GENITAL INFECTION IN ASSOCIATION WITH TRIC VIRUS INFECTION OF THE EYE

III. CLINICAL AND OTHER FINDINGS PRELIMINARY REPORT*

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The aim of this part of the study was to investigate infection of the genito-urinary tract by TRIC virus and to determine what changes the infection produces.

Material

Two groups of patients were studied.

- (1) The parents of five babies (Families D, E, I, C, H) who had developed neonatal TRIC virus conjunctivitis.
- (2) Eight adults (F, K, M, O, J, L, G, P) who had developed TRIC virus infection of the conjunctiva of various types, and four of their consorts.

Race.—One mother and baby (E) were Pakistanis (the husband was not examined), one family (I) were Caribbeans—all the remaining patients were white (Mr H was Maltese).

Method

It was essential both to detect infection by TRIC virus and, at the same time, to recognize the presence of other forms of genital infection.

A detailed history was taken. In male cases, the urethra was examined after the patient had refrained from passing urine either overnight or for a period of hours. Urethral scrapings, taken with a platinum loop, were examined microscopically as Gram-stained smears and as wet films. Material was taken from the urethra for culture using Stuart's swabs which were placed in transport medium (Stuart, Toshach, and Patsula, 1954) and in Feinberg-Whittington trichomonas culture medium (Feinberg and Whittington, 1957). If there was no obvious urethral discharge, the centrifuged deposit of the first part of the voided urine was also examined microscopically, as were wet films of the prostatic-vesicular secretion.

After the patient had refrained from passing urine for at least 3 hours, the urethra was scraped with a long thin curette (Fig. 1*a, b*). This was lubricated with the solution of sucrose potassium glutamate (S.P.G.) and antibiotic used for collecting specimens for inoculation into eggs (Jones, Al-Hussaini and Dunlop, 1964); the urethral meatus was cleaned with a dry swab and, with the patient lying on his back, the curette was allowed to drop by its own weight down the urethra to the bulb. It was then slowly and gently withdrawn with the spoon facing the 12 o'clock position. After re-insertion, it was next withdrawn with the spoon facing 3 o'clock, and so on until each quadrant had been scraped from bulb to meatus at least once. This procedure caused only mild discomfort and was carried out without any anaesthetic. In all, a minimum of six smears were made for Giemsa-staining and two bottles of the solution of S.P.G. and antibiotic were inoculated: the prostate and seminal vesicles were then massaged and the first drops of secretion were allowed to run into one of the bottles.

Women were examined in the lithotomy position. When the cervix had been exposed, using a Cusco speculum, it was carefully swabbed to remove secretion and then examined using a Zeiss colposcope. The appearances were recorded and photographed. A smear of endocervical mucus was made with a loop and allowed to dry for Gram-staining. Scrapings for virus tests were then taken from the marginal area of the cervical os using various patterns of scrapers, the most successful being a shallow spoon with a linear gap cut parallel to the edge (Fig. 1*c, d*). This gap provided additional scraping edges and served to hold material which would otherwise have been pulled off by the cervical mucus. At least nine smears were made for Giemsa-staining and two bottles of S.P.G. and antibiotic were inoculated. Material for culture was then taken from the endocervix using a Stuart's swab and transport medium. A smear for Gram-staining and a specimen for culture were also taken from the vaginal vault, from the urethra, and from the rectum. A proctoscope was passed in every case and the mucosa

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FIG. 1.—(a) Urethral curette. (c) Cervical scraper.
(b) Urethral curette (detail). (d) Cervical scraper (detail).
Note.—Centimetre scale refers to a and c only.

was inspected with the colposcope. In addition, a scraping was taken from the vaginal vault, with a loop, for microscopic examination as a wet specimen for *Trichomonas vaginalis*, and a Stuart's swab was rubbed on the vaginal wall and then placed in Feinberg-Whittington medium.

A specimen of blood was taken from all adult patients. This was tested for syphilis by means of the Reiter protein complement-fixation test, cardiolipin Wassermann reaction, and Price's precipitation reaction. A complement-fixation test for gonorrhoea was performed in all cases. A complement-fixation test for antibody to the psittacosis-lymphogranuloma venereum-TRIC group antigen (L.G.V.C.F.T.) was performed in all but the first four cases. The Frei test was performed in most cases and, in some, a similar intradermal test was carried out using an acid-extract which had been prepared by Prof. C. F. Barwell from the LB 1 isolate of TRIC virus.

Findings

A. Neonatal TRIC Virus Infection (Tables I and II).

Table I (opposite) summarizes the findings in the cases of the parents of five babies suffering from neonatal TRIC virus conjunctivitis. TRIC virus was grown from the conjunctiva of three of these babies, inclusions were present in all five cases.

Table II (overleaf, pp. 36 and 37) gives details of the findings in the case of each family.

Mothers.—Four gave a history of vaginal discharge in pregnancy. With the illumination and magnification provided by the colposcope, signs were found in the cervix in the case of each of the five, which resembled those produced in the conjunctiva by TRIC virus infection. Thus there were "follicles" in the marginal area or within the os, or in both sites, in all five cases, and these were accompanied by larger nodules in one.

In another of the five cases oedema of the cervix was replaced by "follicles", and in another fine scarring occurred. There was an excess of pus in the cervical secretion in four cases, the maximum count of polymorphonuclear leucocytes per high-power field (H.P.F.) being respectively 37, 40, 75, and 60. One white patient (Mrs C), with a maximum count of only 20 per H.P.F., had cervical "follicles" and mild proctitis; the L.G.V.C.F.T. showed complete fixation to 1:16, and partial to 1:64; intradermal tests were not done. Mr C had inclusion urethritis but a negative Frei test and a negative L.G.V.C.F.T. Virus was grown (isolate IOL-4/GCx)* and inclusions were found in the case of Mrs D, 4 weeks after delivery.†

* Designation of TRIC virus isolates is explained in Jones, Al-Hussaini, and Dunlop (1964), see p. 20.

† Inclusions in cervical cells from Mrs D are illustrated in Al-Hussaini, Jones, and Dunlop (1964), see pp. 28 and 29.

TABLE I
STUDY OF THE PARENTS OF FIVE BABIES WITH NEONATAL TRIC VIRUS CONJUNCTIVITIS

Parents		Mothers	Fathers
No. of Subjects		5	4
Genital Infection	History	4	2
	Signs	Cervix "follicles" 2 Cervix oedema, then "follicles" 1 Cervix "follicles", then scarring 1 Cervix "follicles" and nodules 1 Cervix pus 3 Cervix pus, then salpingitis 1 Mild proctitis 1	Urethritis 4 (1 meatitis) Urethral pus 3/3
TRIC Culture + Inclusions + Inclusions +		1	1 } 3/3 2 }
Fixation to L.G.V.C.F.T.		5	2/4
Skin Tests	FREI+	1/4	0/3
	LB 1 +	1/3	0/2
Associated Infection		Trichomoniasis 2	0/3

LB 1 = intradermal test using acid-extract of LB 1 isolate of TRIC virus
L.G.V.C.F.T. = Complement-fixation test for lymphogranuloma venereum

There was then no sign of salpingitis, but 8 weeks later a laparotomy was carried out elsewhere and the patient was found to have acute right-sided salpingitis; the L.G.V.C.F.T., performed 44 weeks after delivery, showed only partial fixation to 1:8.

There was some fixation to the L.G.V.C.F.T. in all five cases: Partial fixation to 1:8 (Mrs D and E), complete fixation to 1:16 and partial to 1:64 (Mrs C), complete to 1:32 and partial to 1:64 (Mrs I), complete to 1:128 and partial to 1:512 (Mrs H).

FIG. 2.—Follicular conjunctivitis due to TRIC virus ($\times 5$ approx.).

FAMILY										D
MOTHER	Nationality									British
	Baby's Age (wks) when Virus Tests Made									4
	Genital Infection	History of Vaginal Discharge								None
		Signs—Cervix								Oedema (salpingitis and “follicles” later) 37 Inclusions + TRIC culture + (IOL—4/GCx)
		Smears—Cervix								
		Virus Tests								
	Fixation to L.G.V.C.F.T.									Partial to 1:8
Skin Tests ..	Frei LB 1								— —	
		Associated Infection							—	
BABY	Inclusions									+
	TRIC Culture									— (at 44 wks)
	Frei and LB 1									
	Fixation to L.G.V.C.F.T.									
FATHER	Nationality									British
	Baby's Age (wks) when Virus Tests Made									4, 15
	Genital Infection	History								Previous painful micturition; pain on ejaculation developed
		Signs								
		Smears—Urethra								
		Virus Tests								
	Fixation to L.G.V.C.F.T.									Partial to 1:8
	Skin tests ..	Frei LB 1								— —
			Progress							Meatitis and tenderness of prostate developed

Smears = Maximum number of polymorphonuclear leucocytes per high-power field.
 LB 1 = Intradermal test using acid-extract of LB 1 isolate of TRIC virus.
 L.G.V.C.F.T. = Complement-fixation test for lymphogranuloma venereum.

The Frei and LB 1 intradermal tests were positive in the case of the one Pakistani patient (Mrs E), with partial fixation to the L.G.V.C.F.T. to 1 : 8, but were negative in the case of Mrs D, who developed salpingitis, and in the case of Mrs H who had complete fixation to the L.G.V.C.F.T. to 1 : 128 and partial to 1 : 512. The one Caribbean mother (Mrs I) had a negative Frei test, although her L.G.V.C.F.T. showed complete fixation to 1 : 32 and partial to 1 : 64.

There was associated trichomonal infestation in two cases (Mrs E and Mrs H).

Fathers.—Two of the four fathers seen had a history of urethral discharge or dysuria, and all showed signs of urethritis. Three attended for further tests: one of these (Mr D) developed meatitis and pain on ejaculation due to prostatic-vesiculitis, and all three had an excess of pus in the urethra and inclusions in urethral scrapings.* From Mr H,

whose wife had the L.G.V.C.F.T. titre of 1 : 128 with partial fixation to 1 : 512, a virus (isolate IOL-9/GU) was grown. The L.G.V.C.F.T. in his case showed only weak fixation to 1 : 8 and the Frei and LB 1 skin tests were negative; similar results were obtained with these three tests in the case of Mr D, whose wife had developed salpingitis. In two cases, including that of Mr I, the one Caribbean, the L.G.V.C.F.T. gave negative results.

Fig. 2 (p. 35) shows conjunctival follicles due to TRIC virus infection.

Fig. 3 (opposite) shows the cervix of Mrs E.

Fig. 4 (overleaf, p. 38) shows the cervix of Mrs I.

Fig. 5 (overleaf, p. 38) shows the cervix of Mrs H, the patient with the high L.G.V.C.F.T. titre (1 : 128, partial fixation to 1 : 512), whose husband had urethritis from which TRIC virus was grown (isolate IOL-9/GU), and whose baby had neonatal conjunctivitis from which TRIC virus was also grown (isolate IOL-8/ON).

* An inclusion body in a urethral cell from Mr C is illustrated in Al-Hussaini and others (1964), see p. 28, *Fig. 6* (c).

I
TRIC VIRUS CONJUNCTIVITIS

E	I	C	H
Pakistani	Caribbean	British	British
7, 10, 12, 34	5, 19	15	8, 11
Yes	Yes	Yes	Yes
"Follicles" (scarring later) 40 — each time	"Follicles", nodules 75 — twice	"Follicles", Proctitis 20 — (cervix and rectum)	"Follicles" 60 — twice
Partial to 1:8	1:32, partial 1:64	1:16, partial 1:64	1:128, partial 1:512
+	N.D.	N.D. N.D.	—
Trichomoniasis	—	—	Trichomoniasis
+ (IOL— 5/ON) — (at 33 wks)	+	+ (IOL— 3/ON)	+ (IOL— 8/ON) — } (at 15 wks)
Pakistani	Caribbean	British	Maltese
N.A.	19	3, 27	9
	None	Recurrent urethritis	—
	Urethritis, urinary shreds at 1 hr N.D. N.D.	Urethritis Urethral pus Inclusions { + at 3 wks — at 27 wks	Urethritis 15, 1st urine 60 in thread Inclusions+ TRIC culture+ (IOL— 9/GU)
	—	—	Weak to 1:8
	N.A. N.A.	— N.D.	—
	N.A.	Gonorrhoea at 27 wks with meatitis Inclusions —	

N.A. = Not available.

N.D. = Not done.

FIG. 3.—Cervical os (Mrs E), showing "follicles" ($\times 10$ approx.). f = "follicle." m = characteristic mottling due to trichomonal vaginitis.

FIG. 4.—Cervical os (Mrs I), showing “follicles” and nodules.

f = “follicle”.
nod = nodule ($\times 11.5$ approx.).

FIG. 5.—Cervical os (Mrs H), showing “follicles” ($\times 6$ approx.).

f = “follicle”.
n = Nabothian ovule.

B. TRIC Virus Infection of the Eye in the Adult (Table III, overleaf, pp. 40 and 41).

Eight adults (all British) suffering from TRIC virus infection of the eye were examined, as were five of their consorts; tests for virus were not performed in one of the latter because he had already been treated for "non-specific" urethritis.

Females.—The ocular syndromes resulting from TRIC virus infection were trachoma (Miss F and Mrs O), inclusion conjunctivitis (Mrs K), and punctate kerato-conjunctivitis (Miss M). Inclusions were demonstrated in conjunctival scrapings from all patients except Mrs O, who had received considerable local treatment before the test. The initial genital tests were taken between 5 and 80 weeks after the onset of conjunctivitis. All four women gave a history of recent vaginal discharge. Changes in the cervix, similar to those produced in the conjunctiva by TRIC virus infection, were seen with the colposcope in all four cases: "follicles" were present initially in two and developed in a third; there was scarring in two. There was pus in the cervical secretion in all four, the lowest count being 40 polymorphonuclear leucocytes per H.P.F. (Mrs K, Miss M). Virus was not

demonstrated in scrapings from the cervix. Trichomonal vaginitis was present in two (Miss F, Miss M).

The L.G.V.C.F.T. was done in three cases. The result was negative in one (Mrs K), showed fixation to 1:8 and partial to 1:32 in another (Miss F), and weak fixation to 1:8 in the third (Mrs O).

The Frei test was done in two cases (Miss F, Mrs O) and was negative in both. The LB 1 skin test was negative in one (Mrs O) and positive in the other (Miss F).

Consorts of the Female Patients.—That of Miss F was treated for urethral discharge by a general practitioner. That of Mrs K would not attend for examination. That of Miss M had already received treatment for "non-specific" urethritis. Mrs O's husband was found to have asymptomatic urethritis; virus was not demonstrated and the Frei and LB 1 skin tests were negative, but the L.G.V.C.F.T. showed complete fixation to 1:32 and partial to 1:64. Mrs O had developed trachoma during pregnancy, and her baby had been treated empirically elsewhere for "ophthalmia neonatorum"; it seems possible that this was in fact a case of neonatal TRIC virus infection. Fig. 6 shows the appearance of the cervix of Mrs O.

FIG. 6.—Cervical os (Mrs O), showing "follicles" ($\times 15$ approx.).

f = "follicle".
n = Nabothian ovule.

TRIC VIRUS INFECTION OF THE EYE IN

Sex		Female		
Patient		Miss F	Mrs K	Miss M
Eye	Syndrome	Trachoma	Conjunctivitis	Punctate keratoconjunctivitis
	Virus Tests	Inclusions + Culture + (IOL-6/OT)	Inclusions +	Inclusions +
Interval between Onset of Eye Disease and Genital Tests (wks)		5	80	32
Genital Infection	History	Vaginal discharge	Vaginal discharge	Vaginal discharge
	Signs	Cervix "follicles"	Cervix "follicles", scarring	Cervix scarring
	Smear	Cervix 60	Cervix 40	Cervix 40
	Virus Tests	— three times	— twice	— twice
Associated Infection		Trichomoniasis	—	Trichomoniasis
Fixation to L.G.V.C.F.T.		1:8, partial 1:32	—	N.D.
Skin Tests	Frei	—	N.D.	N.D.
	LB 1	+	N.D.	N.D.
Consort		Urethral discharge treated by general practitioner	N.D.	"Non-specific" urethra already treated and cured

L.G.V.C.F.T. = Complement-fixation test for lymphogranuloma venereum
 LB 1 = Intradermal test using acid-extract of LB 1 isolate of TRIC virus
 Smear: Maximum number of polymorphonuclear leucocytes per high-power field

Males.—The ocular syndromes resulting from TRIC virus infection were conjunctivitis (Mr J and Mr P), trachoma (Mr L), and punctate keratoconjunctivitis (Mr G). Inclusions were demonstrated in conjunctival scrapings in each case. Genital tests were taken between 2 and 7 weeks after the onset of conjunctivitis. In no case was there any history of discharge or dysuria, but Mr J, Mr G, and Mr P had urethritis, and Mr J also had meatitis (Fig. 7). Mr L had a gross excess of pus in the prostatic-vesicular secretion. There was thus evidence of genital infection in all four cases, and pus in the prostatic-vesicular fluid in all.

Virus was not demonstrated in material from the urethra.

The L.G.V.C.F.T. showed complete fixation to 1:16, partial to 1:32 in one case (Mr J), and complete fixation to 1:8, partial to 1:32 in another (Mr G). The Frei test was negative in the two cases in which it was performed (Mr J and Mr G), and the LB 1 test was positive in one (Mr J).

Consorts of Male Patients.—Mr J's wife had a nodule on the cervix, only 11 polymorphonuclear leucocytes per H.P.F. in the cervical mucus, trichomoniasis, partial fixation to 1:16 to the L.G.V.C.F.T., and a positive LB 1 test.

FIG. 7.—Urethral meatus (Mr J), showing meatitis ($\times 10$ approx.).

II

ADULT: EIGHT PATIENTS AND SIX CONSORTS

	Male			
Mrs O	Mr J	Mr L	Mr G	Mr P
Trachoma	Conjunctivitis	Trachoma	Punctate kerato-conjunctivitis	Conjunctivitis
-- (treated)	Inclusions +	Inclusions +	Inclusions + Culture + (IOL - 7/OPK)	Inclusions +
28	7	2	4	4
Vaginal discharge	--	--	--	--
Cervix "follicles" Cervix 68	Urethritis, Meatitis Urethra 120, P-vesic. pus -- twice	Urethra --, P-vesic. pus --	Urethritis Urethra 40, P-vesic. pus --	Urethritis Urethra 40, P-vesic. pus --
--	--	--	--	--
Weak 1:8	1:16, partial 1:32	N.D.	1:8, partial 1:32	N.D.
--	--	N.D.	--	N.D.
--	+	N.D.	N.D.	N.D.
<i>Husband:</i> Asymptomatic urethritis, smear 120 Virus tests -- Frei and LB 1 -- L.G.V.C.F.T. 1:32, partial 1:64 <i>Baby:</i> Treated for "ophthal- mia neonatorum" Frei and LB 1 --	Cervix nodule, smear 11 Virus tests -- Trichomoniasis Frei -- LB 1 + L.G.V.C.F.T. Partial 1:16	N.D.	Cervix granular erosion and nodule, smear 35 Proctitis Trichomoniasis Virus tests -- (cervix and rectum) Frei and LB 1 -- L.G.V.C.F.T. 1:8, partial 1:16	No sign of infection Cervix smear 5 Virus tests -- L.G.V.C.F.T. N.D.

P-vesic. = Prostatic-vesicular
N.D. = Not done

Mr G's wife had a cervical granular erosion and nodule, 35 polymorphonuclear leucocytes per H.P.F. in the cervical mucus, trichomoniasis, proctitis, negative Frei and LB 1 tests, and an L.G.V.C.F.T. showing complete fixation to 1 : 8 and partial to 1 : 16.

Mr P's wife showed no sign of infection.

That of Mr L was not examined.

Characteristic cellular changes in scrapings, which are associated with TRIC virus infection (Al-Hussaini and others, 1964), were found in genital tests from both groups of patients, namely the parents and the infected adults and their consorts. There were only three exceptions out of the twenty patients tested: Mr L who had prostatic-vesiculitis but no urethritis, and Mr P and his wife. Mr P had prostatic-vesiculitis but urethral scrapings contained only normal epithelial cells. The transient urethritis which followed was almost certainly due to the trauma of scraping for virus. The overnight urethral secretion, which was tested the day after the virus tests, contained some pus, epithelial cells, few bacteria, and no blood. Later, his tests were normal as were those of his wife. In the other males, if the overnight urethral

secretion was tested, this was done before the tests for virus were carried out.

We can conclude that TRIC virus infection is a cause of so-called "non-specific" urethritis in the fathers of babies suffering from neo-natal conjunctivitis due to the virus. The mothers of such babies show changes in the cervix which may be due to the virus, but further investigation is needed of this point. There is some evidence to suggest that TRIC virus infection of the eye in the adult may also be associated with genital infection by that organism.

It is clear that in a selected group of cases, so-called "non-specific" genital infection is due to TRIC virus. But what relation this group has to the whole field of non-specific genital infection remains to be established.

Summary

(1) Signs of genital infection were sought in the parents of five babies suffering from TRIC virus conjunctivitis. TRIC virus was grown from the cervix of one of the five mothers, and she later developed salpingitis. Four mothers gave a history of vaginal discharge in pregnancy. On examination with a colposcope all five had signs in the cervix

resembling those produced in the conjunctiva by TRIC virus infection. There was fixation to the L.G.V.C.F.T. in all five cases:

- Partial fixation to 1 : 8 (two),
- Fixation to 1 : 16, partial to 1 : 64 (one),
- Fixation to 1 : 32, partial to 1 : 64 (one),
- Fixation to 1 : 128, partial to 1 : 512 (one).

The Frei and LB 1 intradermal tests were performed in three cases with negative results in two; in one case both tests were positive; the Frei test was performed alone in one case with negative result.

One of the fathers was not examined, but the other four showed evidence of urethritis. Three of these, one of whom developed meatitis and prostatic-vesiculitis, were tested for TRIC virus: inclusions were found in urethral scrapings from all three and TRIC virus was grown from one. The L.G.V.C.F.T., performed in four cases, showed fixation to 1 : 8 in two and was negative in two.

The Frei and LB 1 intradermal tests gave negative results in two cases; the Frei test was performed alone in one case with negative result.

(2) A similar examination was carried out in the cases of eight adults (four men and four women) suffering from TRIC virus infections of the eye, and four of their consorts. In no case was TRIC virus found in the genital tract but there was evidence to suggest genital infection by that organism.

(3) It appears that in certain selected cases so-called "non-specific" genital infection is due to TRIC virus. The relation of this group to the whole field of non-specific genital infection remains to be established.

We are grateful to Professor C. F. Barwell of The London Hospital for kindly supplying the materials for the intradermal tests and for carrying out the complement-fixation tests for lymphogranuloma venereum, and to Dr A. E. Wilkinson, Director of the V.D. Reference Laboratory of The London Hospital, for carrying out serological tests and cultures.

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Infection génitale avec infection oculaire due au virus TRIC III. Observations cliniques

RÉSUMÉ

(1) On chercha l'infection génitale chez les parents de 5 enfants atteints de conjonctivite due au virus TRIC.

Mères.—On cultiva le virus du col de l'utérus d'une des 5 mères qui fut plus tard atteinte de salpingite; 4 mères avaient eu une suppuration vaginale lorsqu'elles étaient enceintes. La colposcopie montra des signes au col de l'utérus de chacune semblables à ceux produits par la conjonctivite due au virus TRIC.

Le test fixant le complément du lymphogranulome vénérien fut positif pour les 5 mères, avec:

- Fixation incomplète à 1:8 (2),
- Complète à 1:6, incomplète à 1:64 (1),
- Complète à 1:32, incomplète à 1:64 (1),
- Complète à 1:128, incomplète à 1:512 (1).

Les tests Frei et LB 1 furent positifs dans un cas sur trois. Le test Frei seul fut négatif dans un cas.

Pères.—Un des pères ne fut pas examiné, mais les 4 autres montrèrent les symptômes de l'urétrite.

On chercha le virus TRIC chez trois d'entre eux, dont un fut atteint de méatite et prostatico-vésiculite; on trouva des inclusions dans les frottis urétraux des trois et on cultiva le virus TRIC dans un cas.

Le test fixant le complément du lymphogranulome vénérien fut positif à 1:8 dans deux cas et négatif dans les deux autres.

Les tests Frei et LB 1 furent négatifs dans deux cas. Le test Frei seul fut négatif dans un cas.

(2) On examina de la même façon 8 adultes (4 hommes et 4 femmes) atteints d'infection oculaire due au virus TRIC et 4 de leur partenaires sexuels. On ne trouva pas le virus TRIC dans les organes génitaux, mais il y eut des signes indicatifs d'infection génitale par cet organisme.

(3) Il se peut qu'en certains cas l'urétrite "non-spécifique" soit due au virus TRIC. Il faut étudier la relation entre ce virus et l'infection génitale non-spécifique en général.