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med. Wschr. 84, 393, 1954. - 4. Hemsworth B. N., Jackson H.: *Nature*, London 195, 195, 816, 1962. - 5. Hemsworth B. N., Jackson H.: *J. Reprod. Fertil.* 5, 187, 1963. - 6. Hemsworth B. N., Jackson H.: *J. Reprod. Fertil.* 6, 229, 1963. - 7. Heller R. H., Jones H. W.: *Amer. J. Obstet. Gynec.* 89, 414, 1964. - 8. Rugh R., Jackson S.: *J. Exp. Zool.* 138, 209, 1958. - 9. Beaumont H. M.: *Int. J. Radiation Biol.* 3, 59, 1961. - 10. Muratori G.: *Contr. Embryol. Carneg. Instn.* 26, 59, 1937. - 11. Chiquoine A. D.: *Anat. Rec.* 118, 135, 1954. - 12. Pugliatti V., Puglisi Allegra S.: *Riv. Anat. Pat. Oncol.* 29, 413, 1966. - 13. Pugliatti V., Puglisi Allegra S.: *Riv. Anat. Pat. Oncol.* 29, 439, 1966. - 14. Pugliatti V. e Puglisi Allegra S.: *Riv. Anat. Pat. Oncol.* 30, 758, 1966. - 15. Pugliatti V. e Puglisi Allegra S.: *Riv. Anat. Pat. Oncol.* 30, 461, 1966. - 16. Pugliatti V. e Puglisi Allegra S.: *Riv. Anat. Pat. Oncol.* 30, 631, 1966. - 17. Mintz B.: *J. Embryol. Exp. Morphol.* 5, 396, 1957. - 18. Pinkerton J. H. M., McKay D. G., Adams C. E., Hertig A. T.: *Obst. & Gynec.* 18, 152, 1961. - 19. Witschi E.: *Contr. Embryol. Carneg. Instn.* 32, 67, 1948.

The Toluidine Blue Test in the early diagnosis of neoplasia of the vulva

by

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Malignant tumours of the vulva, although developing in an area readily accessible for normal means of diagnosis, are among the gynaecological neoplasias that are diagnosed at a very late stage.

The causes for this delay are attributed both to the absence of a serious and alarming symptomatology and to the difficulty of distinguishing clinically malignant lesions from those that are benign. Moreover, it is considered that this type of neoplasia is characteristic of old age, a time at which a woman's interest in her own genital area has greatly diminished.

The most frequently occurring symptom is pruritus, a symptom moreover that is very general and which for that reason is often underestimated by the patient and the doctor himself, who tends almost always to attribute it to a benign pathological condition.

This phenomenon is crearly of a certain seriousness since, as is verified by neoplasias in other organs as well as by cancer of the vulva, early diagnosis provides an important guide both for the therapeutic indications and above all for the prognosis. In fact survival for more than five years with invasive cancer, despite treatment that is both mutilating and complex, does not exceed 50%, while in intraepithelial cases, also with more restricted intervention, the survival rate varies between 92.5% ([1]) and 100% ([2]).

In order to achieve these objects it is absolutely necessary that the retarding factors mentioned above are reduced; in addition it is important that all the dystrophic lesions of the vulva are diagnosed and adequately treated, in parti-

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cular the hypertrophic forms, such as leucoplakia whether or not associated with sclero-atrophic lichen or progressive sclero-atrophy ([3]), and the chronically infectious conditions such as condyloma acuminatum and granuloma of the groin, which as has been noted, evolve in malignant form in 5-10% of cases ([4, 5]).

The staightforward clinical evaluation and biopsy check of the lesions mentioned above proved on most occasions to be insufficient for early diagnosis, given the extent of the area to be examined and the multifocal nature of the lesions themselves ([6]).

With the aim of overcoming these limitations Collins et al ([7]), after the experiences of Richart ([8]) on cervical carcinoma in situ, introduced a clinical colouring test for the diagnosis of neoplasia of the vulva which enables lesions that are still not clinically detectable to be pin-pointed.

The method makes it possible to find the areas of epithelial neoplasia, in which, unlike what is found with the normal epithelium, there remains a more or less intense blue colouring even after applying an acetic acid solution. This phenomenon is due to the strong and selective nuclear affinity of this substance which causes colouring in the tissues, the intensity of which is in direct proportion to the number of nuclei per unit of area ([9]).

However, the areas where the colour persists are not always an indication that these areas are neoplastic, in that the test will also be positive in the presence of inflammatory infiltrates. It is possible, however, to differentiate the neoplastic areas from those that are falsely positive by the quality of the stain; in fact while the inflammatory areas take on a diffused dark blue tone that gradually fades into the adjoining tissue, those that are neoplastic show an intense royal blue colour that is uniform and clearly defined.

With this method a true and characteristic map of the lesions in the vulva region is obtained, in which the toluidine positive areas are indicative of those to be subjected to biopsy. In this way it is possible to carry out focal biopsy in pathology of the vulva, a fact that is specifically regarded as being very important in view of the frequently multifocal nature of these lesions ([6]).

The initial research of Collins et al. was carried out with 242 patients and enabled ten forms of intraepithelial carcinoma to be detected, to establish the absence of false negative findings and a 7% incidence of false positive ones, mainly attributable to inflammatory forms.

The examination of the toluidine positive areas was also established by colposcopy, by which means Broen et al. ([10]) succeeded in pinpointing six cases of intraepithelial carcinoma in the examination of 1071 patients basically showing no symptoms.

The extremely interesting results obtained by various authors ([1, 2, 3, 7, 11]) led to this test being used as a matter of routine in our centre for pathology of the vulva with the aim of avoiding all the difficulties met with in the early diagnosis of these neoplasias.

MATERIALS AND METHOD

We carried out the toluidine blue test on 164 patients suffering from pathology of the vulva clinically attributable to dystrophic phenomena or neoplastic processes (seven cases). In general these showed as the main symptoms pruritus that had been present for a minimum of a month or a maximum of 14 years, and in 40% of cases associated with dyspareunia. The age of the patients ranged from 28 to 79 with an average of 54.8 years.

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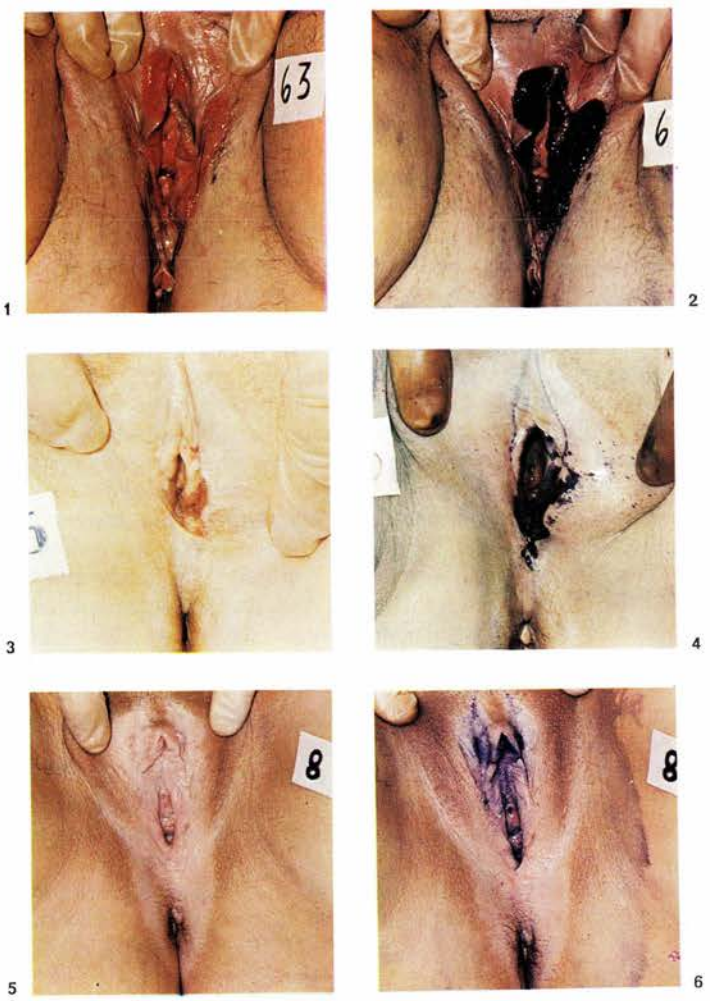

FIG. 1

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

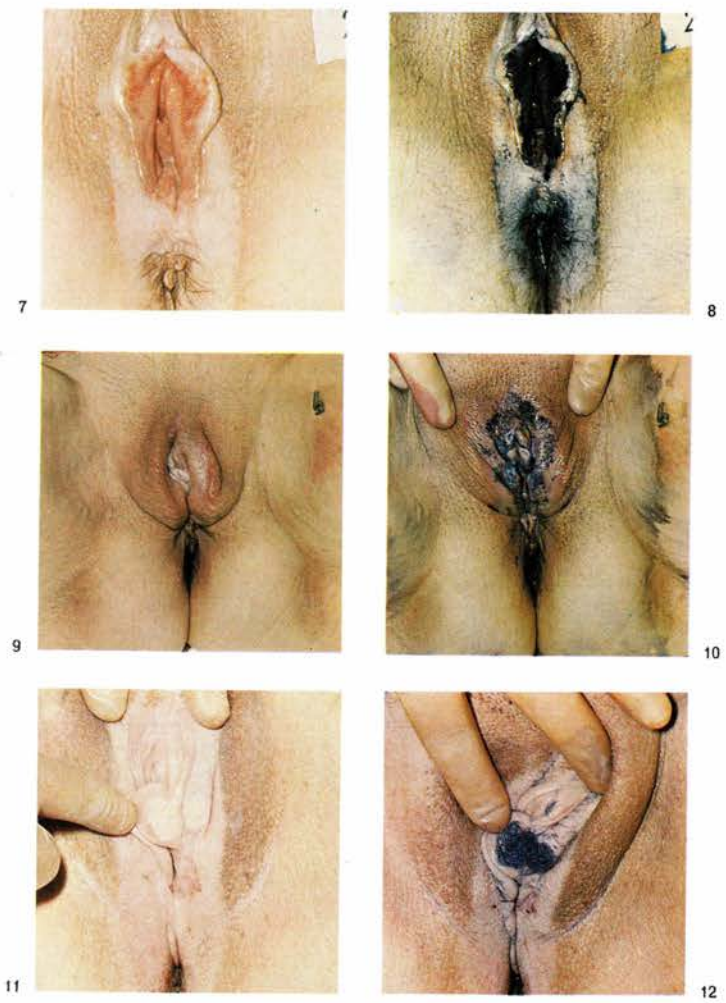

FIG. 1

[7, 8]: Acute phogosis. - [9, 10, 11, 12]: Dystrophy.

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The test was organised in accordance with the directions of Collins et al. as follows:

— the vulval region, having been divided into three areas, is coloured with a 1% solution of toluidine blue using a cotton wool swab. After about five minutes a 1% acetic acid solution is applied which causes an immediate and complete discolouration where the epithelial layer is normal, while a more or less intense colouring persists in the pathological area.

The samples for biopsy are taken following a local anaesthetic in all the most intensely coloured toluidine positive areas with a two and four mm Keyes needle, which enables a 5-6 mm long cylinder of tissue to be taken. With the patients without any toluidine positive area the biopsy was carried out in the clinically most significant areas.

The toluidine positive areas and those subjected to biopsy were all drawn on a special vulvar diagram and photographed, both for photographic record purposes and to be able show up any possible clinical and chromatic changes to the area during subsequent checks.

RESULTS

In those patients examined the test proved positive in 54 cases (32.9%) (Tab. 1).

The biopsy sample, taken in the toluidine positive area, enabled the discovery in 12 (7.3%) of the presence of a malignant neoplasm of the vulva, which in seven cases turned out to be of the invasive type and in five of the intraepithelial type (Tab. 2).

Histologically the invasive forms comprised four epidermoidal carcinomas and three basocellular epitheliomas while the intraepithelial ones were one

Table 1

Lesions	Toluidine Blue Test	
	Positive	Negative
Malignant	12	0
Benign	42	110

Table 2

Histological Diagnosis		Toluidine Blue Test	
		Positive	Negative
Phlogosis	Acute	5	0
	Chronic	2	0
Dystrophy	Leucoplakia 1st degr.	15	28
	Leucoplakia 2nd degr.	6	0
	Sclero-atrophic lichen	4	29
	Senile atrophy	9	53
	Keratoancantoma	1	0
Intraepithelial carcinomas	Carcinoma in situ	3	0
	Bowen's disease	1	0
	Erythroplasia	1	0
Invasive carcinomas	Epidermoid carcinoma	4	0
	Basocellular epithelioma	3	0

Queyrat erithroplasia, one Bowen's disease and three simplex intraepithelial carcinomas. With the three basocellular epitheliomas the toluidine positive areas affected regeneration in a limited manner.

The multiple samples taken from patients with malignant neoplasia enabled us to determine leucoplakia as the most frequently associated vulvar lesion.

In one patient with epidermoidal carcinoma and in one with basocellular epithelioma there was found to be associated with this a carcinoma of the cervix, this also being of the invasive type; while a third patient with invasive carcinoma of the vulva had undergone surgical and radiation treatment for carcinoma of the cervix three years previously.

In the remaining 42 patients (25.6%) where the test was positive the biopsy showed benign pathological forms predominantly of the dystrophic type.

When forms of atrophy and hypertrophy occur together in the multiple histological findings for the same patient, we attributed greater importance to the hypertrophic form and regarded these as hypertrophic cases.

In 110 patients where the test was negative the biopsy that was carried out in the zones most sensitive clinically only showed forms of dystrophic pathology.

In seven patients with inflammatory pathology the control test, carried out after therapy, was negative in all cases.

DISCUSSION

In our research work the toluidine blue test showed positive results in all the forms of neoplasia, whether invasive or in situ.

The invasive forms (seven) had been suspected clinically, while the intraepithelial ones (five) were determined solely on the basis of the indications of the text in the context of dystrophic lesions.

The biopsy samples carried out on lesions clinically negative to the test always showed histologically a pathology of the benign type. This absence of false negative findings in our work is confirmed by the experience of other writers ([1, 7, 10]) and enables us to regard the test as valid above all in the identification of preinvasive lesions.

Collins et al. ([1]) using this method succeeded in discovering 20 cases of intraepithelial carcinoma over a six year period among 41 patients diagnosed during 23 years.

As regards the incidence of false positives in the cases we studied, it should be pointed out that this reached a level of 25.6% and is thus clearly above the level of previous researchers. Collins et al. ([7]) had 17 false positive results in 242 patients examined, equal to 7%, while Broen et al. ([10]) had 33 in 1071 patients who were predominantly asymptomatic, equal to 3%.

In our findings false positive results related mainly to first and second degree leucoplakia and inflammatory forms. In these latter cases the fact that the test showed positive is attributed to the selective nuclear affinity of toluidine as a result of which the areas with inflammatory infiltration necessarily show positive. As regards the forms of leucoplakia it is interesting to note that all the second degree ones showed positive in the test as a result of which it is necessary to carry out repeated checks to be able to follow the development of these forms, it being noted that the leucoplakia, especially when second or third degree, can develop into malignant neoplasia in 5-10% of cases ([12]).

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The use of this test enabled us not to have to carry out with the dystrophic forms clinically more serious and damaging surgery, as was frequently the case in the past.

The observation of two patients with carcinoma of the vulva associated with carcinoma of the cervix on the one hand confirms the multicentral nature of gynaecological cancer ([13, 14]), whilst on the other it leads us to keep in mind such an occurrence in the diagnosis of genital neoplasia through a differentiation in primary and secondary lesions. The discovery then of a carcinoma of the vulva in one patient who has previously received radiotherapy for carcinoma of the cervix led us to consider the possible onset of neoplasia of the vulva as a result of this type of treatment, as has been indicated also by other writers ([15]).

All these points are given fair consideration when it is a question of assessing the type of patient to give the test to.

CONCLUSIONS

Our results enabled us to agree with other writers on the usefulness and validity of the toluidine blue test in the early diagnosis of cancer of the vulva. In fact this test makes it possible to pin-point with accuracy neoplastic areas both in the context of clinical lesions that are not attributable to neoplastic pathology and in apparently normal tissues, and is characterised by the absence of false negative findings, as is sufficiently demonstrated in our research work and in that of other writers ([1, 7, 10]).

The method is quick to carry out and easy to interpret, so much so that it can be used on outpatients; the following are the indications:

— patients with a pathological condition of the vulva, that must be checked regularly;

— patients with other forms of gynaecological cancer, for possible associations;

— patients treated with radiotherapy for gynaecological neoplasia, in which has been shown the successive appearance of neoplasia of the vulva;

— patients who have undergone vulvectomy, for the early diagnosis of relapse of which there can ben an incidence of 25% after partial vulvectomy ([2]);

— in all patients aged over 50, where there was an absence of troubles of the vulva, in that intraepithelial carcinoma was seen to occur also in women with an absence of symptomatology ([10]).

As regards carrying out a programme of screening all women over the age of 50, for the early diagnosis of neoplasia of the vulva, this is still being hoped for, but we are fully aware of the difficulty that would be encountered in carrying out such a programme with out-patients and in working class areas. We consider on the other hand that this will have to become routine in diagnostic investigations in gynaecological wards and in all these health centres where there is a high percentage of old age patients.

SUMMARY

In 164 patients with a pathological condition of the vulva the toluidine blue test was undertaken. The method was quick to carry out and the results were easy to interpret. They are also valid in early diagnosis.

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BIBLIOGRAPHY

1. Collin C. G., Roman-Lopez J. J. e Lee F. Y. L.: *Am. J. Ostet. Gynec.*, 108, 1187, 1970. - 2. Boutselis J. G.: *Am. J. Obstet. Gynec.*, 15, 733, 1972. - 3. Di Paola G. R. e Biliña L. M.: *Enfermedades de la vulva.* Editorial Medica Panamericana. Buenos Aires, 1970. - 4. McAdams A. J. e Kistner R. W.: *Cancer*, 11, 740, 1958. - 5. Underwood P. C. e Hester L.: *Am. J. Obstet. Gynec.*, 15, 849, 1971. - 6. Hodgkinson C. P., Patton R. P. B. e Ayers M. A.: *Ford Hospit. Med. Bull.*, 11, 279, 1963. - 7. Collins C. G., Hansen L. M. e Theriot E.: *Am. J. Obstet. Gynec.*, 28, 158, 1966. - 8. Richart R. M.: *Am. J. Obstet. Gynec.*, 86, 703, 1963. - 9. Cohn H. J.: *Biological Stains.* Williams & Wilkins, Baltimore 1961. - 10. Broen E. M. e Ostergard D. R.: *Obstet. Gynec.*, 38, 775, 1971. - 11. Kaufmann R. H. e Gardner H. L.: *Clin. Obstet. Gynec.*, 8, 1035, 1965. - 12. Woodruff J. D.: *Precancerous lesions of the vulva in treatment of cancer and allied disease.* Pack, G.T., Hoeber New York, 1966. - 13. Rutledge F. e Sinclair M.: *Am. J. Obstet. Gynec.*, 102, 806, 1968. - 14. Abell M. R. e Gosling J. R. G.: *Cancer*, 14, 318, 1961. - 15. Woodruff J. D. e Hildebrandt E. E.: *Obstet. Gynec.*, 12, 414, 1958.

Reliability of the methods for early diagnosis of carcinoma of the uterine cervix

by

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For the discovery of carcinoma of the cervix, which is the most common site of cancer of the female genital tract (4 out of 5 cases) and which constitutes the most common form of cancer when women alone are considered, we traditionally rely upon colposcopic and colpocytological examination, supported by histological studies if the results of these tests are positive.

The present report aims at a critical assessment of the work of the Centre for Colposcopy and Citology of the Second Obstetric and Gynaecological Clinic of the University of Padua, from its beginning (23-2-1970) to 31-5-1974. The advantages and limitations of each of the methods used are outlined.

1) *Colposcopy*

Advantages: a) changes in the exocervical epithelium, which are not obvious to the naked eye, can be differentiated with greater accuracy; b) a colposcopically benign lesion obviates the need for further tests, and in particular for a biopsy; c) precancerous or malignant epithelial atypia alerts the investigator early and necessitates a biopsy, thus leading to early and adequate treatment; consequently the finding of a suspected and non-positive lesion in case of cancer

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