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Abstract

Papanicolaou's smear test is a method based upon the morphological study of the cancer cells exfoliated from the epithelium, whereas T.P.T. is a method for examining the intracellular metabolism, the glycolysis, by a supravital staining of the cancer cells. The latter, therefore, can be called as a cytochemical diagnosis. Since, by the T.P.T. method, even a beginner can obtain the result of approximately 80% in correct and the skilled ones as high as 95%, the clinical diagnosis can be made all the more accurate by using Papanicolaou's test in combination with T.P.T. method. As for the entity of these granular cells, there remains a room for discussion, but Misonou feels that Cell Type A arises from necrobiosis of the carcinomatous tissues while Type B would be a certain wandering cell. This reaction, however, should not be employed to the cases in the puerperium, because the similar cells are exfoliated from the puerperal uterus. Thus, I can say that the T.P.T. is not a specific reaction to cancer. From this study, I would recommend T.P.T. as a method that is quite simple and is servicable for saving a great deal of effort and time on the part of clinicians, and I would like to encourage you to use it as one of tools for the diagnosis of carcinoma of the uterus, especially for an early diagnosis.

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T.P.T. STAINING OF VAGINAL SMEARS FOR DIAGNOSIS OF CARCINOMA OF THE UTERUS *

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INTRODUCTION

For the detection of carcinoma of the uterus, biopsy is a decisive method, and *Papanicolaou's* smear test and Colposcopy are employed as auxiliary methods of diagnosis in combination with it. There is, of course, no question about that the more numerous are the methods used concomitantly, the more accurate will be the diagnosis. Even in the case of carcinoma of the cervix or body, the earlier is the stage, the more difficult is the diagnosis. Therefore, for the purpose of early detection, it is all the more desirable to use as many of these various methods as possible.

In this sense, T. P. T. staining is a method available in the routine cancer laboratories. It is much easier in technique of preparing a slide, the time being almost 1/10, and also easier and simpler in microscopic examination, the time being 1/10-1/20. Even untrained beginners can screen it quite easily while they need more experience for diagnosing the *Papanicolaou* smear. It is, therefore, very recommendable for screening tests, in which many slides are to be examined in a short time. T. P. T. (2, 3, 5-triphenyl tetrazolium chloride, sometimes called T. T. C.) reaction was first reported by PECHMAN and RUNGE¹ (1894). It is a colorless needle-shaped crystalline substance, soluble both in water and alcohol. It participates in the glycolysis of tissue cells and in turn is reduced itself to formazan which is a red colored substance insoluble in water and soluble in fat. Straus and Cheronis², using this reaction in the human and animal (1948), mentioned that the stainability of cancer cells differs from that of normal epithelial cells. MACKENZIE and FULLER³ (1950) applied this reaction to the staining of vaginal smears for the diagnosis of carcinoma of the cervix. In Japan, MISONOU⁴ (1952) is the first one who studied it. Perhaps, as long as there is a difference in their metabolism of glycolysis between malignant cells and normal cells, there results a phenomenon in which malig-

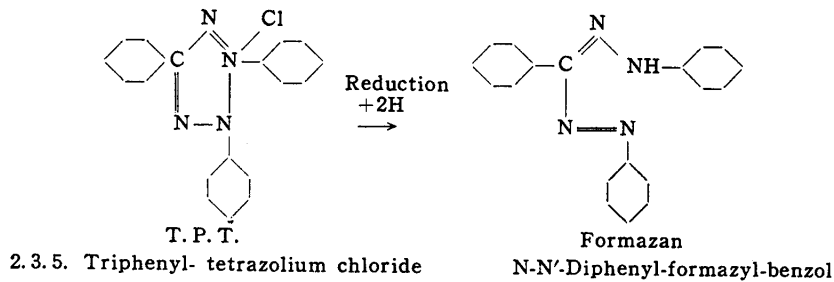
* Read before the Seventh International Cancer Congress at London, 1958.

Table 1

The Chemical Formula and Nature of T. P. T.

2.3.5. Triphenyl-Tetrazolium chloride (T. P. T.), colorless needles shaped Crystalline, soluble in water and alcohol, participates in oxidation and reduction in tissue as one of the H-acceptors.

In this process T. P. T. suffers reduction and turns into Formazan, red colored, soluble in fat, but insoluble in water.



nant cells are stained in deeper red because of more thorough reduction and normal cells are difficult to be stained in red because of less thorough reduction, on the basis of the difference in the degree of respective T. P. T reduction. Moreover, this red coloration appears in the cells as strawberry-like large granules which are so easily distinguishable even for the clinicians at their glance that it is obvious to make differential diagnosis. Thus, a value lies in the application of this color reaction.

T. P. T. STAINING

1. Method of staining. A drop of fresh vaginal discharge is placed on a slide, to which a drop of 0.5-1.0% T. P. T. solution of physiologic saline water is added. Then, both drops are mixed well, making a smear. The smear is covered with a coverglass and its rim is sealed up with vaseline. Being kept it at 37°C, in daylight only 20 minutes, it will be ready for usage.

2. Microscopic examinations. There appear those large or medium-sized cells (30-40 microns in diameter) possessing many red granules (Fig. 1). These are called T. P. T. positive cells. Detection of these is easy and a low magnification (100×) of the microscope is sufficient. When observed under a high magnification (400×, 1,000×); (1) they are round or oval, medium-sized cells full of granules shining like rubies, appearing beautifully brilliant as if they are fine strawberries and are very easily recognizable. These are called Cell Type B (Fig. 2); (2) the others are irregularly-shaped cells (tadpole-like, slender and round) with a few granules in the cell and a few in number as well, and in some these granules



Fig. 1. T. P. T. Reactio (two positive cells in the center)



Fig. 2. Cell Type B. (strawbery like figure)

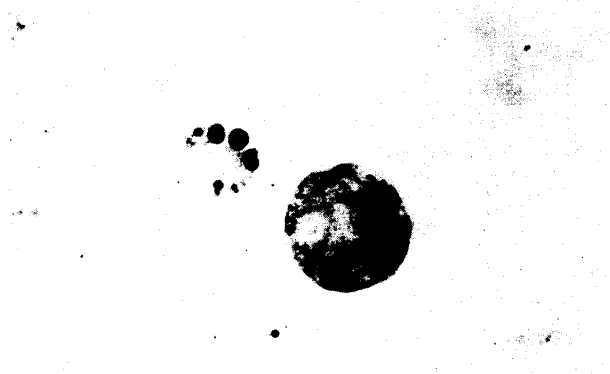


Fig. 3. Cell Type A. (granules surrounding the nucleus)

are scattered in such a way as to surround the nucleus. These are called Cell Type A (Fig. 3); (3) occasionally there are still other smaller cells (15 microns or so in diameter), and they are smaller and the number of granules in the cell is less though similar to the Type B cells. These are called Doubtful Type. When there appears the cell Type A or B, in the examination of slides, it can be diagnosed as positive.

RESULTS OF EXPERIMENT

In the screening test performed on 1,794 patients at the Department of Gynecology and Obstetrics, Okayama University Hospital, the detection of the cancer patients proved to be 80.2 per cent (Table 2).

When this is compared with *Papanicolaou's* test and with the results of histological examination of the biopsy specimens, their relations are as follows:

1. T. P. T. of cancer patients. The results on 495 cases of the cancer patients who had the final diagnosis of biopsy are shown in Table 3.

In other words, of the 476 cases of cervical carcinoma, 82 cases (10.9%) proved to be false negative, and, hence, the rate of reliability in this case is $100\% - 10.9\% = 89.1\%$. Misonou, who is one of the most experienced worker on T. P. T., had false negative of 5.7% in 87 cases of cervical carcinoma, yielding the reliability rate of 94.3%.

In comparing this with the results of *Papanicolaou's* test, as the rate of reliability of *Papanicolaou's* test on the cancer during the same period of time is 92.5% (false negative 7.5%), this is somewhat superior. Misonou, on the contrary, states that the reliability rate of *Papanicolaou's* test 87.3% and that of T. P. T. is 94.3%, and, therefore, the latter is superior. In any event, it may be said that this method of T. P. T. certainly yields the reliability rate superior to that in *Papanicolaou's* test for the skilled person, but, for the unskilled, the result by this method, is somewhat inferior. Next, in 160 cases of non cancerous case (histologically verified), 29 cases, namely, 18.1% was found false positive, while Misonou obtained 11 cases, namely, 10%, in 110 non cancerous cases.

However inferior the result may be, there is a great advantage which amply covers its shortcoming, and that is the characteristic of the T. P. T. method. Namely, it only requires one tenth of the time in diagnosing the case and one fourth the time in the preparation of the specimen, compared to the other method; and it is just an ideal method for giving a rough diagnosis on many smears in a short given time, so that, for the

T. P. T. In Carcinoma of Uterus

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Table 2 T. P. T. in Screening test (1794 cases)

Clinical Diagnosis	No.	(+)	(±)	(-)
Carc. of Cervix ?	23	9	1	13
Carc. of Cervix Stage I	37	30	1	6
" " Stage II	75	64	2	9
" " Stage III	13	9	0	4
" " Stage IV	2	2	0	0
Carc. of Corpus ?	9	4	0	5
After Treatment of Carc.	13	3	1	9
Malig. Ovar. Tumor	5	2	0	3
Tumor of Cervix	2	1	0	1
Carc. of Vagina	2	1	0	1
Carc. of Vulva	1	1	0	0
Gynec. Normal	89	0	0	89
Retroflexio Uteri	66	0	0	66
Myoma Uteri	66	0	0	66
Ovarian Cyst	37	1	0	36
Adnexitis	85	1	2	82
Endometritis	6	0	0	6
Pregnancy	410	0	3	407
Abortion	26	3	2	21
Retentio Chorrii	8	1	0	7
Hypoplasia Uteri	60	0	0	60
Sterility	56	0	0	56
Erosion of Cervix	140	4	5	131
Hemorrhagic Erosion of Cervix	54	8	1	45
Colpitis	97	0	6	91
Functional Hemorrhage	85	8	4	73
Endocervicitis	44	1	1	42
Polypus of Cervix	29	3	0	26
Menstruation	19	2	1	16
Hydatid Moles	4	0	0	4
Chorioepithelioma	3	0	0	3
Ovarian Dysfunction	53	0	0	53
Ectopic Pregnancy	5	1	0	4
Climateric Troubles	27	0	0	27
Postoperative Adhesion	10	0	1	9
Rupture of Cervix	1	1	0	0
Cystitis	12	1	0	11
Ectropium of Cervix	6	1	0	5
Others	114	0	0	114
Total	1,794	162	31	1,601

130 of Carcinoma among 1,794 screening cases 130 : 162=80.2%

Table 3 T. P. T. in Carcinoma (Histologically verified)

	No.	Positive	Doubtful	Negative (%)
Cervix	476	379	15	82(10.8)
Coups	11	7	2	2(18.0)
Vagina	7	5	0	2(28.5)
Urethra	1	1	0	0
Total	495	392	17	86(17.3)

clinician it is sufficiently worth applying this method for the detection of carcinoma of the uterus.

Summing up the facts above mentioned, this method can be used in combination with other methods in diagnosis of carcinoma, in order to secure all the more accurate determinaton, and, therefore, if one gets a rough diagnosis by T. P. T., at first, and then applies *Papanicolaou's* test only for those cases that are suspicious of cancer, one would actually save much time and effort. This is, therefore, a most ideal way of combination on the part of clinicians.

T. P. T. DURING RADIATION TREATMENT

From the daily examinations of T. P. T. in 100 patients with carcinoma of the cervix undergoing the roentgen and radium treatment, it becomes obvious that there is a difference of the radiation reaction from case to case. In the present experiment, roentgen is given 12 times (300r air dose \times 12, total doses of 3600r) to each case. Total of the cases may be divided into two groups, according to their reaction; namely, the ones in which granule cells increase as three times as the initial number (A group), and those in which, an increase being uncertain, but conversely granule cells decrease (B group); and cancer in A group takes a better course of cure than B group, indicating that those responding better to radiation. However, whether or not there is any difference in the five year cure, it was not yet been decided

T. P. T. IN FOLLOW-UP

In the group that received both radiation treatment and radical hysterectomy, there are 702 cases who had the follow-up examinations of T. P. T. (grand total of 1,433 examinations) after the treatment.

During the examination in the radiation group, T. P. T. turns gradually negative from the fourth month after the treatment, and if it should transfer to positive from negative later, one must take it for a sign of recurrence of cancer. The sign usually appears in two months, in

advance, before it can be diagnosed clinically as recurrence, and therefore, it is a great help as an auxilliary method.

In the operatoin group, granulomatous tissues appear often at the vaginal stump, so that the similar positive cells may be observed in the smear. The method is not available for this group.

CONCLUSION

Papanicolaou's smear test is a method based upon the morphological study of the cancer cells exfoliated from the epithelium, whereas T. P. T. is a method for examining the intracellular metabolism, the glycolysis, by a supravital staining of the cancer cells. The latter, therefore, can be called as a cytochemical diagnosis. Since, by the T. P. T. method, even a beginner can obtain the result of approximately 80 % in correct and the skilled ones as high as 95 %, the clinical diagnosis can be made all the more accurate by using *Papanicolaou's* test in combination with T. P. T. method.

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