completely efficacious in the various vulvovaginal infections in 70 out of the 72 cases examined. In the other 2 cases, consisting of intense Tricomonas infections, a clinical cure was achieved even though at ceck-up examination the persistence of a common asymptomatic flora was encountered. The good tolerability and local application, the lack of absorption at the level of the vaginal mucosa render the use of clotrimazole particularly effective, even under those condition which counterindicate the use of drugs (pregnancy, allergic diathesis, etc.).

BIBLIOGRAPHY

1. Bret A. J., Coffard P.: *Rev. du Praticien*: 17, 52, 4515/4527; 1967. - 2. Norreel J., Rousseau Ch.: *Rev. Fr. Gyn. Obst.*: 60, 7/8, 557/562; 1965. - 3. Siboulet A.: *Ency. Medico-Chirur.*: 3 - 1962, Gynec. 370 A 10. - 4. Stewart R. H.: *Am. J. Obst. Gyn.*: 82, 525-529; 1961. - 5. Lecca U., Canfora S.: *Riv. Ost./Gin. Prat. e Med. Perin.*: 54, 77, 1973/74. - 6. Legal H. P.: *Geburtshilfe u. Frauenheilk.*: 33, 1, 1973. - 7. Schnell J. D.: *Geburtsh. u. Frauenheilk.*: 32, 787; 1972. - 8. Weuta H.: *Arzneimittel Forsch.*: 22, 1295; 1972. - 9. Duhm B. e Coll.: *Arzneimittel Forsch.*: 22, 1276; 1972.

The tetracycline induced fluorescence test in the diagnosis of neoplasia of the vulva

by
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The tetracycline induced fluorescence regarded as a viable diagnostic test in oncology since the chance discovery of the selective affinity of this drug for neoplastic tissues. In 1957 Rall et al. ([1]) during research into an antagonist of riboflavin noticed under a Wood light a particular yellow-green fluorescence in the autopsy samples of breast cancer in a patient, to whom tetracycline had been administered for therapeutic purposes. Subsequently, in studies carried out on animals (rats and mice) that were carriers of neoplasia (sarcoma 37), these authors showed that tetracyclines induce a yellow-green fluorescence that persists over a period in neoplastic tissue, while normal tissues display an autofluorescence that is thought to be a reflex phenomenon.

Similar research work carried out on patients suffering from neoplasia enabled confirmation of all that Rall had demonstrated in animals. Neoplastic tissues were studied immediately after surgery ([2, 3]), in vivo ([4, 5, 6, 7, 8, 9, 10]) and at autopsy ([3, 4]); in this way the properties of tetracyclines and the means of using these as a diagnostic aid were determined.

The properties of tetracyclines are as follows:

— they are distributed in all healthy tissues without showing affinity for any of them in particular;

— they accumulate in the excretory organs: liver, kidneys, intestines;

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— they cause yellow-green fluorescence in all tissues, that can be seen in ultraviolet light;

— after six hours there is fluorescence in all the tissues except the brain; this diminishes rapidly, remaining for up to 24 hours in the excretory organs and for 20 days in bone and teeth;

— the fluorescence is particularly intense in the urine, 6-12 hours after administration of the drug, but disappears after some 30 hours.

What distinguishes neoplastic tissue from normal tissue is that the tetracyclines remain considerably longer in the same tissue: 10-20 days in experimental animals and up to 3 months in humans. Furthermore it should be noted that fluorescence seems to be in proportion to the degree of anaplasia of the tumour ([2, 11]).

Tetracyclines become localised in the macrophages, in necrotic tissues and in the stroma of malignant tissues, but not in the neoplastic cells ([1, 3, 4, 12, 13, 14]). As above, the persistence of the tetracyclines' fluorescence also extends to centres of inflammation.

The process by which they are preserved is not well understood. It is presumed that, due to biological conditions as yet undetermined, a lipoprotein-tetracycline complex is formed in the neoplastic tissue that precipitates in the presence of calcium ions.

Tetracyclines for diagnostic purposes can be given orally, intramuscularly, intravenously, intralymphatically and topically (Tab. 1).

Research into the fluorescence induced by tetracycline administered in these ways was carried out using a Wood lamp, that is with an ultraviolet light of 3600 Å wavelenght, but not until 24 hours after the last administration of the drug. Only a few authors ([2, 15, 16]) have carried it out after 1-12 hours. In gynaecology the study of neoplasia by means of this test has been carried out on specimens from operations, on aspirations of the uterine canal ([14]), in vivo ([3, 4, 14, 15, 17]) and in conjunction with colposcopic examination ([8]).

The method is at present primarily practiced by the profession in France for investigating tumours of the vulva. This policy is justified both because of the affinity of the drug for the epithelial type of tumour and because of their situation they are easily accessible to examination with ultraviolet light.

SUBJECTS AND METHOD

The study was carried out on 30 patients with a pathological condition of the vulva, due to dystrophic phenomena or neoplastic processes, who had been admitted to our clinic. The ages of the patients ranged from 45 to 72 years, with an average of 59.2 years. In the majority of cases pruritus was the main symptom.

We administered the tetracyclines intravenously, 500 mg per day for five days, together with 1000 μg of vitamin B_{12} intramuscularly on the first and third days for the reason that between tetracyclines and cyanocobalamine there is a complex and not well understood chemotaxis by which the antibiotic, bonded to the vitamin B_{12}, would be directed with a high karyokinetic activity to the tissues, which are known to have a great need for vitamin B_{12} for the synthesis of nucleic acids ([6, 7]).

Before placing the patients under treatment we examined the area of the vulva with the Wood light to exclude the presence of fluorescence caused by

other substances such as the acridine derivatives and a control photograph was taken in white light.

Examination of the fluorescence was carried out almost 36 hours after the last administration of the drug in a dark room with a Philips 125 HP ultra-violet lamp.

The patient was placed in the position for gynaecological examination and the regions around the area of the vulva covered with dark material to avoid reflection from the Wood lamp on light clothing or on the bedding obscuring the display of the fluorescent area. The ultra-violet lamp is held at a distance of 40-50 cm from the region of the vulva.

With all patients we recorded photographically all fluorescent areas on first examination and on each subsequent control examination, so as to determine any changes that occurred during that time.

The photographic technique required the following measures:

— use of a Kodak Wratten gelatine screening filter type No. 2A, to exclude ultra-violet rays and allow better display of the fluorescent area;

— use of highly sensitive film as very long exposure times are necessary.

Later the toluidine blue test was used as a control. Biopsy was then carried out in accordance with the technique used for the Collins test both on the most intensively fluorescent areas and on those that were only positive to the above test, when these areas did not correspond with the fluorescent ones. In patients who showed negative in both tests biopsy was carried out in the region clinically most significant.

RESULTS

In the 30 patients examined the tetracycline test proved positive in seven. In these cases the fluorescent areas coincided perfectly with those that were toluidine positive (Tab. 2-3).

The biopsies undertaken in these areas indicated four invasive carcinomas, three epidermoid and one basal cell, one simplex intraepithelial carcinoma, one leucoplakia and a sclero-atrophic lichen.

In three cases where the two tests had not matched up (negative to fluorescence and positive to the toluidine blue), biopsy showed the presence of erythroplasia (Queyrat) in one case and leucoplakia in two others.

In patients where both tests were negative, the sample for biopsy was taken

Table 1. *Methods of using tetracyclines*

Administration	Dose
Oral	600-2700 mg for 2-5 days
Intravenous	250-1000 mg for 1-4 days
Intramuscular	750 mg for 2 days
Intralymphatic	2 mg./Kg.
Topical	
	Sol. A: 150 mg TC + 150 mcg vit. B_{12} in 15 cc. dist. water
	Sol. B: 4.9% trichloroacetic acid
	Sol. C: Saturated sodium bicarb. solution

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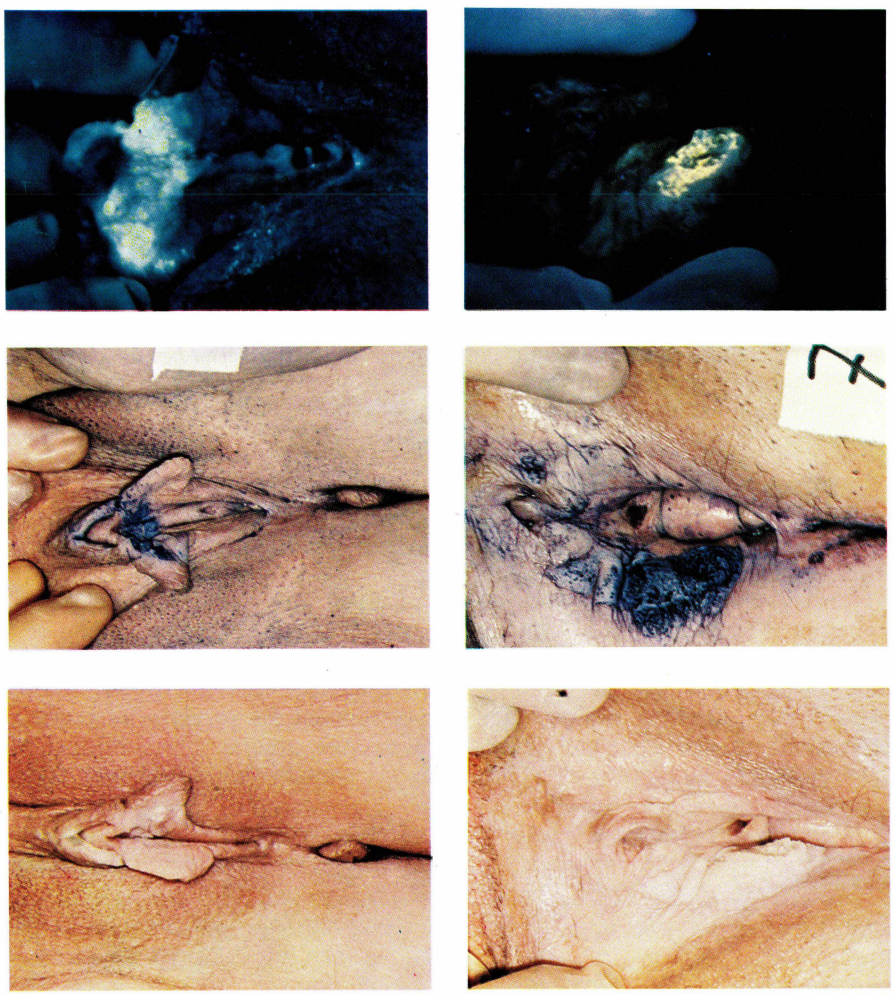

FIG. 1 - [1, 2, 3] Intraepithelial Carcinoma. - [4, 5, 6] Invasive Carcinoma.

Table 2

Histological Diagnosis		Toluidine Blue Test		Test with Tetracyclines	
		Positive	Negative	Positive	Negative
Dystrophy	Leucoplakia	3	9	1	11
	Sclero-atrophic lichen	1	6	1	6
	Senile atrophy	0	5	0	5
Intraepithelial Carcinoma	Carcinoma in situ	1	0	1	0
	Erythroplasia	1	0	0	1
Invasive Carcinoma	Epidermoid carcinoma	3	0	3	0
	Basocellular epithelioma	1	0	1	0

from the most clinically significant area and showed in each case pathologically benign forms, exclusively of the dystrophic type: nine cases of leucoplakia, seven of sclero-atrophic lichen and five of senile atrophy.

DISCUSSION

In our investigations all forms of invasive carcinoma showed positive in the tetracycline test.

According to various authors ([8, 15]) similar results were obtained when administering the drug intravenously and applying it topically, thus confirming the high affinity of tetracyclines for neoplastic tissue and in particular for carcinomas. Only Nirandorn et al. ([14]) make reference to fluorescence that was not clearly significant in three cases of neoplasia of the vulva.

However, as regards the two kinds of intraepithelial carcinoma included in the cases we have studied, the test showed a positive result in only one case, while in the case of erythroplasia there were false negative findings. This patient was one of the first examined and consequently we are not in a position to estimate to what extent the result is attributable to our limited experience.

When using tetracyclines Arrighi et al. ([17]) had negative findings in two cases of Bowen's disease and one that was marginally positive.

Bethoux and Pasquier ([15]) had in ten cases of Bowen's disease nine positive findings and one doubtful in a case of hyperkeratosis. There were negative findings in three cases of Paget's disease. These negative findings were interpreted as being a result of the arrangement of the large clear cells, that are characteristic of this neoplasia, in the deeper layers. However, these authors found in 50% of the patients with Bowen's disease positive to the test areas of fluorescence that were more extensive than the clinical lesions.

This possibility should always been borne in mind whenever proceeding to surgery so as to avoid the possibility of relapse.

The above mentioned authors make no mention of false positive findings, although we observed two, one having a leucoplakia and the other a sclero-atrophic lichen.

The incidence of false positive results obtained with such a method is less than with the toluidine blue test even though a proper comparison is not possible because of the restricted number of cases.

Nevertheless in diagnosing neoplasia at other sites, false positive findings

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have been mentioned by various authors as occuring in 3-7% of cases ([9]) and false negative ones to the extent of 4%.

CONCLUSIONS

The test using tetracyclines is particularly indicated in the diagnosis of tumours of the vulva, both because of the affinity of the drug for neoplasia of the epithelial type and because the site in which these develop is easily accessible for examination with ultra-violet light.

It should be noted, however, that the tetracycline test is not totally free from false negative findings, so that its routine use for early diagnosis would leave us in some doubt even though our experiences are still too limited to offer any final opinion.

To this must be added the fact that there are some inconvenient aspects such as the need for treating the patients for some days with a biologically active substance, not to mention the difficulty of interpreting the results that requires adequate experience in the technique.

At the present state of our knowledge and on the basis of personal experience we do not consider that this test can be used as an alternative to the toluidine blue test, which offers, apart from the greater reliability of the results, the notable advantage of being easier to use and to interpret, factors which make it more appropriate within the framework of early diagnosis for carrying out with a considerable number of patients.

SUMMARY

The test tetracycline induced fluorescence was carried out with 30 patients with a pathological condition of the vulva. The technique proved to be limited due to some technical difficulties and to results only in part reliable.

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BIBLIOGRAPHY

1. Rall D. P., Loo T. L., Lane M. e Kelly M. G.: *J. Nat. Cancer Instit.*, 19, 79, 1957. - 2. McLeay J. F.: *Am. J. Surg.*, 96, 415, 1958. - 3. Ackerman N. B. e McFee A. S.: *Surgery*, 53, 247, 1963. - 4. Vassar P. S., Saunders A. M. e Culling C. F. A.: *A.M.A. Arch. Path.*, 69, 613, 1960. - 5. Berk J. E. e Kantor S. M.: *Geriatrics*, 18, 787, 1963. - 6. Lipnik M. J.: *Arch. Dermat.*, 87, 575, 1963. - 7. Moroni P.: *Arch. Ital. Derm. Vener.*, 32, 406, 1964. - 8. Stoppelli I., De Palo G. M. e Marconato A.: *Attual. Ostet. Ginec.*, 10, 565, 1964. - 9. Bondi S., Cacciaguerra G., De Paoli F. e Severi A.: *Min. Chir.*, 20, 141, 1965. - 10. Whitmore W. F. e Bush I. M.: *J. Urol.*, 95, 201, 1966. - 11. Phillips J. W., Cobb E. G., Richards V., Rhodes W. D., Loehner D. C. e Ritchie J. L.: *Am. J. Surg.*, 100, 384, 1960. - 12. Ludford R. J.: *Proc. Roy. Soc. S.B. London*, 104, 493, 1929. - 13. Hakkinen I. e Hartiala K.: *Ann. Med. Exper. Biol. Fenniae*, 37, 115, 1959. - 14. Nirandorn C. e Hauck J.: *Munch. Med. Wschr.*, 110, 1199, 1968. - 15. Bethoux A. e Pasquier F.: *Pathologie de la vulve, Masson et C^ie Ed.*, 1970, pag. 363. - 16. Hewitt J.: *Comunicazione Proc. First Int. Cong. International Society for the study of vulvar disease, Estepona*, 8 Agosto 1973. - 17. Arrighi L. A., Di Paola G. R., Gomez Rueda N. M., Baliña L. N., Belardi G. e Illescas L. L.: *Rev. Argent. Ginec. Obstet.*, 2, 207, 1971. - 18. Fabiano A.: *Min. Urol.*, 17, 231, 1965. - 19. Testi A. e Barletta O.: *Sett. Med.*, 54, 761, 1966. - 20. Socci M., Bresadola F., Tallero G. e Cavaliere P.: *Chir. Pat. Sper.*, 17, 514, 1969. - 21. Fersini M. e Di Vittorio V.: *Giorn. Ital. di Chirurgia*, 6, 593, 1964.