

Presented with 6 months' weight loss and dyspepsia.

Ba. meal - very extensive infiltration and filling defects of body of stomach.

TC 1000 mg. intravenous over 4 hours.

Interval 48 hours

Lavage fluorescence - brilliant smears.

Cytology - "neoplastic cells present".

No operation and died of carcinomatosis with hepatic metastases.

9. Mr. A.K. Age 56.

Presented with 3 months' weight loss and dyspepsia.

Ba. meal - very extensive malignancy of body of stomach.

TC 1000 mg. intravenous over ½ hour.

Interval 40 hours

Lavage fluorescence - few flecks of moderate intensity.

Cytology - not performed.

Laparotomy - inoperable malignant growth of stomach.

No biopsy.

10. Mr. F. Wa. Age 80.

Presented with 6 months weight loss and dyspepsia.

Ba. meal - large fundal filling defect.

Lavage before TC - no fluorescence.

TC 500 mg. q.d.s. for 4 days. Total 8000 mg.

Interval 36 hours

Lavage fluorescence - small spots of moderate intensity.

Cytology - not done.

No operation as too ill. Died after clinical course of carcinoma.

11. Mr. P.M. Age 60. (= Case 23, Appendix 2.)

Presented with 6 months' dyspepsia.

Ba. meal - mid-gastric tumour.

TC 250 mg. q.d.s. for 5 days. Total 5000 mg.

Interval 36 hours

Lavage fluorescence - NIL.

Cytology - not performed.

Gastrectomy - much connective tissue with many separate "signet-ring" type of cancer cells. Dubious fluorescence of excised tumour.

12. Mrs. M.C. Age 67. (= Case 23, Appendix 2.)

Presented with iron-deficiency anaemia.

Ba. meal - mid gastric plaque.

Gastroscopy - irregular ulcer probably malignant.

TC 500 mg. q.d.s. for 2½ days. Total 5000 mg.

Interval 36 hours

Lavage fluorescence - NIL but vigorous lavage not performed because of bleeding.

Cytology - no malignant cells.

Gastrectomy - well differentiated ulcerated Adenocarcinoma. No fluorescence of excised tumour.

13. Mr. T.S. Age 49.

Presented with 1 year's anorexia, weight loss and dyspepsia.

Ba. meal - large antral papilliferous neoplasm.

Lavage fluorescence before TC - NIL.

TC 1000 mg. IV over 4 hours.

Interval 36 hours

Lavage fluorescence - few spots of moderate intensity.

Cytology - no malignant cells.

Laparotomy - inoperable neoplasm. Biopsy poorly differentiated mucoid Adenocarcinoma.

14. Mr. E.B. Age 55.

Presented with 2 years' dyspepsia.

Ba. meal - large flat lesser curve ulcer.

Gastroscopy - mid-gastric contraction with plaque.

TC 250 mg. q.d.s. for 5 days. Total 5000 mg.

Lavage fluorescence - NIL.

Cytology - no malignant cells.

Gastrectomy - malignant ulcer with poorly differentiated mucoid Adenocarcinoma.

15. Mr. F. Wo Age 60.

Presented with recent epigastric pain and weight loss. Gastroenterostomy 15 years earlier.

Ba. meal - fundal filling defect.

Gastroscopy - good view not obtained.

TC 500 mg. q.d.s. for 4 days. Total 8000 mg.

Interval 36 hours

Lavage fluorescence - few flecks of moderate intensity.

Cytology - no malignant cells seen.

No operation. Died of progressive anaemia, cachexia and hepatic metastases.

16. Mr. F.E. Age 69.

Presented with 6 months' dyspepsia and weight loss.

Ba. meal - probable gastric carcinoma with rigid lesser curve.

TC 250 mg. q.d.s. for 5 days. Total 5000 mg.

16. cont.

Interval 36 hours

Lavage fluorescence - brilliant smears.

Cytology - not performed.

Laparotomy - inoperable malignant tumour of stomach. No biopsy.

17. Mr. J.S. Age 73. (= Case 22, Appendix 2.)

Presented with 4 months' dyspepsia and weight loss.

Ba. meal - rigid stomach.

TC 250 mg. q.d.s. for 5 days. Total 5000 mg.

Interval 30 hours

Lavage fluorescence - NIL.

Cytology - not performed.

Gastrectomy - Adenocarcinoma of stomach of linitis plastica type. Fluorescence of excised stomach - cut surface brilliant but only faint blotch of reddish yellow in mucosal surface.

18. Mrs. L.J. Age 50.

Presented with 18 months' anaemia and 2 months' pain and vomiting.

Ba. meal - NAD.

Gastroscopy - rigid antrum with an ulcer.

TC 500 mg. q.d.s. for 2½ days. Total 5000 mg.

Interval 30 hours.

18. cont.

La Lavage fluorescence - weak flecks.

Cytology - no malignant cells.

Gastrectomy - poorly differentiated ulcerated Carcinoma of Stomach.

19. Mrs. D.H. Age 68.

Presented with 4 months' abdominal pain and weight loss.

Ba. meal - extensive malignancy of stomach.

TC 500 mg. q.d.s. for 2½ days. Total 5000 mg.

Interval 30 hours.

Lavage fluorescence - few flecks of moderate intensity

Cytology - no malignant cells.

Gastrectomy - poorly differentiated ulcerated Adenocarcinoma of Stomach.

20. Miss S.M. Age 72.

Presented with 8 months' loss of weight and recent haematemesis.

Ba. meal - extensive tumour of stomach.

TC 250 q.d.s. for 5 days. Total 5000 mg.

Interval 32 hours. Cytology - malignant cells present.

Lavage fluorescence - brilliant flecks and smears.

Laparotomy - extensive inoperable malignant tumour of stomach. No biopsy.

21. Mr. S.L. Age 68.

Presented with recent malaise. Treated pernicious anaemia for 5 years.

Ba. meal - tumour involving fundus and pars media.

TC 250 mg. q.d.s. for 5 days. Total 5000 mg.

Interval 30 hours

Lavage fluorescence - NIL.

Cytology - not performed.

Gastrectomy - poorly differentiated Adenocarcinoma of Stomach of the ulcerated polypoidal type.

22. Mrs. H.F. Age 55.

Presented with 10 months' dyspepsia.

Ba. meal - extensive infiltration of upper part of stomach.

TC 500 mg. q.d.s. for 2½ days. Total 5000 mg.

Interval 30 hours

Lavage fluorescence - flecks of moderate intensity.

Cytology - malignant cells present.

Palliative oesophago-jejunal anastomosis. No histology of the malignant disease.

23. Mr. J.O. Age 58.

Presented with 4 months' loss of weight and vomiting.

Ba. meal - constant pyloric narrowing and irregularity.

Lavage fluorescence before TC - NIL.

TC 1000 mg. intravenous over 1 hour.

Interval 30 hours

Lavage fluorescence - occasional weak flecks.

Cytology - no malignant cells.

Laparotomy - inoperable malignancy of stomach with peritoneal deposits. No histology.

24. Mr. S.A. Age 63.

Presented with 5 months' loss of weight, epigastric pain and ascites.

Ba. meal - extensive filling defects of body of stomach.

Gastroscopy - ulcerated neoplastic mass.

TC 250 mg. q.d.s. for 5 days. Total 5000 mg.

Interval 30 hours

Lavage fluorescence - moderately intense smears and flecks.

Cytology - no malignant cells.

No operation in view of ascites.

25. Miss L.D. Age 75.

Presented with 9 months loss of weight, pain and vomiting.

Ba. meal - polypoidal mid-gastric mass.

TC 1000 mg. intravenous over 1 hour.

Interval 32 hours

Lavage fluorescence - brilliant smears and flecks.

Cytology - many clumps of malignant cells.

No operation in view of hepatomegaly.

26. Mrs. F.W. Age 80.

Presented with 1 years' cachexia, anorexia, abdominal pain and vomiting.

Ba. meal - extensive neoplastic ulcer.

TC 1000 mg. intravenous over 1 hour.

Interval 30 hours

Lavage fluorescence - moderate flecks.

Cytology - not performed.

No operation in view of age and poor condition.

Died shortly after but no autopsy.

27. Mr. A.C. Age 73.

Presented with 9 months' anorexia and loss of weight.

Ba. meal - infiltrating neoplasm of body of stomach.

TC 250 mg. q.d.s. for 5 days. Total 5000 mg.

Interval 30 hours.

Lavage fluorescence - brilliant smears.

Cytology - not performed.

No operation in view of hepatic metastases.

28. Mr. A.L. Age 44.

Presented with 6 months' anorexia and dyspepsia.

Ba. meal - suspicious of mid-gastric tumour.

Lavage fluorescence before TC - NIL.

TC 250 mg. q.d.s. for 5 days. Total 5000 mg.

Interval 32 hours

Lavage fluorescence - brilliant flecks and smears.

Cytology - not performed.

Laparotomy - widespread peritoneal metastases from neoplasm of stomach.

No biopsy.

29. Mrs. M.W. Age 65.

Presented with anaemia and 3 months' loss of weight.

Ba. meal - obvious large fungating tumour of body of stomach.

TC 500 mg. q.d.s. for 2½ days. Total 5000 mg.

Interval 30 hours

Lavage fluorescence - brilliant smears and flecks.

Cytology - not performed.

Laparotomy - inoperable tumour of stomach.

No biopsy.

30. Mr. J.J. Age 69.

Presented with 6 months' dyspepsia and anorexia.

Ba. meal - mid-gastric malignant ulcer.

TC 250 mg. q.d.s. for 5 days. Total 5000 mg.

Interval 30 hours

Lavage fluorescence - NIL.

Cytology - not performed.

Gastrectomy - well differentiated

Adenocarcinoma.

PHOTOGRAPHS

Photograph A taken under ordinary photographic illumination compared with ultraviolet illumination in a dark room, illustrates the difficulty of obtaining contrast on black-and-white film between golden-yellow fluorescence and reflected points of light from the small amount of visible light allowed through the filter paper of the ultraviolet lamp.

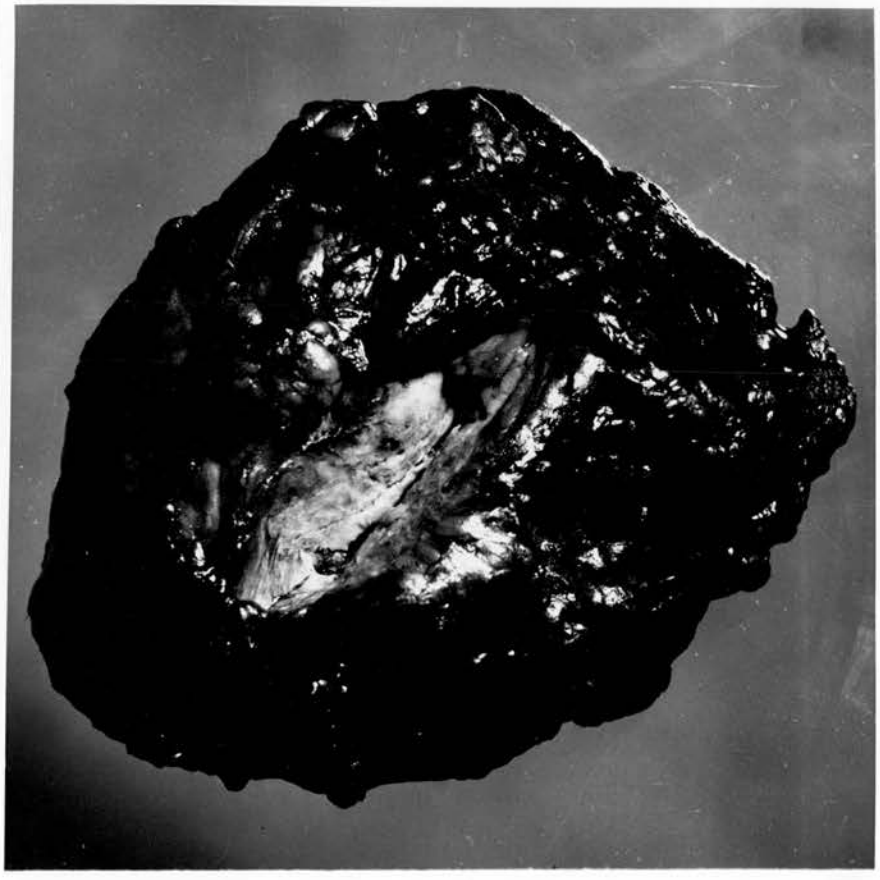
Colour photographs of the gastric lavage smears were attempted by the Hospital Photographer at King's College Hospital, using Kodachrome 2 film, a Wratten 85B filter over an f5.6 lens situated immediately behind the ultraviolet lamp, which was 4 to 6 inches from the smears. Colour processing and printing does not demonstrate the true golden-yellow fluorescence which appears pale green on the prints.

PHOTOGRAPH A

Under visible light.

Cut tumour seen as pale area in centre of fatty mass.

Case 21. (Appendix 2.)
Adenocarcinoma of Breast
- prepared with 2.0 gm. TC orally.

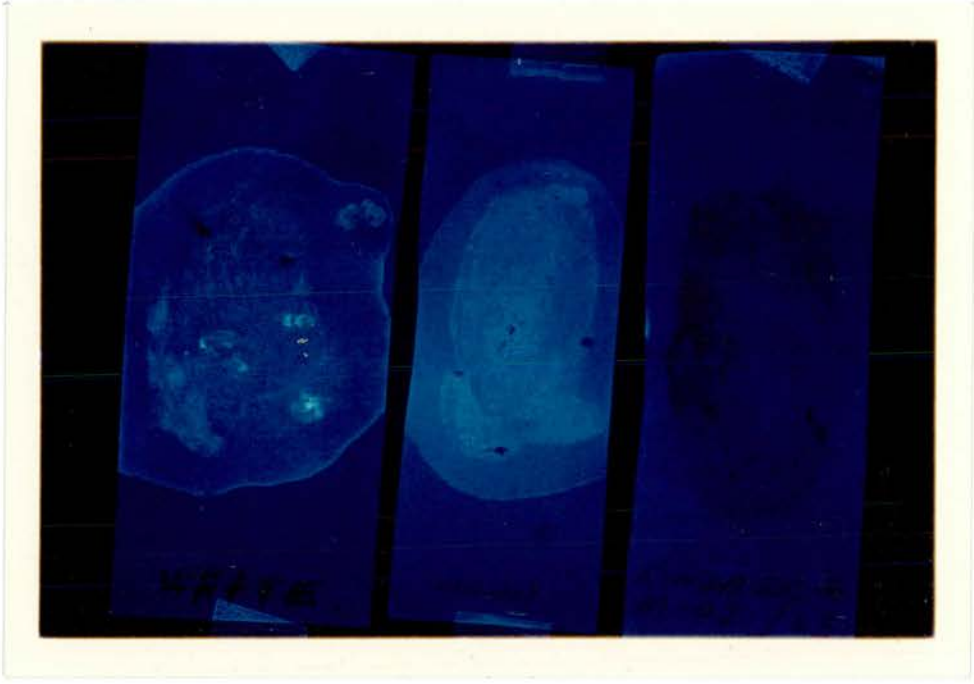


Under ultraviolet light.

Cut tumour seen as central greyish-white area representing golden-yellow fluorescence, but peripheral white spots are bluish-white reflection from ultraviolet lamp.

PHOTOGRAPH B.

Letters "TC" drawn on white paper with a weak solution of TC. When viewed by ordinary light, the letters were invisible, but under ultraviolet light, fluoresced a bright golden-yellow, although colour reproduction gives a rather green appearance.



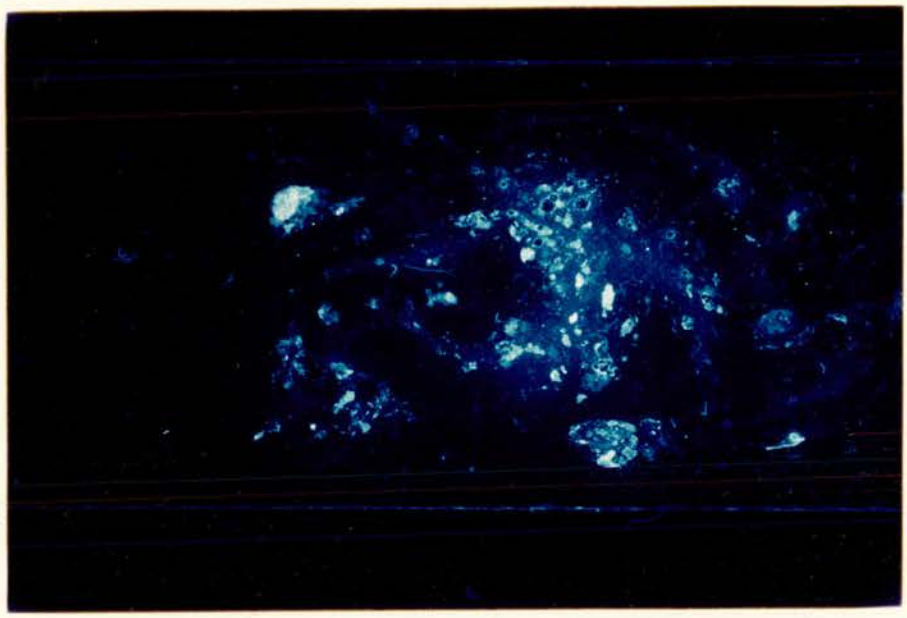
PHOTOGRAPH C.

Three smears from gastric lavage on strips of filter paper. All three patients previously prepared with TC. From left to right:-

Smear 1. Two small smears and two flecks of characteristic TC fluorescence of moderate intensity From Case 15, (Appendix 3) - from patient with carcinoma of fundus of stomach.

Smear 2. White fluorescence - not golden-yellow - from patient with benign gastric ulcer. Negative result.

Smear 3. No fluorescence - from patient with benign gastric ulcer. Negative result.



PHOTOGRAPH D.

Smear on glass slide from gastric lavage of Case 20 (Appendix 3.), a patient with extensive inoperable gastric malignant disease, showing positive fluorescent result of brilliant intensity.

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