

# *Trichomonas vaginalis* and Trichomoniasis

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## I. INTRODUCTION

*Trichomonas vaginalis* was described by Donné in 1836 and has long been regarded as a harmless commensal of the human vagina. In 1916 Höhne described as a clinical unit the so-called "Trichomonadenkolpitis = trichomoniasis" of women whose vaginae contained many of these flagellates. The

concept of *T. vaginalis* as a primary pathogenic parasite was only gradually accepted. In central Europe credit is due to Rodecuret, although various ideas of his on the resistance of *T. vaginalis* were not substantiated. Without a doubt, *T. vaginalis* is the commonest of the human trichomonads and "Trichomonadenkolpitis" does exist despite the sceptical adverse views of some prominent older gynaecologists. The literature on *T. vaginalis* and trichomoniasis is vast. The first monograph (Trussell, 1947) lists 1586 references. At present, the number of publications on *T. vaginalis* far surpasses 3000. The three symposia dedicated to this subject (Monaco, 1954, "Symposium sur les uréthritides non gonococciques"; Reims, 1957, "Symposium international sur les infestations à trichomonas"; Montreal, 1959, "First Canadian symposium on non-gonococcal urethritis and human trichomoniasis" published 1960) confirm the world-wide interest taken in this human infection. In recent years, three symposia were also arranged in Poland by the Polish Parasitological Society (Olsztyn, 1961; Lublin, 1963; Białystok, 1965). Additional monographs on this subject are by R. Peter, "Nákaza bičíkovcem poševním u dětí, panen a mladistvých", Prague 1945; J. Okla, "Rzesistkowe zapalenie pochwy i jego leczenie", Warszawa 1954; J. M. Bedoya, "Tricomonas sexual humana", Valencia 1959; N. E. Sidorov, A. M. Korchemkin and A. P. Kolesov, "Trichomonaz močepolových orgánov člověku", Moscow 1959. Many comprehensive articles have been published in various medical journals, but in most textbooks on parasitology and gynaecology, little attention has been given to the problems of *Trichomonas vaginalis*. An exception is the textbook by Jírovec, "Parasitologie für Ärzte" (Jena, 1960), in which a chapter (26 pp.) is devoted to trichomoniasis. On *T. vaginalis* in the human male there is a monograph by Veynerov and Rozhinski, "Trichomonadny uretrit mužchin", Kiev, 1956; and a comprehensive study by J. Jíra (1961), "Studie o mužské trichomoniase", with a complete bibliography. An abstract of this paper was published earlier (1958) in *Zentbl. Bakt. Parasit. I. Abt. Orig.* 172, 310-329.

The present review deals with more recent knowledge of the morphology and biology, pathology and laboratory diagnosis of *T. vaginalis*, with clinical aspects of trichomoniasis in women, men and children, and with the epidemiology and treatment of this infection. The older literature (up to 1947) can be found in the monograph by Trussell (1947), more recent literature in the Proceedings of the two Symposia (Reims, Montreal). We have generally considered more recent papers, although some of them were inaccessible and in others we found a considerable overlap. Nevertheless, 30 years of personal experience of research on *T. vaginalis* and trichomoniasis provide justification for critical appraisal of the various problems discussed.

## II. TAXONOMIC POSITION OF *Trichomonas vaginalis*

The flagellate *T. vaginalis* belongs to the superclass Mastigophora Diesing, 1866, class Zoomastigophorea Calkins, 1909, order Trichomonadina Kirby, 1947, family Trichomonadidae Chalmers and Pekola, 1918, emend. Kirby, 1946. Honigberg (1963) divided this family into two subfamilies: Trichomonadinae, receiving the genera *Trichomonas* and *Pentatrichomonas*, and the

subfamily Tritrichomonadinae with the single genus *Tritrichomonas*. The characteristics of these three genera are given below.

1. Genus *Trichomonas* Donné, 1836

Four anterior flagella, the fifth (posterior) flagellum terminating with the undulating membrane. No trailing flagellum. Undulating membrane shorter than the body. Capitulum of axostyle only moderately extended, terminating anteriorly in thin pelta, axostyle thin. Parabasal body rod-shaped or with lateral bifurcation. *T. vaginalis* (Donné, 1836) from the human urogenital tract. *T. tenax* (Müller, 1773, emend. Ehrenberg, 1838) Dobell, 1939 (syn.: *T. buccalis*, *T. elongata*) from the oral cavity of man. *T. gallinae* (Rivolda, 1878) Stabler, 1938 (syn.: *T. columbae*) from fowl and pigeon.

2. Genus *Pentatrichomonas* Mesnil, 1914

Five anterior flagella—four in one group and one solitary flagellum beating in independent rhythm. Posterior flagellum passing into long trailing flagellum. Well-developed undulating membrane extending to the termination of the body. Costa well developed. Capitulum of the axostyle widened by lateral membranes, terminating in a large pelta. Axostyle of medium width. Parabasal body composed of one to several granules, surrounded by an elliptical or spherical zone. *P. hominis* (Davaine, 1860) Wenrich, 1931 from the intestines of man.

3. Genus *Tritrichomonas* Kofoid, 1920

Three anterior flagella, one posterior flagellum passing into a long trailing flagellum. Well-developed undulating membrane extended to body termination. Axostyle thick, sharply pointed at posterior end, surrounded by one to several periaxostylic rings at the site where it projects from the body. Parabasal body rod-shaped, often very elongated. *T. suis* Gruby, Delafond, 1943 (syn.: *T. foetus*) from cattle and swine.

For concise morphological and physiological data on four species of this subfamily, see Table I.

### III. MORPHOLOGY OF *T. vaginalis*

#### A. LIGHT MICROSCOPY

Investigations by Honigberg and King (1964), using phase contrast, clarified some morphological details of *T. vaginalis*. The shape of the body is variable in both living and preserved forms. Actively swimming forms are ellipsoidal or ovoidal, sometimes spherical. The flagellates are very plastic and may pass through narrow spaces. All strains have the capacity to form pseudopodia-like extensions, which are used in feeding, for attachment to stationary objects, but not for amoeboid movement. All non-dividing flagellate forms have four anterior flagella, somewhat unequal in length; these originate in an anterior basal granule complex. After protargol-staining they are seen to end in small rods or knobs. The undulating membrane and the costa arise in the basal granule complex postero-dorsal to the anterior flagella. The free margin of the

TABLE I

*Morphological and physiological data on the four species Trichomonas  
(comb. after Kulda, 1965)*

	<i>Trichomonas vaginalis</i>	<i>Trichomonas tenax</i>	<i>Trichomonas gallinae</i>	<i>Pentatrichomonas hominis</i>
Body measurements (average in brackets)	4-32 × 2·4-14·4 $\mu$ (10 $\times$ 7 $\mu$ )	4·2-12·8 × 2·1-14·7 $\mu$ (7·4 $\times$ 5·3 $\mu$ )	6·2-18·9 × 2·3-8·5 $\mu$ (10·5 $\times$ 5·2 $\mu$ )	7-15 $\times$ 4-10 $\mu$
Shape of body	oval	piriform	spherical	piriform
Ratio of body length to width	1·4:1	1·4:1	1·8:1	—
Nucleus after Honigberg,	2·4-6·4 × 1·2-3·2 $\mu$ (4·2 $\times$ 2 $\mu$ )	1·5-3·3 × 1-2·5 $\mu$ (2·5 $\times$ 1·7 $\mu$ )	1·8-3·1 × 1-2·1 $\mu$ spherical without nucleoli	spherical with central nucleoli
Ratio of nucleus length/width	2·1:1	1·5:1	1·8:1	—
Chromosomes	5 (Hawes) (Powell)	3 (Hinshaw)	?	5 (Bishop)
Flagella	short, approx. 1/1 of body length	approx. $\frac{1}{3}$ of body length	—	long, terminating freely at level of 6th flagellum
Number of anterior flagella	4	4	4	5
Paraxostylar granules	present	absent	present	—
Shape of parabasal body	mostly V-shaped	exclusively rod-shaped	mostly hook-shaped	—
Optimal pH	5·8-6	7·0-7·5	7·0-7·5	7
Optimal temperature	37°C	31-32°C	37°C	30-37°C
Isolation in axenic culture	easy in standard media	not possible in standard media	easy in standard media	easy in standard media
Pathogenicity for mice	pathogenic	non- pathogenic	pathogenic	?
Cytopathogenic effect on tissue culture	strong	none	strong	?

membrane consists of the accessory filament and the recurrent 5th flagellum, which are about equal in length and diameter. The posterior end of the costa is usually obscured by the terminal segment of the undulating membrane. The two rows of paracostal granules are visible quite clearly in hematoxylin-stained preparations and in living organisms viewed in a phase contrast system.

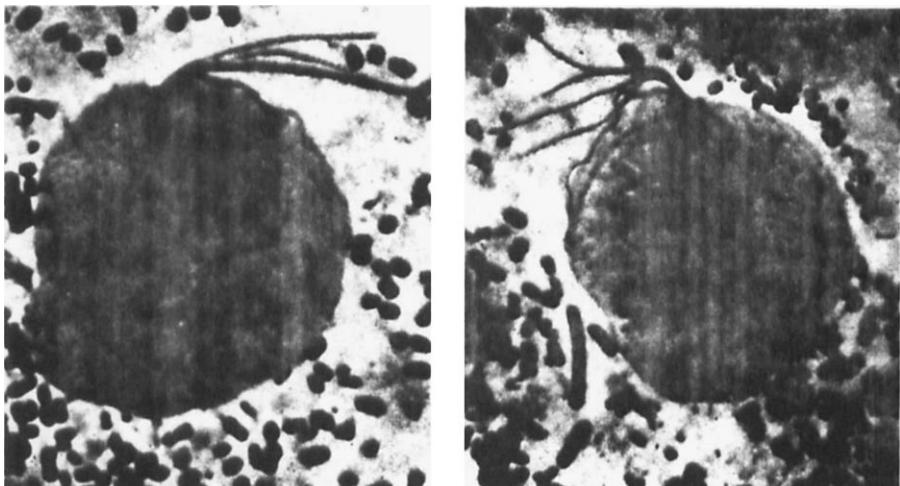


FIG. 1. *Trichomonas vaginalis*. (Giemsa staining. Photo Professor J. Fiala.)  $\times 3000$ .

The axostyle has a spatulate capitulum about one-third of its total length and this extends anteriorly into a small, crescent-shaped pelta, seen most clearly after protargol staining. The trunk of the axostyle is a thin hyaline rod passing through the centre of the organism and projecting slightly from the posterior surface. Swellings or ridges are noted along the axostylar projection part and may be mistaken for periaxostylar rings, which do not exist in *T. vaginalis*. Constantly present are three rows of paraxostylar granules, a typical arrangement for *T. vaginalis*. The parabasal apparatus consists of the parabasal body associated with one or more filaments. In most strains forms with a V-shaped parabasal body are the more common. The ellipsoidal or ovoidal nucleus is situated near the anterior end of the body, often containing a small spherical nucleolus in a chromatin-free area. In fixed and stained preparations the nuclei appear rather elongate. In the cytoplasm there are chromatophilic inclusions of varying size.

A cytostome seems not to exist and food particles are ingested in the posterior region by the fine, pseudopodia-like processes.

The biometric characteristics of *T. vaginalis* were worked out by Kurnatowska (1964, 1966) as parameters such as: length, breadth, surface of projection volume, shape index (length/breadth ratio) and the plasmonuclear surface and volumetric indices. Statistically significant differences exist in the biometric

characteristics of flagellates from women with asymptomatic, acute and chronic infection, the smallest in acute cases, the largest in chronic infection. *T. vaginalis* from women treated with arsenical drugs are much under average size, though the nucleus retains its original size. Differences in length, breadth and shape of *T. vaginalis* obtained from women at various stages of infection, disappeared when strains were cultured for one month in Pavlova's medium.

Schmidt-Gross (1958) described changes in the shape of the body during metabolic activity.

#### B. ELECTRON MICROSCOPY

The first studies on the ultrastructure of *T. vaginalis* were made by Shimada (1959) and Inoki *et al.* (1960), the latter writers describing a double-layered nuclear membrane with pores, a lamellar Golgi apparatus, and flagella with 9 peripheral double fibrils and one central fibril.

Ludvík *et al.* (1961) studied ultra-thin sections and total preparations shadowed with beryllium and chrome, and gave this description of the ultra-structure of *T. vaginalis*: four anterior flagella of equal length (15–22  $\mu$ ), not exceeding the length of the cell body, their ends sharply pointed or terminating in a hook. They consist of 10–11 fine fibrils forming a bundle, which is surrounded by a plasmatic sheath. Nine fibrils are arranged in a circle, one or two stronger fibrils being central. The undulating membrane is 1–1·6  $\mu$  thick, its exterior border formed by a fine marginal fibril. In the middle of the undulating membrane, the centrally situated short flagellum also has 10–11 fibrils; it ends in the first half of the cell body along with the undulating membrane, but is never free and trailing. Flagella and undulating membrane arise in the group of the five basal granules situated in the terminal part of the axostylar capitulum. The four costal fibrils also arise in this group, all of them directed towards the centre of the cell. Two are thicker and longer, two thinner and shorter; they are composed of several disks 55 m $\mu$  thick.

Anteriorly the axostyle forms a thick capitulum, thin in its median part (0·5–0·7  $\mu$  in diameter); its termination outside the cell body is like a delicate thorn 5–7  $\mu$  long. The parabasal body (5–6  $\mu$  long, 1  $\mu$  wide) lies in the anterior part of the cell body, mostly dorsal to the nucleus. Most cytoplasmic, osmophilic granules (average size 0·4  $\mu$ ) are crowded round the spindle or drop-shaped nucleus and along the axostyle; finer granules occur along the costal fibrils, especially along the costa beneath the undulating membrane. Nielsen *et al.* (1966) completed these studies on the ultrastructure of *T. vaginalis*. The nuclear membrane is an ordinary, three-layered membrane, about 7 m $\mu$  wide. Most nuclei contain several large electron-dense granules, which either represent nucleoli or are simply clusters of chromatic material (chromosomes?). The parabasal body is morphologically a Golgi zone located near the anterior nuclear pole opposite the axostyle, measuring about 1·5 × 0·5  $\mu$  and composed of more or less flattened cisternae with a layering almost parallel to the nuclear surface. Vesicles seem to have arisen from the individual cisternae by a process of constriction or budding. All of them are limited by a triple-layered membrane 7–10 m $\mu$  wide. The sheath of the axostyle consists of a monolayer of

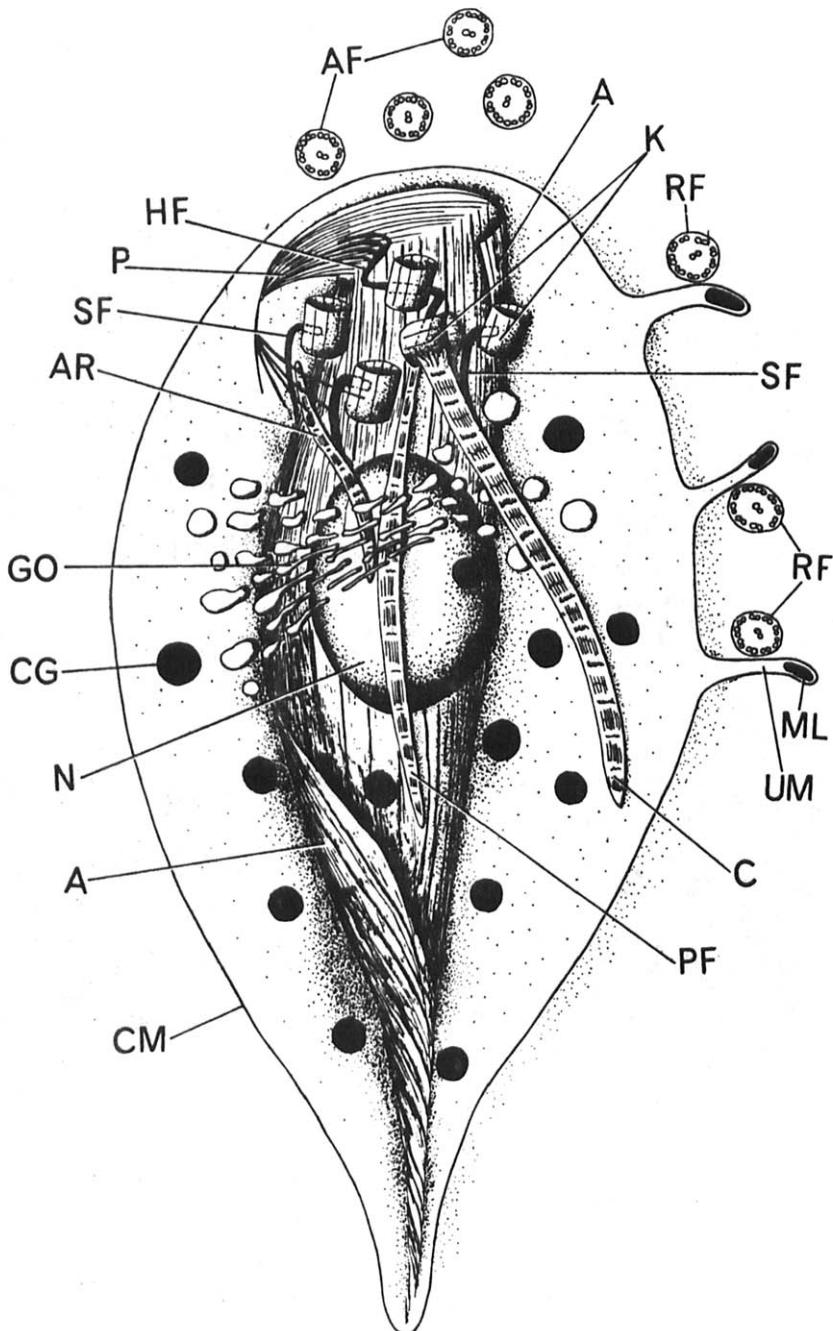


FIG. 2. *T. vaginalis*—semiperspective diagram based on electronmicrographs. CM—cell membrane; A—axostyle; P—pelota; N—nucleus; GO—Golgi or parabasal body; C—Costa; PF—Parabasal filament; AR—accessory rootlet fibre; SF—sickle-shaped fibres; K—Kinetosomes; AF—anterior flagella (in T.S.); RF—recurrent flagellum (in T.S.); UM—undulating membrane (in T.S.); ML—marginal lamella (in T.S.); CG—paracostal and parastylar granules. (After Nielsen *et al.*, 1966.)

about 50–55 parallel, tubular fibres, the external tubules measuring 20 m $\mu$ , the internal ones 7 m $\mu$  in diameter. Four of the basal granules (kinetosomes) have parallel long axes, distributed radially around the fifth granule, to which the recurrent flagellum is attached. Each individual *T. vaginalis* has at least two rootless flagellar fibres (costae) and a parabasal filament attached to the kinetosomes by sickle-shaped fibres without cross striations. The costa is longer and wider than the filament, its body being characteristically flattened near the kinetosome. It is located near the periphery close to the attachment

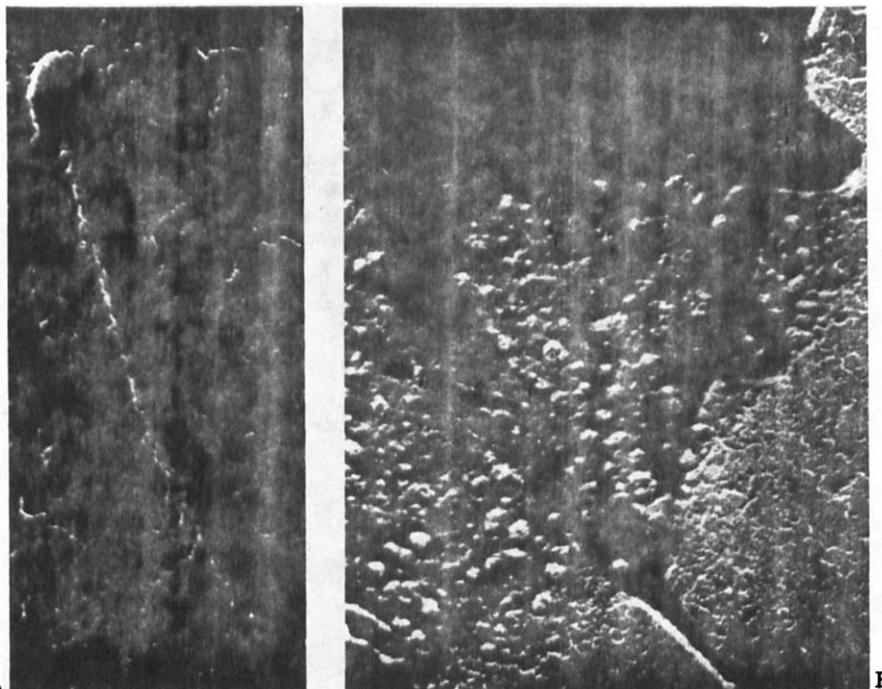


FIG. 3. *T. vaginalis* electronmicrographs. A—End of a flagellum. B—End of the axostyle and undulating membrane. (Photographed by J. Ludvík.)

of the undulating membrane. The parabasal filament lies nearer the centre of the plasma. In rare cases the costa is 13  $\mu$  long and 1.5  $\mu$  broad. Each of the periods of cross striation in the costa and the filament are subdivided by a less dense cross line, the subfibril inside the costa being arranged longitudinally. The recurrent flagellum is along its entire length attached to the undulating membrane. The endoplasmatic reticulum is found frequently as a corona around the nucleus and is always abundant in the cytoplasm inside the capitulum of the axostyle. Free ribosomes are distributed all over the cytoplasm. Vesicles of different size and small tubules limited by a three-layered membrane are frequently observed at the cell periphery. Larger vesicles and vacuoles, some containing electron-dense material, are situated in the caudal end of the

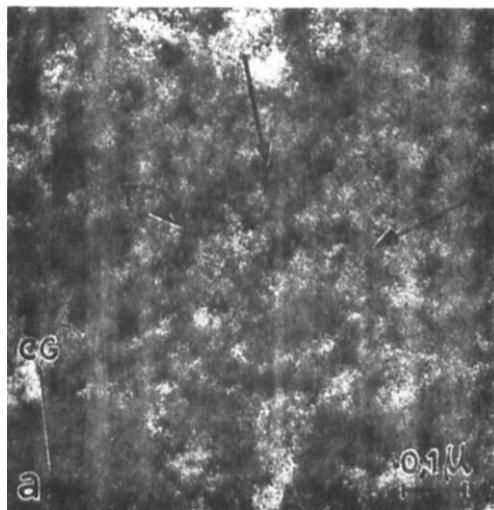


FIG. 4. E.M. section of *T. vaginalis* showing individual fibres (TF) of caudal part of axostyle (A). Tubules connected side by side by a delicate membrane (arrows).  $\times 90000$ . (After Nielsen *et al.*, 1966.)

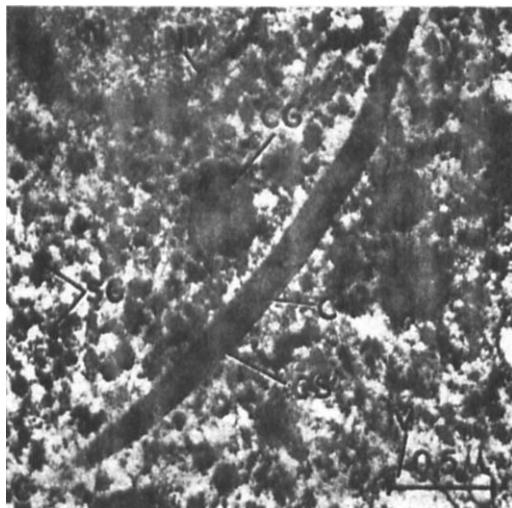


FIG. 5. E.M. section of *T. vaginalis*. Costa (C) shown in L.S. Subfibrils (CS) within costa arranged almost in parallel to long axis of organelle and at right angles to cross striation. Fibrils approximately  $10\text{ m}\mu$  apart. Segmentation of costa indicated by a single major cross line, but a less dense intermediate line can also be distinguished.  $\times 90000$ . (After Nielsen *et al.*, 1966.)

body. Most of them seem to be food vacuoles. The paraxostylar granules, measuring about  $0.5\text{ }\mu$ , are limited by a triple membrane and consist of a coarse, granular, electron-dense matrix. There are also small granules ( $0.05\text{--}0.1\text{ }\mu$ ) with a dense globular matrix but no limiting membrane.

Smith and Stewart (1966) confirmed Inoki's original observation that there are no mitochondria in *T. vaginalis*. The axostyle appears to be a cup-shaped structure comprised of a single row of 35 fibrils, each about  $200\text{ \AA}$  in diameter and a constant distance apart. A dense-cross-banded costa with a regular

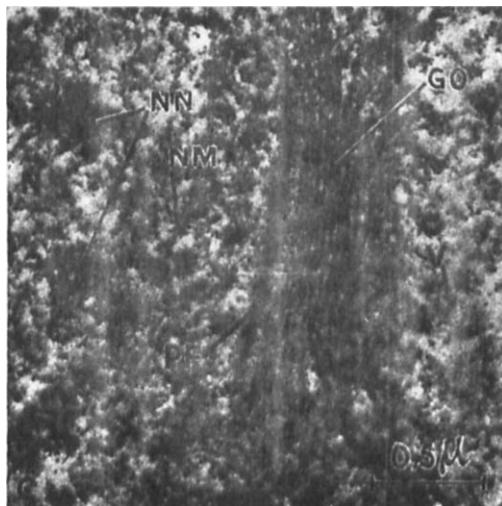


FIG. 6. E.M. section of *T. vaginalis* showing location of parabasal filament between nucleus and parabasal body (Golgi zone). The nucleus (*N*) with nuclear membrane (*NM*) contains nucleoli or clusters of chromatin (*NN*) in the nucleoplasm. *PF* denotes parabasal filament and *GO* parabasal body. Note vesicles (*V*) with parabasal body are increasing in size towards cell periphery.  $\times 31000$ . (After Nielsen *et al.*, 1966.)

periodicity of about  $400\text{ \AA}$  is closely associated with a kinetosome of an anterior flagellum. By differential staining, glycogen deposits were demonstrated. The lack of mitochondria is in keeping with the normally anaerobic life cycle of *T. vaginalis*.

Samuels (1961) found two general types of variation in *T. vaginalis*: (a) drug resistant mutants, (b) clonal strains with heritable differences of morphology. Some clones had almost exclusively small cells, in others the cells were larger; many giant cells were found in these cultures and, in addition, these organisms contained many granules and vacuoles. The possible sources of these variations may be either nutritional or metabolic differences or an infection with some agent, possibly a virus.

#### C. REPRODUCTION

*T. vaginalis* multiplies by bipartition, and the nuclear division is mitotic. According to Hawes (1947), five chromosomes are formed outside the nucleoli.

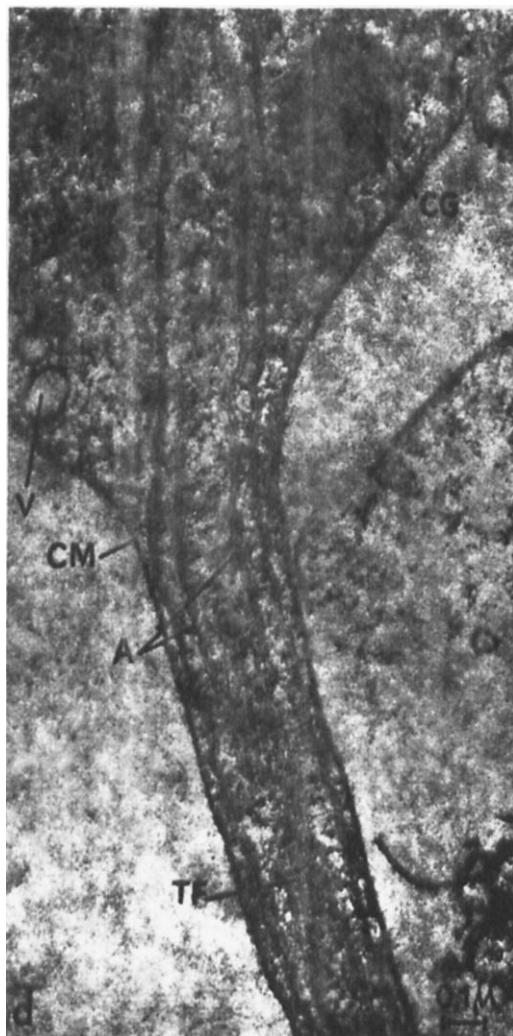


FIG. 7. L.S. through caudal part of axostyle of *T. vaginalis*. A chromatic granule with a paraxostylar location (CG) and vesicles (V) are found close to the cytoplasmic membrane (CM). Axostyle (A) consists of several layers spirally wound (cf. Fig. 1). Individual tubular fibres (TF) of axostyle tangentially cut, their helical course is clear.  $\times 52000$ . (After Nielsen *et al.*, 1966.)

The basal corpuscles pass to the opposite poles of the mitotic spindle, but no details are yet available on this process. After division of the nucleus, the locomotory apparatus is completed and finally the plasma divides. The fact that nuclear division is by mitosis is well known to protozoologists, but mitoses are difficult to find and may become indistinct or appear to be atypical after fixation and staining—and dry smears are useless.

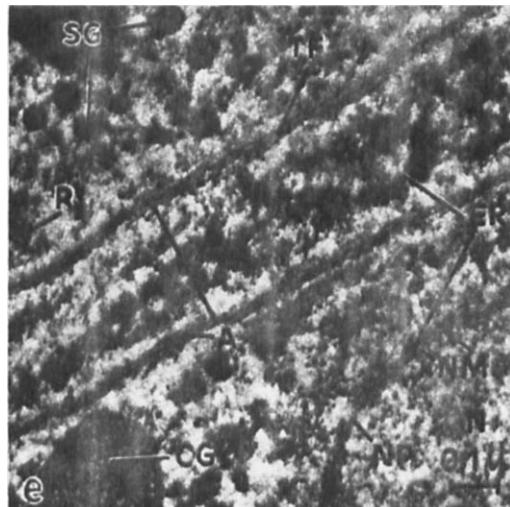


FIG. 8. Section of *T. vaginalis*, axostyle (A) in region of lower nuclear pole. N denotes nucleus with nuclear membrane (NM) and nuclear pore (NP). At nuclear pore there seems to be continuity from nuclear membrane to innermost layer of rough endoplasmic reticulum (ER). Between NM and ER a narrow perinuclear space (arrow). TF indicates obliquely cut tubular fibres of axostyle and R a cluster of free ribosomes in cytoplasm. Part of a chromatic granule lined by triple-layered membrane at CG and small cytoplasmic granules intensively stained with lead salts at SG.  $\times 54000$ . (After Nielsen *et al.*, 1966.)

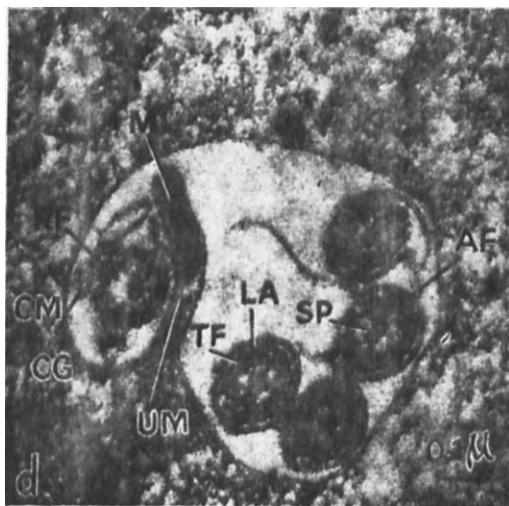


FIG. 9. Section of *T. vaginalis* showing flagella (AF) and (RF), undulating membrane (UM); cytoplasmic membrane (CM), lining recurrent flagellum (RF) is torn. Marginal lamella (ML) of undulating membrane (UM) is visible, also nine pairs of peripheral fibres (TF) and (LA) and two single central fibres (SP). CG denotes chromatic granulum.  $\times 54000$ . (After Nielsen *et al.*, 1966.)

Multiple division in *T. vaginalis*, as described by Dyroff and Michalzik (1954), is quite exceptional. The liberation of young forms from the "cysts", as observed by both authors, may be ascribed to erroneous observation. Malyszko (1964) found multiple division of *T. vaginalis*, grown on Roiron medium and also in samples collected directly from patients. Also Hoffman and Malyszko (1966) described multiple division and polynuclear *T. vaginalis* in one out of the 22 women examined before treatment and in 6 women examined on the third day of treatment with flagyl, aminitrozol or nitrofuranon. In the urethral discharge of males, polynuclear *T. vaginalis* were found in 10 patients before treatment and in 12 on the third day of treatment. Unfavourable conditions of the environment may explain increase in numbers of polynuclear forms.

There is no exact confirmation of sexual reproduction of trichomonads including *T. vaginalis*. The drawings by Grimmer (1950) depict either an accidental adherence of two completely independent flagellates or the mentioned constriction of protoplasmic particles which, under certain circumstances, may again become regressive. Dyroff and Michalzik (1954) consider the possible existence of permanent forms without confirming this assumption.

#### D. THE CYST PROBLEM

The problem of cyst formation by trichomonads has been studied by many protozoologists but the opinion prevails that cysts are not formed by *T. vaginalis*. Some spherical, motionless trichomonads can be found in vaginal discharges and especially in the urethral secretion of the male, recognizable by their sky-blue plasma and the moderately spindle-shaped, reddish-violet, finely granulated nucleus (Giemsa-stained), but these never have any flagella or a distinguishable cyst membrane, well known characteristics of *Amoeba* or *Lamblia* cysts. These formations seem to be degenerate trichomonads destined to die unless they are in time transferred into a culture medium or even into the genital ducts of the opposite sex. Such motionless, unflagellated trichomonads, covered with a mucous layer, can be found in the intestine of various rodents. These also are not cysts but are trichomonads dying on clusters of excrement. They are also transmissible, to a certain degree, perorally to other hosts or can grow in appropriate culture media, but otherwise are also condemned to death. This is never the case with true cysts. Also in the culture cysts are never formed, and the spherical trichomonads are degenerate forms. In some instances it could be proved that the formations, previously considered to be trichomonad cysts, belonged either to other flagellates (*Retortomonas dobelli* a.o.) or were the cells of *Blastocystis*. The stage drawn by Grimmer (1950) is certainly not a cyst but is a decomposed host cell. The cysts observed by Holz (1953) may be either degenerate host cells or yeast-like organisms covered with a thick membrane and bearing large vacuoles. The data by Holz could not be confirmed by Reusse (1955). Studies on *T. foetus* and *T. muris* have shown that these parasites never reach a strictly cystic stage. In old cultures, however, and under adverse conditions they may take on a rounded form, and by the loss of their flagella and undulating membrane they become entirely motionless. As observed in cultures of single trichomonads, rounded-off forms are labile organisms with

very little motility of the endoplasm and with hardly any sign of life, yet still able to regain their former vitality and to reproduce. In such cases amoeboid movement sets in with the protrusion of pseudopodia, soon to be followed by transverse division. Revitalization is also possible without the amoeboid stage, but then the tendency for an early bipartition is missing (Reusse, 1955).

Although Mandoul *et al.* (1946) do not consider the rounded forms of *T. vaginalis* to be true cysts, they ascribe to them an analogous role without exactly proving this assumption.

In Giemsa-stained smears it is sometimes possible to observe rounded, blue-staining formations without nucleus, flagella or axostyle, which may originate from torn-off pieces of flagellates trying to pass through crevices. Hereby, the anterior end with the nucleus, the flagella complex and the axostyle are torn off and the posterior portion, consisting of almost homogenous plasma, may give the erroneous impression of being a cyst. To prove trichomonad cysts, observations of native preparations under the light field, dark field and phase contrast microscope have to be completed with examinations of the conditions of the nucleus, the flagella and the axostyle by cytological methods on wet smear preparations and on protargol-stained trichomonads. Such cysts would also have to be regularly present in the vagina, like the cysts of intestinal *Lamblia*, *Amoeba*, *Chilomastix* and other Protozoa. This may be especially misleading for beginners who are trying to solve the cyst problem without having yet acquired sufficient knowledge of protozoan morphology.

#### E. SPECIFICITY OF HUMAN TRICHOMONAD SPECIES

The taxonomic position of *T. vaginalis* as an independent species will be discussed in this Section. The conception that *T. vaginalis* is only a certain aspect of *Pentatrichomonas hominis* or of *T. tenax* has been suggested by various authors (e.g. Grollet and Montaugé, 1957), but not agreed by protozoologists who have studied the morphology of *T. vaginalis* for the past 20 years or by the two symposia (Reims and Montreal). The differences in the three *Trichomonas* species are as follows (Table I):

(1) Morphologically, *T. vaginalis* is the largest of the three species. The undulating membrane extends along one half of the body, to which both ends are connected by a fibril. The nucleus is spindle-like or oval. The four free flagella terminate in a hook. *T. tenax* is much smaller, its nucleus is rounded and the ends of the short undulating membrane are connected by an indistinct fibril. The flagella terminate also in a small hook. *Pentatrichomonas hominis* is smaller, its undulating membrane is long, and there is a sixth free trailing flagellum. All five flagella terminate in a sharp point without a hook. These differentiating features are constant and are retained for several years even in cultures or after inoculation into various experimental animals by different methods (unpublished experimental results by Jírovčová, 1963). Cysts are not formed by any of these three species.

(2) Biological differences concern the location in the human body: *T. vaginalis* occurs only in the genito-urinary system. *T. tenax* is found, as a rule, only in the human buccal cavity, but occasionally in the stomach

during achlorhydria; *P. hominis* only in the large intestine. The least resistant of the three *Trichomonas* species to lower temperatures, desiccation, hypo- or hypertonic media and other influences of the external environment is *T. vaginalis*, most resistant is *P. hominis*; the resistance of *T. tenax* lies in between that of the other two species.

(3) Experimental transmissions of the individual species to unnatural environments were performed by Westphal (1936) and Bauer (1943), with negative results.

(4) The epidemiology of the three *Trichomonas* species also favours the view that they are independent species. Long ago, Jírovec *et al.* (1942) emphasized the fact that *T. tenax* does not coincide with *T. vaginalis* in *T. vaginalis* infected women. In fact, *T. tenax* is practically absent in young women with healthy teeth and becomes more common past the age of 40, when teeth generally start to decay; while *T. vaginalis* reaches its peak between the age of 20–40.

Červa and Červová (1961), examining 609 adult women in Prague coprologically and vaginally by cultivation and microscopical methods, found 25% of them to be infected with *T. vaginalis*. *P. hominis* was not observed in any of the examined women. Only in one instance was the flagellate *Enteromonas hominis* and, in one other, *T. vaginalis* isolated from the stool. In central Bohemia, *P. hominis* has been recorded only from children's homes (age limit 6 years). In central Europe, the incidence of *P. hominis* infection in adults is so low that it may easily be disregarded in view of the high hygienic standards of the present. The findings of *T. vaginalis* and *P. hominis* are not explicitly connected. De Carneri and Giannone (1964) statistically studied the eight possible combinations of the three infections (*T. vaginalis* 30·2%; *T. tenax* 43·3%; *Entamoeba gingivalis* 37·6%) of 367 North Italian women, but did not reveal any relationship of association or exclusion among the three parasitoses.

#### IV. CULTIVATION OF *T. vaginalis*

After it became possible to cultivate bacteria-free strains of *T. vaginalis* by adding penicillin and streptomycin to the media (Adler and Pulvertaft, 1944; Johnson and Trussell, 1944; Jírovec and Peter, 1948; Magara *et al.*, 1953; etc.), cultivation has become easy and cultures can be used standardly even when diagnosing latent trichomoniasis and controlling therapeutic results. *T. vaginalis* grows under anaerobic conditions when a native serum is present. The redox potential can be decreased by adding L-cysteine-chloride or Na-thiogluconate; the pH of the medium is adjusted to 5–6. Glucose or maltose are used for supplying the source of energy. An additional 0·1% of agar stabilizes the cholesterol suspension and slows down oxygen diffusion. Bacteria-free cultures are obtained by adding mostly 400–1000 units of penicillin and 100–1000 µg of streptomycin per 1 ml of culture fluid. Any yeasts occurring in the original material can be removed within 24–72 h by adding 300 γ myco-statin/ml (see Honigberg, 1957). The most frequent media used are Johnson's CPLM, and the Vf bouillon after Magara *et al.* (1953), after Diamond (1957), after Feinberg (1953) and after Roiron-Rattner (1957, 1958), either in the

original formula or in various modifications. Another improvement is the use of Hall's test tube with an attenuation in the posterior one-third of its length (Fig. 10b); a glass ball, stoppering this attenuated portion, to some extent prevents the diffusion of oxygen from above, providing conditions for better and prolonged growth of the trichomonads under anaerobic conditions. The number of *T. vaginalis* is 10 000–1 000 000 per ml of culture fluid. However, it is not always possible to obtain a free isolate (Holečková-Červová, 1960, records positive results in only about 60–70%), and also pure strains have

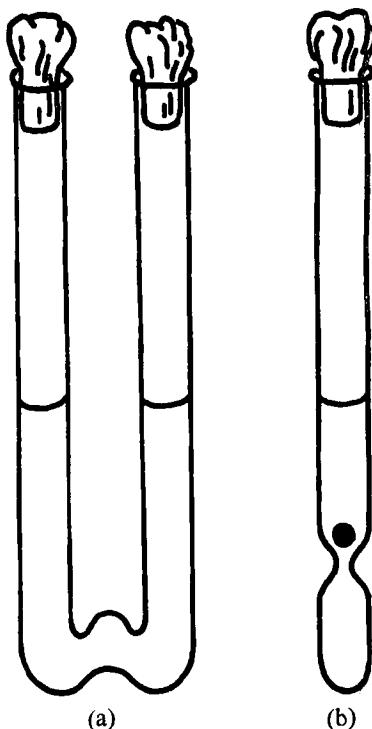


FIG. 10. Culture tubes: W-shaped tube (after de Carneri) and single tube (after Hall).

been found to die for unknown reasons after several months. The same author observed differences in the growth of various strains of *T. vaginalis*, when cultured e.g. in the Vf-bouillon with 10% rabbit serum; some of the strains grow constantly in coarse flakes along the posterior half of the fluid column, others in vertical or diagonal lines.

When used for diagnosis the culture must be observed for 10–12 days every 24 h (Teras *et al.*, 1963). For further breeding the culture must be reinoculated after 3–5 days at a temperature of 35–37°C. Sometimes it is difficult to remove the bacteria. De Carneri (1956) used for this purpose a W-shaped tube (20 × 1 cm) (Fig. 10a) filled under sterile conditions with 15 ml of the CPLM medium,

10% human serum and 1000 units of penicillin and 1000 µg streptomycin per ml. The contaminated material, containing trichomonads, fungi and bacteria, is seeded into one arm of the "W" tube. The agar in the medium prevents conversion streams and the fungi are confined to develop in the upper part of the other arm, while the trichomonads penetrate the whole medium. After a 2-day incubation period at 37°C, some drops are removed with a pipette from the second arm and transferred to the same medium in a normal tube.

By increasing oxygen tension it has been possible to induce the formation of multinucleated cells in a very high proportion of 50–80% "giant somatella" (Wirtschafter, 1954). Inoculation into a flask containing 100 ml of medium at 37°C shows after 48 h an oxidized state with reference to methylene blue indicator in the CPLM medium. Such culture incubated under reduced oxygen tension produces, in 48 h, a completely normal culture without giant cells.

Many investigators have cultivated *T. vaginalis* on solid media (Magara *et al.*, 1953; Wirtschafter, 1954; Asami and Nakamura, 1955; Filadoro and Orsi, 1958; Ivey, 1961; Samuels, 1962). The solidity of the culture (CPLM, Diamond etc.) is obtained by an addition of 1–2% of agar; anaerobiosis can be achieved either by cultivation on Fortner plates with *Serratia marcescens*, by feeding the medium with N<sub>2</sub> or CO<sub>2</sub>, or by the absorption of oxygen through a mixture of pyrogallol–NaOH. The colonies attain a size of 0·5–2 mm and remain viable in e.g. a N<sub>2</sub> atmosphere for about 11 days. Colonies of 1 mm diameter are composed of about 100000 trichomonads. The advantage of cultures on solid media is the longer viability of the flagellates, the possibility of better isolation from the clones and an easier observation of the inhibition by various *Trichomonas* controlling preparations.

Schoenherr (1958) developed a method for quick identification of the number of cells in *T. vaginalis* and *T. foetus* cultures. He determined the amount of sediment after centrifugation in graduated pipettes under constant g-figure and standard times. Lash's modified nutritive medium with addition of liver proved to be significantly superior. The vaginal secretion with trichomonads contains a "growth factor" for *T. vaginalis*, which is favourable for cell reproduction also in more diluted and normally unsuitable nutritive media. The generation time of *T. vaginalis* was found to be approximately 3·88 h, for *T. foetus* 3·47 h. Axenic culture of *T. vaginalis* in hens' eggs incubated for 11 days was achieved by Müller (1967).

The resistance of *T. vaginalis* to changes in temperature was studied by different authors. Whittington (1951b) found that in temperatures fluctuating between 5·9–15·5°C two strains survived for 3 days but no longer. Another strain resisted 9·2–10·6°C for 2 days and another 2·2–8·9°C only 1 day. In vaginal exudate diluted with water 1:1 one strain survived 4·4–6·7°C only 2 days, another strain in undiluted vaginal exudate –4–0°C for 2 days. MacEntegart (1954, 1959) conserved *T. vaginalis* in culture with 5% glycerol for 26 months at –79°C, and with 5–15% dimethylsulphoxide for 35 months at –170°C.

Stabler *et al.* (1964) found that *T. vaginalis* and *T. gallinae* kept their original pathogenicity for natural and experimental hosts and for tissue cultures for many months in glycerol-containing media at below –70°C in dry ice. Typi-

cally only a small percentage of the flagellates survived. Honigberg *et al.* (1965) conserved both species in the Diamond medium in the presence of dimethylsulphoxide in liquid nitrogen.

Diamond *et al.* (1965) conserved axenically cultivated *T. vaginalis* in the presence of 5% dimethylsulphoxide by freezing and then stored at -170°C in vapour above liquid nitrogen. After 2 years, thawed and subcultured samples of trichomonads showed no significant differences between these organisms and the ones tested before freezing, in view of the virulence for mice after i.p. inoculation.

Pray (1952) divided the bacteria influencing the growth of *T. vaginalis* into three groups:

- (1) Those prolonging the life of the culture beyond that of bacteria-free controls (*Staphylococcus aureus* and *S. albus*).
- (2) Those having a moderately inhibiting effect (*Brucella suis*, *Streptococcus lactis*, *Pseudomonas fluorescens*, *Alcaligenes faecalis*, *Sarcina lutea*, *Bacillus subtilis*).
- (3) Those which greatly curtail multiplication of the flagellate and the life of the culture (*Escherichia coli*, *Aerobacter aerogenes*, *Pseudomonas aeruginosa*, *Salmonella schottmuelleri*, *Proteus mirabilis*, *Salmonella paratyphi*).

Filtrates of bacterial cultures gave no indication that specific metabolites or antagonistic substances were responsible for the effects observed. Maltose and glucose were found equally effective for prolonging the life of *T. vaginalis* cultures in the presence of bacteria which had an inhibiting effect. Changes in the pH and in the oxidation-reduction potentials gave no indication that the effects of bacteria were due to such factors. Other studies on this point were made by Sorel (1954), de Carneri (1956), Feo (1958) and others.

A 2-3-day old *T. vaginalis* culture grown in the CPLM medium after Hitchcock (1948) produced no inhibition of different *Salmonella* sp., *Aerobacter aerogenes*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Shigella* sp. and *Staphylococcus aureus*. *T. foetus*, however, produced inhibition of *Salmonella pullorum*, *S. schottmuelleri* and *Corynebacterium renale*.

## V. BIOCHEMISTRY OF *T. vaginalis*

Exact biochemical investigation of *T. vaginalis* becomes possible only after the development of a method for cultivating this species in bacteria-free cultures. The first experiments were performed by Trussell and Johnson; later the problems were pursued by Kupferberg, Baernstein, Asami and many other investigators. Yet our present knowledge of biochemistry is still fragmentary.

*T. vaginalis* is generally considered to be an anaerobic protozoan parasite; it contains no cytochrome c, no mitochondria and is adversely affected by a high oxygen tension; yet under certain conditions it does take up oxygen. It is capable of utilizing a number of polysaccharides, but only those which contain an  $\alpha$ -1·4-glycosidic linkage provide those properties essential for good growth (Wellerson and Kupferberg, 1962).

*T. vaginalis* uses glucose as one of the essential sources for the production of energy-rich compounds necessary for living and multiplication. Growth in the culture is markedly stimulated by an addition of glucose, fructose, maltose, glycogen and dextrin, and considerably stimulated by sucrose and soluble starch; lactose and galactose did not stimulate multiplication (Asami, 1956). The amount of glucose consumed by the multiplying organisms (from 9000 to 3000000 individuals) was 4.5 mg; lactic acid was produced in an amount of about 20 mg/100 ml during 96 h incubation. In *T. vaginalis*, succinic-, malic- and citric-dehydrogenases have been identified, indicating the probable existence of a TCA cycle during metabolism. Dehydrogenase activities are strictly inhibited by monooiodoacetate; cyanide and malonate had no effect upon the activities. While in *T. vaginalis* catalase activity was found to be low, it was high in *T. foetus* and *T. gallinae* (Asami, 1956). Washed intact cell preparations of *T. vaginalis* oxidize pyruvate and malate. They are unable to utilize other intermediates of the Krebs cycle. Attempts to inhibit pyruvate oxidation with such Krebs cycle blocking agents as malonate, arsenite, parapyruvate and fluoroacetate, were unsuccessful. The Krebs cycle is not the pathway for the oxidation of pyruvate in *T. vaginalis* (Wirtschafter *et al.*, 1956). The principal product of anaerobic glycolysis—lactic acid—was produced in high concentration (about 40% of the total amount of acids). *T. vaginalis* had a high glycogen content—17% of the dry weight of the cell. *T. vaginalis* as an anaerobic protozoan parasite is one of the few nonchlorophyll-containing Protozoa capable of fixing CO<sub>2</sub>. The entire amount of radioactive CO<sub>2</sub> supplied during the course of growth was found to be fixed in lactic acid. No significant portion of radioactivity remained within the cell. Labelled CO<sub>2</sub> appeared only in the carboxyl group of lactate and none was found in malate or succinate (Wellerson *et al.*, 1959). This seems to exclude the possibility of CO<sub>2</sub> being incorporated by pyruvate carboxylation. In the degradation of glucose, through the various phosphorylated intermediates ending with pyruvate, which is subsequently converted to lactic acid, the classical Embden-Meyerhof scheme seems to be followed. No cytochrome C is present in *T. vaginalis*. Cyanide resistance of the respiration does not suggest the participation of cytochrome oxidase.

Glycolytic enzymes were most studied in *T. vaginalis* (Baernstein, 1955; Wirtschafter, 1954; Wirtschafter and Jahn, 1956; Kupferberg, 1960; Wellerson and Kupferberg, 1962). In cell-free extracts prepared from mass cultures of *T. vaginalis* were found aldolase, lactic acid dehydrogenase, trioso-phosphate dehydrogenase, trioso-phosphate isomerase, phosphoglucoisomerase, phosphofructokinase, phosphoglucomutase, pyruvate kinase system, and hexokinase. Phosphorylase and alcohol-dehydrogenase were not demonstrated. Functional sulphhydryl groups remain active in KCl-solution for a month or more when stored at 2°C (Baernstein, 1955).

*T. vaginalis* grown aerobically after Kunitake *et al.* (1962) slowly metabolizes uniformly labelled glucose-U-C<sup>14</sup> and succinate-2,3-C<sup>14</sup> to CO<sub>2</sub> and to amino-acids which are then incorporated into protein. Analysis of protein hydrolysates from cells grown on glucose-U-C<sup>14</sup> reveals radioactivity in 15 amino-acids. A tricarboxylic acid cycle seems to be operating.

The consumption of oxygen is 162 mm<sup>2</sup> for 100 millions flagellates per hour

—the same number of *T. foetus* consume 215 mm<sup>3</sup> per hour and *T. gallinae* as much as 600 mm<sup>3</sup>.

*T. vaginalis* is not only capable of metabolizing amino-acids but appears to be able to achieve the *de novo* synthesis of nearly all of its amino-acids. The purified DNA from *T. vaginalis* reveals an adenine-thymine type DNA, one which is different from that of most other flagellates (Wellerson and Kupferberg, 1962).

Native serum seems to be indispensable for *T. vaginalis*. Sprince and Kupferberg (1947) obtained from human serum two fractions, both of which are necessary—one fraction soluble in ether, the other soluble in water. It seems that linoleic acid is the active substance necessary for growth. Pantothenic acid is also necessary (Kupferberg *et al.*, 1948).

Cystein and Na-thioglycolate reduce the redox-potential of the medium and are added to the used media. Ascorbic acid, glutamic acid and choline caused a stimulation of the cell multiplications, the first probably by lowering the redox potential of the medium. DL-N- $\langle\gamma$ -glutamyl $\rangle$ -ethyl-amine acts as a comparative antagonist, probably of glutamine rather than of glutamic acid, and it seems that glutamine is obligatory for the development of the culture of *T. vaginalis* (Back *et al.*, 1950). Cortisone and hydrocortisone inhibit the endogenous respiration and simultaneously the oxidation of Na-succinate and fructose. Oestrogen hormones do not influence the growth in culture (Kupferberg and Johnson, 1941).

Analysis of the purified DNA of *T. vaginalis* by thermal denaturation and density gradient centrifugation shows both to be quite rich in adenine and thyamine. *T. vaginalis* and *T. gallinae* are found to have the AT-type of DNA and to display considerable compositional heterogeneity.

Iyori (1959) follows the protein-N, rest-N, amino-N and ammonia-N in the cultures of *T. vaginalis*. The change in the amount of various nitrogen fractions in the culture media during the growth of *T. vaginalis* was very small compared with that of carbohydrates. The decomposition of protein seems to be more active in the period of cultivation when the number of organisms decreases, than in the early period when they actively multiply. This is probably due to the enzymes liberated from the destroyed organisms.

Ninomiya and Suizuoki (1952) demonstrated on washed suspension cells of *T. vaginalis*, by manometric methods, that glucose and maltose are rapidly oxidized whereas pyruvate and lactate are metabolized at half the rate of the sugars. Succinate, citrate, fumarate, acetate, butyrate, allanine, glutamate and gluconate were not suitable substrates. According to Magara *et al.* (1953) their strains of *T. vaginalis* were not capable of utilizing inuline, glycerine, mannite, dulcite and saracine, and utilized saccharose and rhamnose only in a small degree, whereas good utilization of glucose, galactose, levulose, maltose and glycogene was observed. *T. vaginalis* cannot produce haemolysis, indol, or H<sub>2</sub>S, nor liquefy gelatine, produce catalase or decomposed urea. Kupferberg *et al.* (1953) were unable to demonstrate the production of any gas other than CO<sub>2</sub> in any appreciable amount.

*T. vaginalis* has a limited capacity for oxygen utilization, which is sensitive to cyanide and results in hydrogen peroxide accumulation except when catalase

is also present. A flavoprotein terminal oxidase is indicated, but the importance of the oxygen utilization is not known.

Riboflavin and flavin mononucleotide were isolated from *T. vaginalis*, the first in a concentration of 75 mg/g of cell dry weight (Kupferberg, 1960). Some strains produced hydrogen gas which is probably linked to electron transport. The organisms are essentially anaerobic and therefore depend upon coupled reactions mediated by pyridine- and flavoproteins resulting in the production of reduced compounds (Baernstein, 1963).

*T. vaginalis*, *T. foetus* and *T. gallinae* show cyanide and azide insensitive respiration. Wellerson *et al.* (1959) isolated considerable amounts of riboflavin from *T. vaginalis*. *T. vaginalis* cannot oxidize all intermediates of the citric cycle, though *T. gallinae* is able to do so. Oxygen uptake in these three species of trichomonads is probably mediated by flavoproteins. There is no doubt that conventional glycolysis is an important system in their metabolism supplemented with other systems linked to NADP and to succinate to furnish electron donors. Electron transport in anaerobic metabolism is limited to dehydrogenase coupling and to the excretion of the reduced compounds (Baernstein, 1963).

Sonic homogenates of *T. vaginalis* contain a metal activating aldolase. Cobaltous and ferrous ions are effective—their action is greatly enhanced by cysteine or thioglycolate. Ethylene-diaminetetraacetate is more effective as inhibitor than dipyridyl at pH 7. The inhibition may be completely reversed with cobaltous or ferrous salts. The optimum pH for aldolase activity is about 7 and the Michaelis constant is  $0.25 \times 10^{-3}$  M hexose-diphosphate. Aldolase in *T. vaginalis* is a soluble enzyme. Glucose seems in some degree to have restrained the decomposition of proteins which is used as a source of energy. By paper chromatography 11 amino acids were detected in culture media: aspartic acid, glutamic acid, taurine, glycine, threonine, tyrosine, alanine, arginine, valine, leucine and proline. The same applied to material obtained from the control from uninoculated culture media. *T. vaginalis* showed the ability of deamination, though quite small in amount with cysteine, histidine, arginine and methionine.

Kojima has demonstrated the presence of histamine in the vaginal contents of patients infected with *T. vaginalis*. The experiments of Iyori (1959) showed that in culture *T. vaginalis* did not produce histamine and that it had no decarboxylase to produce histamine from histidine.

Ludvík *et al.* (1961) investigated *T. vaginalis* with cytochemical methods (Fig. 11). The DNA is present only in the nucleus as fine granulations at the periphery of the membrane (detected by the Feulgen nucleal reaction and the staining with methyl green). The endosome contains no DNA and is always Feulgen-negative. RNA was detected with pyronine and toluidin blue (after Brachet) as small granules near the nucleus and the axostyle. It is possible to dissolve all RNA with ribonuclease. The silver-impregnation with protargol (after Bodian) coloured the whole locomotion apparatus, the undulating membrane, the costae, the endpoint of the axostyle and also the nucleus. PAS positive granules can be found in the anterior half of the body and along the axostyle, some are also distributed in the plasma. Best's carmine-stained

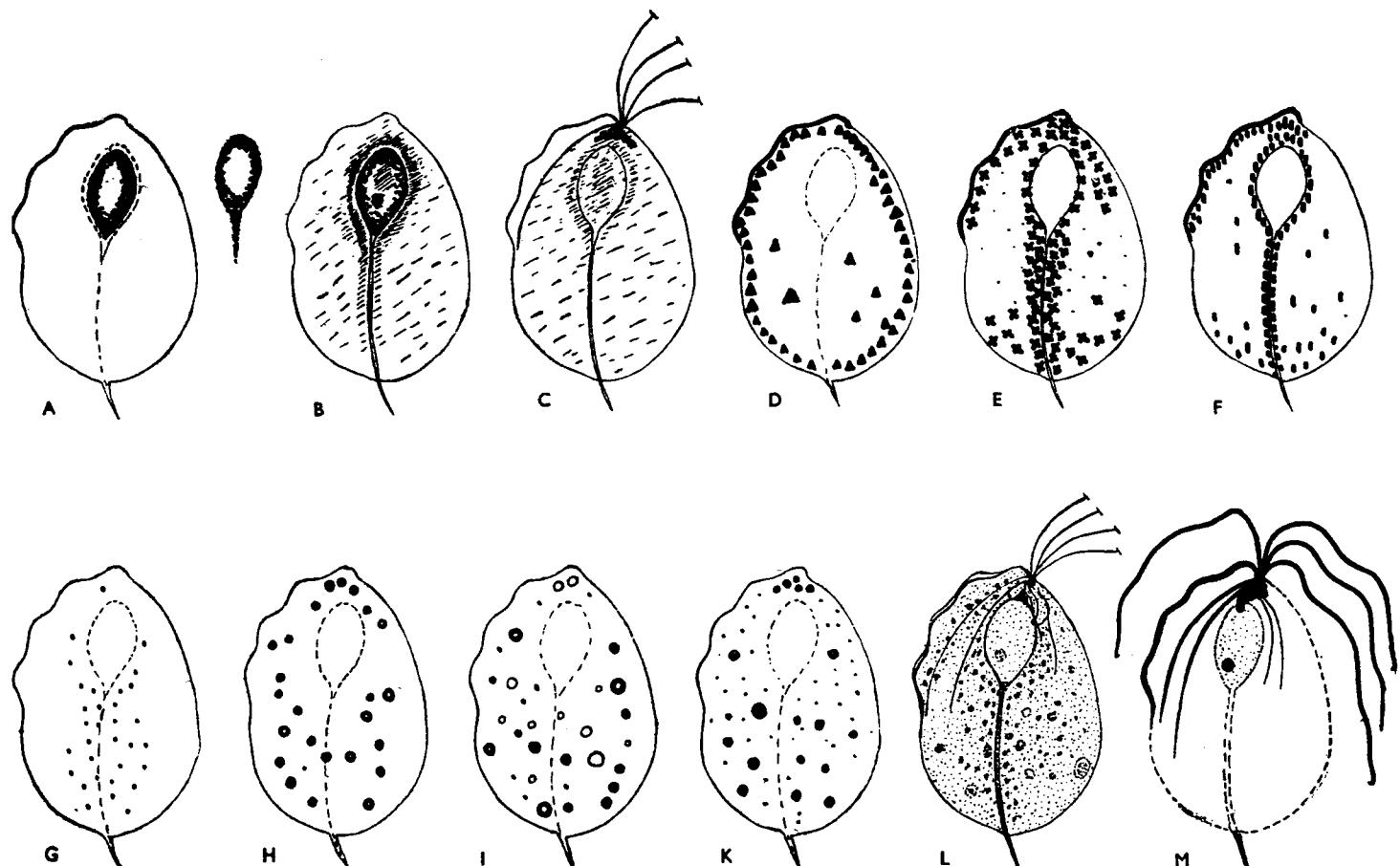


FIG. 11. Some cytochemical reactions on *T. vaginalis*. A: Feulgen nucleal reaction (DNA). B: Brachet staining (RNA-DNA). C: Gram staining. D: Best staining (glycogen). E: PAS staining (MacManus-Hotchkiss). F: Halle staining. G: Ebel staining (volutine). H: Oil red. I: Nile blue sulphate. K: Baker staining. L: Giemsa-Romanowski method. M: Bodian protargol impregnation. (After Ludvík *et al.*, 1961.)

glycogen concentrated mainly under the cell membrane. Solitary glycogene granules are distributed irregularly throughout the plasma. Acid mucopolysaccharides (after Hall) found at these sites are not so plentiful as the PAS positive substances. Lipids are present as small droplets in the plasma; they can be stained with Oil-red O, Sudan III, Sudan IV and Nile blue sulphate.

## VI. SEROLOGY AND IMMUNOBIOLOGY OF *T. vaginalis*

Serological and immunobiological investigations have been only of theoretical interest until the present, because the finding of *T. vaginalis* by cultivation or direct microscopical examination is easier and more reliable. In mostly symptomless trichomoniasis of man, however, such investigations could be important practically speaking. It seems that the divergences reported by different authors, may be due to the antigenic structure of the individual *T. vaginalis* strains. The height of titres also seems to have no connection with the clinical symptomatology (Korte, 1958).

### A. THE COMPLEMENT FIXATION REACTION (CFR)

Riedmüller (1932) was the first to develop weak titres in the CFR in guinea-pigs by repeated i.p. injections of vaginal discharges containing *T. vaginalis*. Wendelberger (1936) used an alcoholic extract from *T. vaginalis* suspension as antigen for the CFR. 68% of the 32 examined women with clinical trichomoniasis were positive, 16% without symptoms negative. Trussell *et al.* (1942), using an aqueous antigen, found a positivity of CFR in 42·3% of 110 women with *T. vaginalis* and in 16·5% of 290 women without *T. vaginalis*. Trussell (1947) wrote: "... no practical importance is now attached to the demonstration of CFR. Certainly the test is of no diagnostic value and it remains to be seen whether there is any correlation with spontaneous or therapeutic cure".

New, more promising investigations were performed by Hoffman *et al.* (1966). Using the CFR in the quantitative technique of Kolmer, he found a positivity in 80% of adult female and in 40% of adult male patients, both infected with *T. vaginalis*, the control groups of both sexes showing positive reactions only in 9–10% of the cases. Patients with chronic trichomoniasis showed a higher positivity (95%) than that found in persons at the acute stage (53·8%). The same could be observed in the males—in chronic stages the positivity was 57%, in acute stages 28·5%. Jaakmees *et al.* (1966) used 8 antigens of a serotype and found no positive CFR in the control group. One hundred and seventy patients with *T. vaginalis* showed complete or strong positive CFR with the antigen of at least one serotype. The authors claimed the necessity of using simultaneously antigens from different serotypes. The only serum in which a partial CFR was observed had been obtained from a woman infected with *T. vaginalis* only 2 weeks before the examination. The presence of CF-antibody as well in the blood of *T. vaginalis*-negative sexual partners of patients infected with trichomoniasis could be due to the fact that regardless of repeated negative examinations, these people might still have been infected with *T. vaginalis*. After treatment with metranidazol and the disappearance of

*T. vaginalis* no later than the fourth day of treatment, followed by a rapid regress of clinical symptoms, the CFR in 24 women showed a continuous decrease in the titre beginning at the third month after treatment; in most cases antibodies disappeared completely from the blood within a year at the most. Similar results were also obtained by studying the dynamics of CFR in 16 male patients treated with metranidazol. There is no explanation why CF-antibodies disappear from the blood of some people considerably sooner than from others.

#### B. AGGLUTINATION AND AGGLOMERATION REACTIONS (AR)

Tokura (1935) probably first indicated the formation of agglomerating and killing antibodies in rabbits inoculated with repeated i.v. injections of *T. vaginalis* mixed cultures killed with formalin. Trussell (1946), studying the development of agglutinins in rabbits injected with axenic cultures of *T. vaginalis*, found macroagglutination less satisfactory than microagglutination. The highest titres obtained were 1:5000 to 1:10000. MacDonald and Tatum (1948) obtained specific antisera against *P. hominis*, *T. vaginalis* and *T. foetus* by injecting formalinized whole cells for demonstration of agglutination and agglomeration of these trichomonads. *T. vaginalis* and *P. hominis* were antigenically identical also in cross-reaction at the same titre. Specific adsorption of antisera by either *T. vaginalis* or *P. hominis* removes the antibody for both. *T. foetus* was antigenically different. Tatsuki (1957), using the agglutination test, obtained positive reactions in 84.4% of infected women, the mean agglutination titre being 1:274. In the control group without *T. vaginalis* the positivity was also high, 52.9%, but the titre relatively low (1:30). The lysis of *T. vaginalis* in sera from non-infected women was positive in 60% of the cases, from infected women in 57%. The author states that trichomonas cannot be diagnosed by the agglutination test because *T. vaginalis* is also agglutinated by blood sera of uninfected persons. Also Lanceley (1958) demonstrated the presence of an agglutinating body against *T. vaginalis*. From 20 strains of *T. vaginalis*, 18 were agglutinated in an immune serum and 2 were not. From 10 strains tested against 2 antisera prepared from different specimens, 6 were agglutinated by the one and 4 by the other, but no strain by both antisera. The titre in the immune serum was 1:320, in the control 1:40. Stepkowski and Bartoszewski (1959) obtained positive results using the agglutinin test in 60% of positive females, using the CFR, in only 30%.

Very important investigations were conducted by Teras and his collaborators. Teras (1961) reported that normal human serum contains agglutinins for *T. vaginalis* in titres 1:40–1:80, also normal rabbit serum in titres 1:20–1:80. Therefore, the titre up to 1:80 cannot be regarded as specific; on the other hand the CFR may be regarded as specific. Teras (1959, 1961, 1966) succeeded in finding a reliable explanation: the agglutinin titre of the sera depends largely on the strains of *T. vaginalis* and of its serotype; one serotype gave 3–4 times higher agglutinin titres than the other. In people without contact with *T. vaginalis*-infected patients the agglutinin titre with an antigen never exceeded the titre 1:160, which is regarded as the limit titre of normal agglutinins. In

TABLE II

*Review of the results of agglutination reaction in females and males suffering from trichomoniasis  
(After Teras et al., 1966)*

Clinical form of tricho- moniasis	Number of patients	Female			Male			
		Agglutinin titre			Number of patients	Agglutinin titre		
		1:480 and higher	1:200– 1:400	up to 1:160		1:480 and higher	1:200– 1:400	up to 1:160
Acute	44	31	13	—	—	—	—	—
Subacute	46	34	12	—	12	10	2	—
Chronic	70	55	15	—	39	22	17	—
Latent	11	11	—	—	32	15	17	—
Total	171	131	40	—	83	47	36	—

patients suffering from trichomoniasis the titre was 1:200–480 and higher (Table II). Specific agglutinins were demonstrable in nearly all men and women suffering from trichomoniasis. This was only due to the fact that the AR was used simultaneously with all serotypes (Table III). Within a year after recovery from trichomoniasis in the male and within 16 months in the female, the agglutinin titre decreased in nearly all cases to its limit in normal blood (1:160). The rate of decrease seems to depend neither on the clinical form of trichomoniasis nor on the initial titre. This supposition was confirmed by Nigesen (1963). There seems to be a temporary infection immunity in the case of genito-urinary trichomoniasis (Teras *et al.*, 1965).

TABLE III

*Dependence of results of agglutination reaction on the serotype of the antigen of T. vaginalis in cases of genito-urinary trichomoniasis (After Teras et al., 1966)*

Clinical form of tricho- moniasis	Number of patients	Female				Male			
		Serotypes			Number of patients	Serotypes			
		TN	TLR	TRT		TR	TN	TLR	TRT
Acute	44	26	22	30	26	—	—	—	—
Subacute	46	28	23	32	29	12	5	5	8
Chronic	70	50	39	53	42	39	17	12	21
Latent	11	10	8	6	8	32	16	12	16
Total	171	114	92	121	15	83	38	29	45

In further experiments, Teras (1961) vaccinated 16 rabbits i.v. with living cultures of *T. vaginalis* 3–4 times in 10-day intervals, and i.v., i.m. or s.c. with cultures killed by heating. The highest agglutinin titre (up to 10 000) was found in rabbits vaccinated with living cultures. After injection of killed trichomonads the titre was up to 1:5 000. It seems most probable that *T. vaginalis* causes only a non-sterile immunity in rabbits, which vanishes soon after the disappearance of the infective agent from the organisms.

Summarizing the investigations by Teras *et al.* (1961–1966), the results obtained in the diagnosis of genito-urinary trichomoniasis by means of CFR and AR can be considered reliable only in cases in which serotypes most common in the given area are used as antigens in both reactions.

#### C. THE INTRADERMAL TEST (IDT)

The results of the IDT with specific antigen prepared from pure *T. vaginalis* were unsatisfactory in cases of genito-urinary trichomoniasis (Adler and Sadowsky, 1947; Lanceley, 1958), because positive reactions were obtained not only from patients suffering from trichomoniasis, but also quite often from persons with no detectable *T. vaginalis* infection. Adler and Sadowsky (1947) found positive reactions in 81% of the Trichomonas-group and in 23% of the control group, without correlation between the severity of the symptoms and the intensity of the skin test). Better results were obtained by Kawai *et al.* (1961), who noted that the IDT becomes positive about 2 weeks after the onset of infection. Two extracts were obtained from *T. vaginalis*, one without specific characteristics, the other, identified by 3 spots on 2-dimensional paper chromatography as being composed of some polysaccharides, causing a specific IDT in women with *T. vaginalis* infection. The IDT was performed on the forearms of 530 out-patients and was considered positive if the area of erythema attained more than 10 mm diameter. An erythema of more than 18 mm always indicated the presence of *T. vaginalis*. The area of erythema decreased with treatment and the test became negative when the trichomonas disappeared from the smears. Therefore this skin test has a diagnostic value for *T. vaginalis* infection.

Jaakmees and Teras (1966) obtained better results with corpuscular antigen than with antigen-lysates, confirming the conclusions drawn by Anita-Radtchenko (1959) and Sinelnikova (1961). The corpuscular antigen prepared from the serotypes TLR, TN, TRT and TR caused either reddening or swelling in every person in the control group (20 men and 20 women), but those symptoms were demonstrable in all women (41) suffering from trichomoniasis. Therefore the IDT can be recommended for the diagnosis of genito-urinary trichomoniasis, although the specific allergy does not depend on the content of CF- and AR-bodies in the blood serum. It is evident that the CFR and AR can be substituted by the IDT and vice versa in the diagnosis of trichomoniasis. The best IDT is obtained with the serotypes TR and TLR. Higher allergic reaction occurred more frequently in women with acute or subacute trichomoniasis, less often in women with chronic and latent forms (Table IV). A positive reaction could also be obtained from persons negative for *T. vaginalis*,

TABLE IV

*Dependence of results of the intradermal test on the clinical form of trichomoniasis in the female and male  
(After Jaakmees and Teras, 1966)*

Clinical form of trichomoniasis	Number of patients	Intensity of reaction			
		++ or +++ for females, only ++ for males		+	
		+	0		
Female	acute and subacute	75	21	38	16
	chronic and latent	51	6	26	19
	Total	126	27	64	35
Male	acute and subacute	8	3	2	3
	chronic	26	7	10	9
	latent	28	1	13	14
	Total	62	11	25	26

but this occurred only in cases where the sexual partners of the patients were ill with *T. vaginalis* (latent stages or infections of some time ago).

#### D. OTHER SEROLOGICAL REACTIONS

*Haemagglutination test (HT):* Lanceley and MacEntegart (1953) were not able to find a positive HT in experimentally infected women.

*Immunofluorescent reaction* was employed by MacEntegart (1958) and later by Kučera and Kramář (1965). In the indirect Coons method sera positive results were obtained from 17 women and 2 men suffering from trichomoniasis at various stages, while sera of the 6 negative persons were also negative.

*Protection test (PT):* in the experiments by Teras (1961) the PT of 19 blood sera, after injections of mice with a pure culture of *T. vaginalis* s.o. from patients with vaginal trichomoniasis, shows two distinct groups: the first comprises 11 sera with a high protective effect (in the injected mice in general *T. vaginalis* did not cause any pathological changes, or they were very slight). In the second group of 8 sera a weaker protective effect was observed, but compared with the unprotected controls the pathological changes in the protected mice were slighter. There was no link between the protective effect of the investigated sera and the results of CFR, between the degree of the protective power and the clinical form of trichomoniasis, the duration of the infection or the age of the patients. In his following work Teras (1963) found

no differences in the PT in mice with antisera from strong virulent and low virulent strains of *T. vaginalis*. Testing the pathogenicity of different serotypes of *T. vaginalis* on monocellular cultures, Teras and Tompel (1963) could find no differences. Only immediately after the isolation from patients was the effect considerably higher.

Vava (1958) immunized mice with three injections at 4-day intervals of heat-killed *T. vaginalis* cultures, followed by an injection of living *T. vaginalis* cultures into the hindleg: only in 24% of the immunized animals were *Trichomonas* abscesses formed, in contrast to 100% in the unimmunized animals. After an immunization with *T. gallinae* and *T. vaginalis*, abscesses occurred in 43% of mice in the first case and in 47% in the second case. This shows that a partial immunity to *T. vaginalis* develops even after an immunization with *T. gallinae* and *T. foetus*.

The *mucous-agglutination reaction* (MAR), after Rom and de Thiery (1958), with the mucous content of vagina from infected women, gives no applicable results. This is an important difference between *T. foetus* and *T. vaginalis* (Florent, 1938).

Weld and Kean (1958) described in the human serum a *cytolytic factor*, destroying *T. vaginalis* in a few minutes. This factor is inactivated by heating for 30 minutes at 56°C. In the experiments of Reisenhofer (1963) all of the examined human sera as well as the sera from horses, cows, dogs, sheep and pigs showed the property of damaging *T. vaginalis*. Their cytolytic powers are reduced by storage and inactivation. While most of the human sera will not agglutinate *T. vaginalis*, the latter will be agglutinated by all the animal sera examined, mostly in the dilution of 1:32 (1:16:164). Samuels and Chun-Hoon (1964) showed that active normal sera or other body fluids from amphibians, reptiles, birds and mammals (e.g. chicken egg yolk, yolk-sac contents, human ascitic fluid and cow milk) cytolise *T. angusta*. Heat-inactivated sera only agglutinated this species.

The *Sabin-Feldman reaction* (SFR) on toxoplasmosis is highly specific for toxoplasmosis and there is no cross-reaction in women infected with *T. vaginalis* (Piekarski *et al.*, 1957). The opinion of Michalzyk (1953) that the SFR is unspecifically positive in such cases has not been confirmed. There is no relationship between toxoplasma and trichomoniasis. Fuchs *et al.* (1964) studied the possible influence of trichomoniasis on the results of toxoplasmin tests in 722 women. No statistically significant difference was found in the incidence of positive tests in infected and non-infected groups of women. CFR on toxoplasmosis in 294 women had the same negative results.

#### E. DIFFERENT SEROTYPES OF *T. vaginalis* IN LABORATORY ANIMALS

Schoenherr (1956), using AR, CFR and precipitation, distinguished not only *T. vaginalis* from *T. hominis*, *T. gallinae* and *T. foetus*, but also some serotypes in *T. vaginalis*. Kott and Adler (1961) proved by simple cross-agglutination and cross-adsorption tests that *T. vaginalis* is not a serological homogenous species, but consists of a number of distinct serotypes, though all

strains have agglutinogens in common. Eight distinct serotypes were found among 19 strains of *T. vaginalis*. The results of both reactions were constant during an observation period of 2½ years. Two strains seem to have changed their antigenic structure after being contaminated with bacteria. Two distinct serotypes could easily be distinguished among 5 strains of *P. hominis*—the serotype I had some agglutinogens in common with *T. vaginalis*, but not the serotype II. Sera prepared against 3 strains of *T. tenax* did not agglutinate any of the strains of *T. vaginalis* and *P. hominis* examined. Specific sera against *T. tenax* contained two types of antibodies: one agglutinating the flagellates and one paralysing their flagella.

Hoffman and Gorczynski (1964), using AR, HR and CFR, could distinguish in the 23 strains of *T. vaginalis* the existence of several serological types. Thirteen strains had the same antigenic structure as that of the three strains used for preparations of the antigens; 5 strains were comparable to that of another TA strain; and the remaining 5 strains were serologically different from all used strains.

TABLE V  
*Distribution of typified strains of T. vaginalis according to clinical forms of trichomoniasis*  
(After Teras et al., 1966)

Clinical form of trichomoniasis	Total strains	Serotype of <i>T. vaginalis</i>			
		TN	TLR	TRT	RT
Acute	9	1	—	2	6
Subacute	25	3	5	8	9
Chronic	45	4	4	16	21
Latent	20	6	4	3	7
Total	99	14	13	29	43

Teras (1959, 1962, 1963, 1965), Nigesen (1963) and Jaakmees (1965) found four additional different serotypes, designated TR, TN, TRT and TLR (this designation is not a good one). The distribution of these serotypes in 99 patients is shown in Table V. Of 100 isolated *T. vaginalis* strains, 43% belonged to the serotype TR, 29% to TRT, 14% to TN and 13% to TLR. It is interesting that in 32 cases of the 34 married couples, the strains isolated from both spouses belonged to the same serotype. In the two not corresponding cases the husbands had extramarital intercourse. Table VI shows that none of the four serotypes was found to be associated with only one particular clinical form, neither could any correlation between the antigenic properties and the virulence be observed. Only in the AR and CFR could the authors find differences in the intensity of these reactions. All four serotypes have an antigenic component in common.

TABLE VI

*Dependence of complement fixation and agglutinin reaction on the serotypes in patients infected with the serotypes TR, TRT, TN, or TLR of Trichomonas vaginalis*  
*(After Teras et al., 1966)*

	Serotypes of <i>T. vaginalis</i>	Total strains	With serotype				With homologous serotypes	With all serotypes
			TR	TRT	TN	TRL		
Complement fixation	TR	43	43	32	33	33	43	24
	TRT	29	24	29	23	24	29	29
	TN	14	12	12	13	13	13	11
	TLR	13	9	12	11	13	13	9
	Total	99	88	85	90	93	98	64
Agglutination 1:320 and higher	TR	43	43	30	27	24	43	15
	TRT	29	17	28	19	11	28	6
	TN	14	7	8	13	4	13	2
	TLR	13	7	8	9	13	13	3
	Total	99	74	84	68	52	97	26

The use of a polyvalent antigen, prepared from all known serotypes of *T. vaginalis*, is necessary to estimate correctly the value of all serological reactions for the diagnosis of trichomoniasis, as well as to check the results of treatment and to investigate different questions concerning immunity (Teras *et al.*, 1965).

## VII. EXPERIMENTAL INFECTION IN LABORATORY ANIMALS

Earlier studies performed with bacteria-infected cultures of *T. vaginalis* or directly with *Trichomonas* containing vaginal secretions are, at present, only of historical interest. As soon as bacteria-free cultures became available, interesting results were achieved in renewed experiments.

### A. INTRAVAGINAL TRANSMISSION

Schnitzer and Kelly (1954) and Uhlenhuth and Schoenherr (1955) transferred a culture of *T. vaginalis* to the vagina of the Golden Hamster, infecting 20–30% of experimental animals after one inoculation, 80–90% after repeated inoculation. The infection persisted for 1 year and longer, and also a transmission from one hamster to another was possible. Cavier and Mossion (1957) describe a successful intravaginal transmission to rats in permanent oestrus. Castrated female rats were injected either daily with 1·2–5 µg oestradiol-benzoate in oil; or every 8th day 5 mg of crystal suspension of this substance was implanted subcutaneously, or 10 mg every fortnight. The consequence was a permanent oestrus. *T. vaginalis* multiplied in the vagina of the rats and

persisted for about 40–76 days after the discontinuation of the hormonal treatment. The same results in non-castrated rats were obtained by Combescot *et al.* (1957b). In this case a permanent oestrus of the rats was induced by a subcutaneous implantation of 20 mg oestradiol. Successful infection was achieved in 80–100% of the cases. Daily injections of 2·5–5 mg progesteron caused the disappearance of *T. vaginalis*. Combescot *et al.* (1957c) observed in castrated rats that the pH in the vagina remained almost neutral (6·6–7·5). In artificially induced hibernation (e.g. with chlorpromazine) the temperature in the vagina was reduced to 20°C. This had no effect on *T. vaginalis*, which has been found capable of surviving even in dead animals at a temperature of 6°C (Combescot *et al.*, 1957a). Mandoul *et al.* (1957) tried unsuccessfully to obtain a lasting infection with *T. tenax* in the vagina of young castrated rats, which had been brought artificially into a state of permanent oestrus; the presence of the parasite in the vagina could be confirmed only 48–96 h after inoculation. This also proves that both parasites are independent species. Vershinskii (1957) intravaginally infected hamsters, this infection lasting from 2 weeks to several months. In two animals, trilflagellate trichomonads, measuring only 6–7 µ, could be seen in the vagina.

Soszka *et al.* (1962) and Ginel (1962) inoculated guinea-pigs with a pure culture of *T. vaginalis*. This successful inoculation was used by these authors in later work (1962) for biochemical studies of *T. vaginalis* infection. The *Trichomonas* inflammation in the vagina of guinea-pigs was expressed by an accumulation of acid or neutral mucopolysaccharides on the surface of the epithelium. In the germinative layer of the epithelium a decrease of the DNA and an increase of the RNA were registered. Following treatment, the RNA slowly receded and the DNA increased significantly in the nuclei in this layer. According to many investigators, long-lasting elevations of the RNA and DNA are significant for neoplastic states. The long-lasting process of infection with *T. vaginalis*, as well as uncompleted treatment, changes the cells and the inter-cellular substance metabolism in the epithelium of the vagina, which may be the starting point for a pre- and carcinomatous state. Similar observations were also made by Kazanowska (1962). Ginel (1962), inoculating the vagina of guinea-pigs with pure cultures of *T. vaginalis*, found on the third day distinct symptoms of inflammation of the reproductive tracts, expressed by a swelling and reddening of the vulva and by the presence of an abundant, yellow, foamy and offensively smelling secretion. Great masses of leucocytes, *T. vaginalis*, and shedded and changed epithelial cells could be demonstrated on the slides. There were also numerous mononuclear macrophages present among the leucocytes. The epithelial cells were characterized by the presence of relatively large hyperchromatic nuclei of two types: one with a coarsely grained chromatin, the second without granular structure. The guinea-pigs aborted usually single foetuses, which were macerated. The entire mucous membrane of the vagina exhibited all the morphological characteristics of inflammation: swelling of the substratum, dilatation and hyperemia of the blood vessels particularly under the epithelium, often giving the impression of endothelial penetration, and the presence of numerous white cells migrating in the connective tissue and epithelium.

## B. PERITONEAL TRANSMISSION

Peritoneal transmission of axenic cultures of *T. vaginalis* in mice produces a purulent fibrinous peritonitis with necrotic foci in the liver, pancreas, spleen, lymphatic nodules and other abdominal organs, with the formation of ascitic fluid (Schnitzer *et al.*, 1950; Hamada, 1953; Yamagata, 1954; Teras, 1954–1966; Iwai, 1957; Vershinskii, 1958; Paronikjan, 1958; Reardon *et al.*, 1961; Honigberg, 1961–1966; and some other recent investigators). The infective dose is 1·5–4 millions (Teras, 1954; Vershinskii, 1958), the  $LD_{50}$  is 1·75 millions of flagellates (Nigesen, 1961). Cultivation on media containing agar, methyl-cellulose or gelatine stimulates the pathogenicity of *T. vaginalis* and *T. gallinae* (Honigberg, 1959). Many mice died during the first week p.i. Also in guinea-pigs, chronic inflammation and formation of granulation tissue could be observed. Iwai (1957, 1959) detected differences in pathogenicity in three strains of *T. vaginalis* with the aid of i.p. inoculation. Reardon and Jacobs (1958) isolated a highly virulent strain C and a low virulent strain R from mice. Strains of *T. vaginalis* isolated from patients with acute and sub-acute colpitis proved to be much more pathogenic than those isolated from patients with chronic colpitis (Bogowsky and Teras, 1958; Reardon and Jacobs, 1958; Reardon *et al.*, 1961; and others).

Teras and Roigas (1966) published studies on 171 freshly isolated strains. Seventy-four of them (43%) were found to be of high pathogenicity, 76 of medium (44%) and only 21 of low pathogenicity (12%). A non-pathogenic strain has not yet been found.

The earliest and most frequent pathological change is fibrinous purulent peritonitis, located particularly in the region of the liver and spleen. One of the most characteristic phenomena, observed especially in mice killed at the end of the observation period, was a conglomerate consisting of liver, stomach, spleen, pancreas and the lymph nodes of the upper part of the abdominal cavity, all these organs being covered with a fibrinously purulent exudate containing many leucocytes and trichomonads. Often foci resembling abscesses and containing an enormous number of leucocytes and *T. vaginalis*, localized on the mesenterium and on the peritoneum covering the intestine, were detected. Strains of high pathogenicity invaded the liver, causing typical necrotic foci. The macroscopic aspect showed grey foci, of a slightly yellowish shade and of cheese-like density. Their size varied greatly—in some cases they were hardly as big as a pin, in others they occupied almost a whole lobe. Trichomonads were detected also in the blood vessels passing through the necrotic foci of the liver. Ordinarily there was a thickening and an inflammation of the intima of the veins. Pathological change, detectable as a rule only in histological investigations, could be observed most frequently also in the pancreas, in the gastric wall and in the lymph nodes of the abdominal cavity. More uncommon was the invasion of trichomonads into the spleen, this being observed generally only in lethal cases. Only in a few cases was an invasion into the suprarenal glands observed. A high death rate (5–8 days at an early stage) was characteristic for these highly pathogenic strains.

Inoculation of strains with a medium pathogenicity was at first also followed

by fibrinously purulent peritonitis, sometimes in the form of circumscribed foci. However, these changes were detectable later and to a smaller extent, the amount of exudate in the peritoneal cavity being smaller in such instances. *T. vaginalis* also invades the liver, but these cases are rare and occur at a later period; in some cases it penetrates the pancreas (atrophy of glandular follicles), and rarely the gastric wall and the lymph nodes. The death rate of infected mice was markedly lower (up to 21 days). Inoculation of strains with low pathogenicity showed, in animals killed during the first days after inoculation, only a weak exudation and simultaneously some proliferative changes. Very rarely trichomonads were found to invade the abdominal organs. The mice died within 4–8 weeks. A comparatively simple method for testing the pathogenicity of strains of *T. vaginalis* has been worked out by the authors: 10–11 mice are inoculated i.p. and killed after 10 days. The results are tabulated as an index with 10 points. The most important and severest injury is the necrosis in the liver. Index 0-mice die spontaneously, index 10-mice are without infection and without any pathological changes. The more invasive strains are more resistant to osarzol, sanazine and uropine (Teras, 1958). Neither the pathogenicity of *T. vaginalis* nor the clinical forms of the genito-urinary infection have any correlation with the serotype of the trichomonads. On the other hand the clinical forms of trichomoniasis depend on the degree of pathogenicity of *T. vaginalis* (Teras, 1965).

From 48 *T. vaginalis* strains isolated by Laan (1966), 26 strains were highly pathogenic for mice (i.p.), 18 were of medium and 4 were of low pathogenicity. The pathogenicity of cultivation in TN-1 medium (up to 4 months) did not change essentially, but decreased constantly in prolonged passage up to 32 months. The pathogenicity of the serotypes TLR and TN disappeared in prolonged passage in medium *T. vaginalis*-1. The virulence of *T. vaginalis* is changeable; it is reduced by stovarsol. On the other hand, the antigenic properties among the strains of *T. vaginalis* are stable *in vitro* as well as *in vivo*. The fermentative activity in the production of acid from maltose and glucose was less intensive in strains with high virulence, than that in the strains of medium and low virulence, but no changes could be observed in the fermentation of lactose, sucrose and manitol. Only the agglutination proved to be stable in prolonged passages *in vitro* and repeated passages *in vivo*. The strains of *T. vaginalis* could be identified only by their antigenic differences.

In sections of different organs of mice infected with *T. vaginalis* cultures, a specific immunofluorescence was observed, which was particularly strong in kidney and lung sections, but less intensive in liver and spleen sections (Karbowski, 1966). These results indicate that the immunofluorescent method is suitable for revealing the distribution of trichomonads in the tissues of experimentally infected mice and probably also in man.

#### C. SUBCUTANEOUS INOCULATION

Subcutaneous inoculation of *T. vaginalis* in mice produced subcutaneous lesions with a great number of flagellates (Schnitzer *et al.*, 1950; Paronikjan, 1958; Honigberg, 1961; and others). The acute stage developed during the

first 8 h, later changing into reparative processes with histiocytic infiltration and giant cell formation. After 1–2 weeks all pathological changes disappeared. *T. vaginalis* were distributed in the edematic tissue and could also be found in more distant macrophages.

Honigberg (1961) developed a very simple method for the determination of the pathogenicity of different *T. vaginalis* strains: subcutaneous inoculation of mice with fresh axenic cultures and subsequent measurements of the volume of lesions caused by the flagellates. The mean volume of 6-day-old lesions served as the basis for evaluating the relative pathogenicity level of a given strain. A statistical comparison (Honigberg, 1961) of subcutaneous lesions produced in mice by the several strains of *T. vaginalis* reveals that these volumes faithfully respect their relative virulence to the natural host. The least virulent strains of *T. vaginalis* are still more harmful to mice than the least pathogenic strains of *T. gallinae*. Maintained in culture the strains of *T. vaginalis* become attenuated in their virulence. The presence of agar must have some influence upon the physiology of the parasites, rendering them more pathogenic, and the presence of methyl-cellulose was found similarly influential upon lesion-production. The lesions were smaller when mucin was substituted for agar. Abscesses produced by the parasite in the presence of gelatin were not enlarged.

In subcutaneous lesions produced by *T. vaginalis* (after Frost and Honigberg, 1962), the mechanisms of progression in all strains were studied orderly and found to involve: influx of polymorphonuclear leucocytes, multiplication of parasites, death of leucocytes, destruction of the host tissues with the lysis of the abscess wall, edema of the surrounding tissues and spreading of the flagellates, influx of leucocytes, remultiplication of the parasites and continuation of the cycle, the progression of which results in pure mantles of either leucocytes or trichomonads formed against the inner wall of the injection pocket. Both the precise picture and time sequence vary with the strain of *T. vaginalis* and are related to its pathogenicity. An excellent correlation was found between the experimental laboratory data evaluated by the mean volumes of subcutaneous lesions produced by the parasites in C-57/B1 mice 6 days after inoculation, and the severity of vaginal and cervical diseases of the female patients (Honigberg *et al.*, 1966). Ivey and Hall (1964) found no relationship between the human host status and the virulence of the strain for mice infected by the intraperitoneal route. Only after subcutaneous inoculation most strains from symptomatic patients showed more marked virulence for mice than did any of the strains isolated from normal persons.

With great probability the results of infestation experiments also depend on the strains of mice used. Kulda (1965), in applying Honigberg's methods, proved that minimal development was noted in the strain C-57/B1 ( $132 \text{ mm}^3$ ) and maximal in the mouse strain A ( $309 \text{ mm}^3$ ). He recommends the strain Balb/c as a standard for testing virulence. The strains DBA/2 and DBA seem unsuitable for these purposes.

Subcutaneous inoculation of germ-free guinea-pigs with *T. vaginalis* cultures shows important differences between the strain C with a strong virulence and the R-strain with a low one. The same was observed when using mice for

i.p. inoculation. The strain C killed the mice in a few days, while strain R did not (Newton *et al.*, 1960).

#### D. OTHER PATHWAYS OF INFECTION

Weld and Kean (1956) succeeded in inoculating *T. vaginalis* into the anterior chamber of the eye and into the vitreous body of rabbits. In 22 out of the 28 inoculated eyes flagellates were found in both sites. The best method is the inoculation into the vitreous body. Even after intramuscular inoculation deep abscesses containing numerous trichomonads are formed after 2–3 weeks. Infection could not be obtained in mice infected intranasally, orally and intra-intestinally (Paronikjan, 1958).

Inoki and Hamada (1954) describe changing experiments with *T. vaginalis*. When inoculating mice i.v. with 0·1–0·5 ml of washed chicken erythrocytes, and additionally with pure *T. vaginalis* culture, 70% of the mice died within 14–40 days. The recultivated trichomonads were found to grow in media which had been considered unsuitable and are described as having 3 flagella and a long undulating membrane. This change from *T. vaginalis* to *T. foetus* has not been confirmed by other authors and there are certain doubts as to its correctness.

#### E. PATHOGENICITY OF *T. vaginalis* FOR CELL CULTURES

This pathogenicity was shown by Hogue, and Honigberg *et al.* (1961–1966) continued the studies with different cell cultures (HeLa cells, chick-liver cells, fibroblasts, epithelial cells, and others) and *T. vaginalis* strains of different virulence. *T. vaginalis* caused degenerative changes in all three cell types and, to a lesser or greater extent, also in the filtrates of rich cultures (Honigberg and Ewalt, 1961). All the effects are more pronounced in infection with more pathogenic strains. The original pathogenicity could be maintained for two years from the time of isolation in axenic cultures by keeping them at about –72°C in the presence of glycerol (Honigberg and King, 1962). Healthy trichomonads of the pathogenic strain are often found within fibroblasts and especially epithelial cells, which then undergo many abnormal changes. Pathological changes occur also in cells which are neither in close contact with nor contain any trichomonads. In fibroblasts the division is stopped, commonly in the prophase, and these cells later degenerate. The introduction of cell-free filtrates of active *Trichomonas* cultures results in the appearance of many abnormal changes in the cell cultures, and these are similar to but typically less extensive than those observed in the presence of the parasites. The effects of filtrates from cultures of pathogenic strains are more pronounced than those of the mild ones. In chick-liver cultures, within 2 h over 70% of macrophages contain one or more parasite. In most instances the intracellular trichomonads degenerate. Occasionally the flagellates can multiply and may ultimately destroy the phagocytes. Only 1% of the epithelial cells in the avian tissue cultures are invaded after 2 h, 6% after 8–12 h. The epithelial cells appear somewhat more resistant than fibroblast-like cells. HeLa cells are rarely invaded by the flagellates. The cytoplasm of uninfected cells is retracted and the

nuclei show signs of degeneration. It is likely that some at least of the substances produced by the parasite either alone or in combination with the degenerating tissue are lytic in nature.

Christian *et al.* (1963) used relatively small inocula (5000 organisms) for infection of HeLa cell cultures. They observed no intracellular parasites, but reported the appearance of lesions that were lined with trichomonads. After massive inocula ( $5 \times 10^5$ ) of a relatively pathogenic strain, Honigberg and Ewalt (1961) observed parasites within some cells, especially in advanced infections. The parasites must have exerted both mechanical and chemical effects.

Trypsin-dispersed chick-liver cultures, after infection with a relatively pathogenic strain, show the following changes (Sharma and Honigberg, 1966). Fibroblasts and epithelial cells gradually lose their cytoplasmic RNA. Nuclear DNA levels of all cell types appear to show no significant changes. Glycogen is not stored in the fibroblasts, but epithelial cells show much of this polysaccharide and macrophages show moderate amounts. Acid mucopolysaccharides are not demonstrable by this method. Progression of the infection leads to a large accumulation of lipids in fibroblasts and epithelial cells. Significant loss of lipids is seen in phagocytes which contain *T. vaginalis*. Phospholipids were not demonstrable.

## VIII. TRICHOMONIASIS AS A CLINICAL ENTITY

Genito-urinary trichomoniasis has been acknowledged as a clinical entity since the description by Höhne (1916). Perju (1957) gave the following definition:

La trichomoniasis urogénitale est une entité morbide parasitaire produite par le flagellé *Trichomonas vaginalis* chez l'homme et chez la femme évoluant sous diverses formes uniques ou associées et présentant un marqué caractère vénérien.

### A. *Trichomonas* IN ADULT WOMEN

The clinical symptoms are very well known and nothing of importance has been published in recent years. Only some observations concerning the pathology must be mentioned.

#### 1. Pathobiology of *T. vaginalis*

The greatest number of trichomonads appear in vaginal secretion in the late luteal phase and in the early oestrogen phase (VII,I) and then in phases II of the cycle according to De Allende, the lowest number appearing in phases IV, V and VI (Kurnatowska, 1958). In climacteric women the population density of *T. vaginalis* varies irregularly. Experiments *in vitro*, using cervical mucus of 30 women and *T. vaginalis* strains from 24 patients, indicate that the cervical mucus may serve as a barrier preventing the trichomonads from entering. In 91% of pregnant women infected with *T. vaginalis* inflammatory manifestations are present in the vagina.

After abortion, during labour and puerperium, complications occurred twice as often in women with *T. vaginalis* as in the control groups. The most common complications in puerperium were: a higher temperature, foetid excretions and puerperal inflammation of the uterine mucous membrane (Jedrzejczak, 1966). In view of the evidence that 90% of the 975 cases of trichomoniasis had erosions, it can be assumed that the inflammatory conditions induced by *T. vaginalis* may have an effect on the occurrence and development of cervical erosion. For that reason antitrichomonal measures should be considered in the prophylaxis of precancerous states (Zawadzki, 1966).

The penetration of other organs by *T. vaginalis*, very common in laboratory mammals inoculated with pure culture, seems to be very rare in women. A case is reported by Hoffman *et al.* (1966):

from a patient suffering from generalized carcinoma with concomitant trichomonads in the vagina, the authors could cultivate *T. vaginalis* from the cancerous foci in the lungs and liver and from section of the spleen and vaginal mucous membrane.

Two isolations of *T. vaginalis* from the oviduct are reported by Zwierz and Klyszejko (1964).

Moore and Simpson (1954) and McEwen (1960) point out psychosomatic symptoms during trichomoniasis, such as sexual disturbances, disparesunia, frigidity, moral laxity, emotional instability, religious obstacles. Certainly every physician must take into account these psychic complications, but we must refuse their postulate: "*T. vaginalis* is a normal contaminator of the vulva and vagina, waiting only for favourable conditions for reproduction and, therefore, not a venereal disease".

Contrary to that, many new investigations proved trichomoniasis to be a true venereal infection and venereal disease.

## 2. *T. vaginalis* in the urinary tract

*T. vaginalis* survives in the female urethra, in the paraurethral glands, in mucous crypts etc., causing apparently less damage than in the vagina, although adequate histological studies are not yet available. Evidence of trichomonads in urine was provided by many authors in the last decade. It is possible that bacteria are transported to the bladder by *T. vaginalis* (Kean, 1955). Some relapses can have their origin in urethral infection. Grys (1966) found *T. vaginalis* in the bladder, accompanied by an increased number of inflammatory elements in the sediment. Catheterization can introduce *T. vaginalis* into the bladder from infected urethra (Grys, 1966). Glebski (1966) could not find any differences in the incidence of *T. vaginalis* in the urine from diabetic patients (4.5% positive) and the controls (4%). In 76% of trichomoniasis cases Durel and Roiron-Rattner (1957) also found trichomonads causing urethritis in women. Glebski (1966) assumed that certain features of the urine such as specific weight, pH, albumin content, presence of cylinders, erythrocytes and leucocytes in the sediment, and presence of bacteria occur in statistically frequent cases in connection with the presence of trichomonads, *T. vaginalis* has no effect on the morphological composition of the blood.

Candiani has found no differences in the content of pregnandiol, estrogen and 17-keto-steroids in the urine during *Trichomonas* infections.

Grys (1964), investigating by cultivation samples collected from the genital organs of 387 women, found *T. vaginalis* in urethra alone in 1·7% of the cases, in the paraurethral glands in 3·2%, in the external orifice of the vagina in 3·2%, in the urethra, paraurethral glands and vagina in 68·8%, in the urethra, paraurethral glands, vagina, cervical canal in 13·1%, in the urethra and paraurethral glands in 3·2%, in the urethra and external orifice of vagina in 0·6% and in paraurethral glands and external orifice of vagina in 12·7%. Out of 32 patients operated who had been infected with *T. vaginalis* before the operation, the presence of trichomonads in the corpus and in the cervical canal was observed in one case only.

Littlewood and Kohler (1966) noted *T. vaginalis* in a female infant of approximately 28 weeks gestation with abdominal distension and readily palpable kidney on the 19th day of life. The flagellates were present in the urine and were associated with significant pyuria. On the 64th day oral treatment with metronidazol (60 mg t.d.s. for 7 days) was started and this rapidly eradicated the parasite and the pyuria.

### 3. The influence of *T. vaginalis* on the vaginal epithelium

Bechtold and Reicher (1952), Řeřábek *et al.* (1953) and Teter and Polachowski (1954) showed that in vaginal smears containing *T. vaginalis* the epithelial cells acquire a malignant aspect as in anisonucleosis, poikilonecrosis, large granulation of the chromatin etc. Pundel (1957), Charvet *et al.* (1957), and others confirmed these findings. Papanicolaou and Wolinska (1955) summarize this problem as follows:

In cases of *T. vaginalis* infestation vaginal smears exhibit a characteristic pattern: a marked increase in the number of the cornified cells of the superficial squamous type. Some of the cells have irregular outlines and are covered with smudges and grayish mucus. The cytoplasm is often dense and intensively acidophilic. The nuclei are deeply stained in well preserved cells, while in degenerating cells they are faintly stained and have a pinkish appearance. Marked keratinization of the cells is occasionally noted. The parabasal cells are often prominent and appear singly or in small clusters. They show some variation in size and a more pronounced vacuolization of the cytoplasm than seen in normal cells and, sometimes, an increased affinity to acidophilic stains. All these changes may in some cases be the result of a concomitant secondary inflammatory condition. In a number of *T. vaginalis* positive smears exfoliated cells may exhibit marked nuclear atypia. This consists chiefly in an enlargement, irregularity in form and hyperchromatia of the nuclei. These changes when pronounced may lead to a false interpretation of the cell as malignant. It is thus important to explore thoroughly every vaginal and cervical smear showing nuclear atypia to rule out the presence of trichomonads. This does not imply that every smear with abnormal nuclear forms, in which trichomonads have been found, should be considered to be negative for malignancy. On the other hand, more conclusive evidence should be sought before such a smear is reported as definitely positive. Nuclear atypia in association with trichomonads may be observed not only in the superficial, intermediate and parabasal squamous cells, but also in cells of

endocervical origin. Extreme cytologic abbreviations are seen in a relatively small number of trichomoniases. In most instances the smear shows only minor morphological changes and in many cases there may be a complete absence of clinical symptoms.

The identification of *T. vaginalis* in smears stained after Papanicolaou is more difficult than by the Giemsa method, because the flagella, the undulating membrane and the axostyle are not stained.

Kazanowska (1962) observed inflammatory changes resembling in appearance mucous membranes observed in precancerous states. Occasionally a coexistence of cancer and trichomonads is observed.

After Frost (1962), the cytology of the *T. vaginalis* infestations can be divided, in view of its cellular reaction, into three parts: the general background, the regressive cellular changes of degeneration due to tissue injury and destruction, and the progressive cellular changes of regeneration in reaction to injury of dysplasia and of neoplasia. All three components of the cellular response pattern may be found. In such cases a clear differentiation is most important.

Holtorff and Krimmenau (1960), studying colposcopy of trichomoniasis, made the following observation: in 4·1% of the patients the vagina was not inflamed in spite of the presence of trichomonads; in 10·5% the irritation was not visible to the eye, but colposcopy revealed a diffuse, flat papillary formation. In 18·1%, grade I of the inflammation was a diffuse vaginitis with formation of papillae spreading over the cervical and vaginal mucous membrane. This became clearly visible after the iodine test. In 23·7% grade II of inflammation was a diffuse reddening or reddish spots on the macroscopic picture. Colposcopy showed very well developed slightly elevated edematous papillae of connective tissue with marked hyperemia of the vessels. Often, the papillae coalesce in the presence of the accompanying edema, appearing as small irregular spots. Inflammation grade III was most frequent (30·9%). Macroscopic symptoms are red spots, which start bleeding at the slightest touch. Colposcopy showed numerous foci of varying appearance—fine, washed out, edematous, highly elevated above the general surface. These round foci are marked by hyperemia, dark-red dense, capillary loops, their margins being moistened with a yellowish secretion. Less frequent are fine fibrinous membranes giving the appearance of fibrinous colpitis. The mucous membrane is highly edematous, most sensitive and bleeds easily (colpitis granularis).

#### 4. Sterility and *T. vaginalis*

Long-lasting infection of *T. vaginalis* is followed by sterility (Gautier and Biguet, 1957; and others). On the other hand, after successful treatment, which has cured the patient or at least has caused the disease to become latent, many can conceive and give birth to a healthy, normal child. Phagocytosis of the sperms by trichomonads, as emphasized by Kolesov (1950), is of no importance; in fact we have never observed it during all these years; but it is possible that toxic metabolites of *T. vaginalis* may reduce the motility of the sperms. According to Hynie *et al.* (1960) this temporary sterility depends on the following factors:

A. In *Trichomonas-colpitis* the mucus of the vagina and cervix is covered with purulent discharge (pseudocervical blockade) so that even short therapeutic measure can cure this sterility. The situation is worse when endocervical inflammation is present and the spermatozoids are caught up in the cervical canal (cervical blockade).

B. In the case of less intensively motile spermatozoids in a more diluted ejaculate it was possible to observe that the spermatozoids were stopping in a medium with more plentiful and active trichomonads. The stopping of the spermatozoids gave the impression that they had come into an area contaminated by toxic substances from the trichomonads. More flagellates had a greater destructive influence on the spermatozoids. In a supernatant from a *T. vaginalis* culture these spermatozoids showed very little motion after 2 h. The crushed trichomonads considerably restrict the motility of the spermatozoids after 1-1½ h. After 3 h there was no movement. Crushed cultures obtained by autolysis for 1-2 days strongly reduced the motility of the spermatozoids within a few minutes, while a 24-hour-old crushed discharge containing trichomonads stopped the intensely motile spermatozoids within 2 h. *T. vaginalis* may be the cause of sterility especially in the culminating stage of very heavy infection. This may apply to cases in which the male's semen is relatively weak and the ejaculate is not sucked into the uterus intensively enough. Thus the spermatozoids remain longer in contact with the vaginal secretion containing trichomonads and find it difficult to penetrate the cervix by their own propelling force.

C. Through inflammatory processes caused by the pathogenic influence of *T. vaginalis* and by pyogenic bacteria, the introitus vaginae often becomes very painful, making cohabitation very difficult and leading to dispareunia.

In the practice every woman suspected of sterility should be examined for the presence of trichomonads and, in positive cases, treatment of both husband and wife should be started before taking other therapeutic measures to cure sterility.

##### 5. *Microbic vaginal pictures and the dynamic aspect of Trichomonas infestation*

Mere bacteriological classification of the cleanliness of the vagina (Manu af Heurlin, 1914) does not satisfy clinical needs, for it omits gonococci, *T. vaginalis* and pathogenic yeast organisms. With regard to the entire microfauna and microflora and to the histological elements, Jírovec *et al.* (1947-1948) proposed a new classification of the vaginal biocenosis in adult women. Their six basic groups (called microbic vaginal pictures) were numbered I-VI. The technique is very simple: 2 smears from vaginal contents are dried in the air, fixed with methyl alcohol, then one is stained with Giemsa 1:10 for 1 h, the other by the Gram method, and both are examined under a microscope ( $\times 1000$ ). The basic microbic vaginal pictures are:

I. Normal physiological picture of a healthy adult woman with many epithelial cells, many *Lactobacillus vaginalis* and few leucocytes.

II. Non-purulent bacterial discharge with many epithelial cells, few leucocytes but many different bacteria—cocci, rods, sometimes *Vibrio vaginalis* but no *Lactobacillus*.

III. Purulent bacterial discharge with few epithelial cells, many leucocytes and a mixed flora of bacteria and cocci.

IV. Gonorrhreal discharge—in the acute phase many gonococci, Gram-negative of typical form, are found inside the leucocytes; in the chronic form the gonococci could be detected mostly by cultivation.

V. *Trichomonas* discharge.

VI. Vaginal mycosis with *Candida* div. sp., *Torulopsis* sp., *Saccharomyces* div. sp., and others.

The period of sexual rest (1–10 years approximately)—an unoestrogenized vagina—is characterized by the physiological picture “O” expressed cytologically only by basal and parabasal cells; microbes of all kinds are absent, or present only in very small numbers, and in particular there are no *Döderlein* bacilli. No other microbes, e.g. *Vibrio*, *Trichomonas* or yeast-like organisms, occur in this period.

This classification has been introduced to Czechoslovakia, Poland, G.D.R., Rumania and Yugoslavia as a simple routine diagnostic method (Table VII).

In microscopical examination of trichomonal discharge the flagellates are frequently accompanied by bacteria and purulent cells. Sometimes solitary trichomonads in the otherwise normal picture of the vaginal biocenosis indicate a latent trichomoniasis (I/V). Some outstanding gynaecologists have been led to believe that trichomonads in the vagina are quite harmless. The dynamic conception of Peter and Jírovec (1950) shows a logical connection of all stages of the infection encountered in the practice, and at the same time explains the incongruities in the literature. In the course of *Trichomonas* infection they distinguish three or four types concerning the V microbial vaginal picture.

#### *V/A-Trichomoniasis acuta* (Fig. 12)

A few days after infection, mostly transmitted by sexual routes, quickly reproducing trichomonads appear in the vaginal secretion, the numbers of epithelial cells decrease, *Döderlein's Lactobacillus* subsides and the inflammation is unfailingly announced by a considerable number of leucocytes. Other bacteria which adjoin later are entirely missing at this stage or can be found only in insignificant numbers. The clinical manifestation, however, does not differ from the subsequent stage. The discharge is strikingly thin, whitish, yellowish or greenish, sometimes dropping from the genitals and often mixed with gas bubbles. This acute trichomoniasis, generally of short duration, is seldom observed in time by the physician as women delay their visit and come for examination when the second stage has set in.

#### *V/B—Culminating trichomoniasis* (Fig. 13)

The smears contain numerous trichomonads, many leucocytes and many different bacteria. There is less epithelium and *Döderlein's Lactobacillus* has disappeared. Clinically, there is a thin discharge as described above, lasting for several weeks, months or even years, of the same or fluctuating intensity. This stage, most frequently encountered by the physician, is followed by a chronic stage.

TABLE VII  
*Frequency of microbic vaginal pictures in women visiting gynaecological ambulatories etc. (sick population)*

Microbic vaginal picture	Dáňa, Prague (1949) (1944-1947)	Žižková (1956) Prague		Kostić, Beograd (1955)		Holtorff, Dresden (1961)	Engelbrecht and Müller, Berlin (1962)	Lipenský and Viehweg, Demmin, GDR (1963)
	%	(1951)%	(1952)%	women (1949)%	men (1958)%			
I	6·0	23·6	24·9	6·0	3·3	4·0	10·1	8·66
II	22·0	21·4	25·1	23·0	18·4	24·0	51·0	15·33
III	24·0	23·2	20·0	22·0	26·1	7·4	24·0	38·0
IV	1·4	0·1	—	3·8	0·56	—	—	0·33
V	38·0	25·0	23·1	38·0	39·0	62·5	14·9	33·0
VI	8·6	6·7	6·7	6·35	12·6	1·9	—	1·0
Number of examinations	3000 = 100%	3255 = 100%		1827 = 100%		625 = 100%	820 = 100%	300 = 100%

TABLE VIII  
Dynamic aspects of Trichomonas infections

Microbic vaginal picture	Dáňa, Prague (1949) %	Holtorff, Dresden (1961) %	Engelbrecht and Müller, Berlin (1962) %
V/A	7·6	0	6·1
V/B	68·0	33·0	59·5
V/C	24·4	37·1	22·3
Transitory	—	11/V 29·9	V/B-C 12·1

V/C—Chronic trichomoniasis (Fig. 14)

Trichomonads can be seen in varying numbers, many epithelial cells, few leucocytes and a mixture of rods, cocci and other bacteria, but no Döderlein bacilli. Women complain of an atypical discharge. This chronic stage is sometimes transformed by a keen reduction of the trichomonads into a latent stage I/V with the normal numbers of Döderlein's *Lactobacilli* and epithelial cells, only very few leucocytes and few flagellates, detected in most cases only by culture (Fig. 15). This stage can be artificially brought about by treatment. Table VIII shows the findings by three investigators from different localities.

*T. vaginalis* is also often associated with different species of yeast-like organisms and yeasts (Microbic picture V/VI). Feo (1953) found in a group of

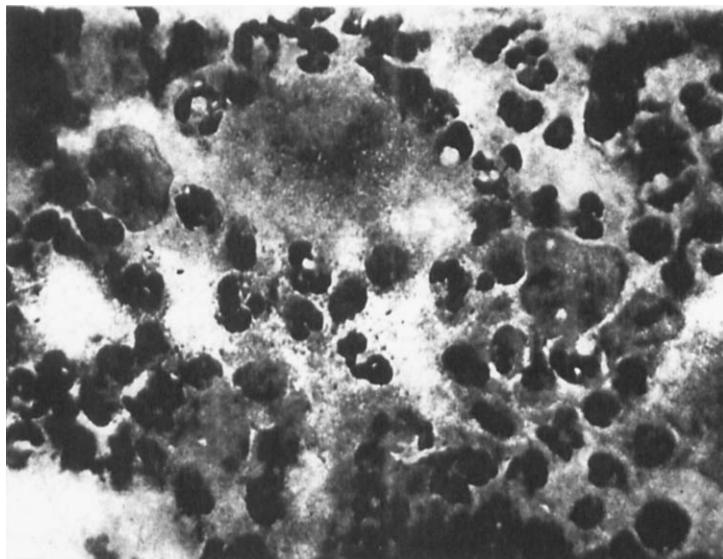


FIG. 12. Microbic vaginal pictures: acute stage of trichomoniasis (V/A). Many trichomonads and leucocytes, few epithelial cells, no bacteria.  $\times 800$ . (Giemsa staining—photo O. Jírovec.)

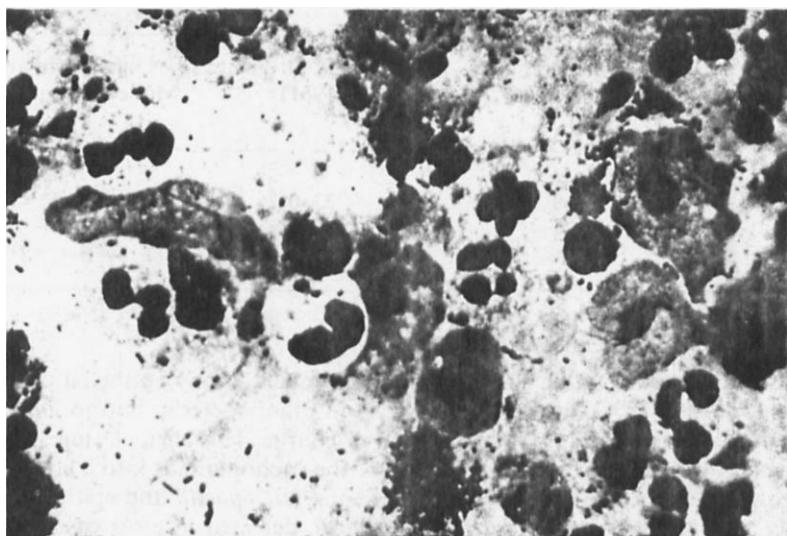


FIG. 13. Microbic vaginal picture: culminating trichomoniasis (V/B). Many trichomonads, leucocytes, bacteria and few epithelial cells. Without Döderlein *Lactobacilli*.  $\times 2000$ . (Giemsa staining—photo Professor J. Fiala.)

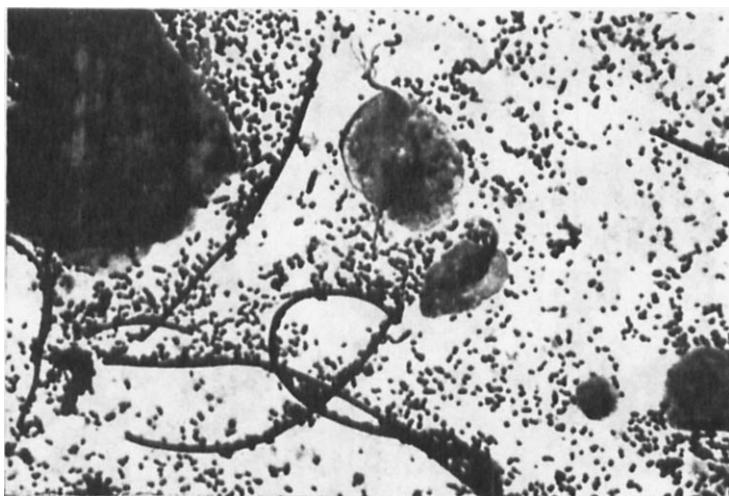


FIG. 14. Microbic vaginal picture: chronic trichomoniasis (V/C).  $\times 1500$ . (Giemsa staining—photo Professor J. Fiala.)

200 white women trichomonads in 16.5% and *Candida* in 12%; in a group of 500 negroes the percentages were 43.6% and 15.2% respectively. Capriora *et al.* (1957) detected 40.8% of trichomonads infection and 11.3% of vaginal mycosis in 1204 women; in 3% both infections were present. Lauras and Garin (1958) found, in 36 women with trichomonads, *Candida albicans* in 12% and *Aspergillus fumigatus* in 6%. Kurnatowska (1958) gave an account of such associated infections (*Candida* sp., *Torulopsis* sp., *Aspergillus* sp., *Trichothecium* sp.) which are particularly resistant to treatment with metronidazol.

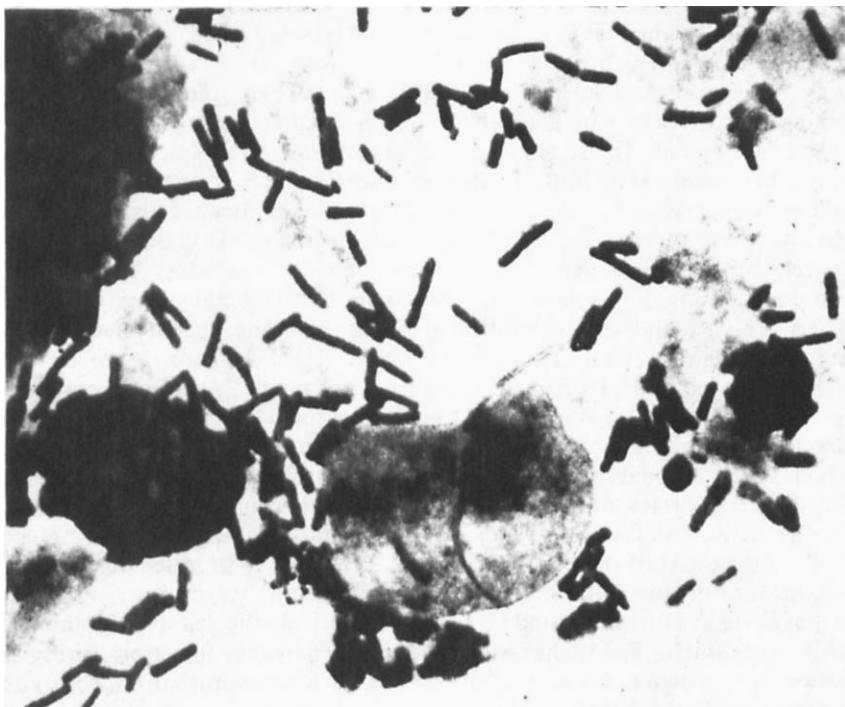


FIG. 15. Microbic vaginal picture: latent trichomoniasis (I/V). Many Döderlein lactobacilli and epithelial cells, few leucocytes. No inflammatory reaction.  $\times 2000$ . (Giemsa staining - photo Dr. J. Lom.)

Lang *et al.* (1960) record the combination of *T. vaginalis* and *Candida* in 31% of cases, of trichomonas alone in 43% and of *Candida*-vaginitis in 21.3%. Peoples *et al.* (1957) isolated in 14.7% of 34 persons a peripneumonia-like organism, but attributed no significance to this association. Petru (1966) subjected the vaginal secretions of 6258 women to mycological examination and isolated yeast-like organisms in 16.3% of a total of 1020 mycologically positive cases; vaginal trichomoniasis was also found in 162 patients (15%). These numbers indicate the low coincidence of vaginal mycoses with vaginal trichomoniases; most of the mycologically negative cases of this group (30%) were found *Trichomonas*-positive. At present nothing definitive can be said

about the antagonistic relations: these may be due to the influence of highly increased acid conditions in the vagina caused by yeasts, to a certain competition for glycogen, or to other and unknown mechanisms.

#### B. *Trichomonas* IN GIRLS

Older and recent investigations have emphasized the fact that *T. vaginalis* infection is very rare in girls and virgins but that the incidence of infection is rapidly increasing after defloration. Peter (1945, 1957, *et seq.*) found, in child records (Prague), that out of a total of 11 500 girls aged 0–14 years there were only 109 recordings of *T. vaginalis*; of these positive cases, 13 were sucklings from mothers infected with trichomonads, 5 were girls aged 4–7 years, 20 girls aged 9–11 years and 71 girls aged 12–14 years. These findings are in accord with the observations by Peter that *T. vaginalis* can settle only in an oestrogenized environment. The sucking is under the influence of its mother's oestrogen for several weeks after birth, Döderlein's *Lactobacillus* is present in the vagina of newborn girls and glycogen is abundant in the epithelial cells, representing the same environment as in a healthy adult woman. Trichomonads are acquired during birth if the mother is infected with flagellates. Another possibility of transmission to newborn babies is due to highly unhygienic conditions. There is no evidence of the duration of infection because the infected sucklings are treated immediately and can be cured relatively soon, even by older therapeutic methods. During the rest period of the child's genitalia, conditions in the vagina are unsuitable for the development of trichomonads, being practically due to the absence of glycogen in the vaginal mucous membrane. Should trichomonads penetrate into such a vagina, they disappear after several days or do not settle at all. Two out of the five cases of *Trichomonas* infection in the girls observed by Peter occurred during the pubertas praecox. Only at the onset of puberty, when the ovaries start to produce hormones, do conditions become suitable for *T. vaginalis*. At this stage also *Lactobacillus vaginalis* infects for the second time, now definitively the vagina of prepubertal or pubertal girls. The higher incidence of *Trichomonas* infection during this period has its own epidemiological reasons, such as masturbation, coitus *ante portas*, etc. (Peter, 1945).

Lang (1959) investigated 110 cases of vaginitis in girls who had not yet started to menstruate and found only four infected with *T. vaginalis*. Crowther (1962) observed two well-documented cases in infants: both children were of low birth-weight and in one there was a congenital abnormality of the urinary tract associated with meningocele. Using the oral route both were cured by giving 50 mg metronidazol three times daily for 7 days. Komorowska *et al.* (1962, 1964) confirmed the opinion of Peter: of 35 newborn babies up to 3 weeks old, 17·2% were *T. vaginalis*-positive, of 1101 girls up to the age of 10 years 0·8% were positive, of 870 girls past the age of 10, 10·4%. Girls without oestrogenic reactions of the vaginal epithelium do not show *T. vaginalis*. The ovulatory cycles occurred in 27% of girls infected with *T. vaginalis* and only in 3% in non-infected ones (Komorowska and Kurnatowska, 1964). Girls sharing a bed or using the same bath and lavatory as adult infected persons very seldom

become infected. *T. vaginalis* occurs in girls who are under the influence of genital hormones; very rarely, the infection occurs during the resting period of the genital organs. Urinary infection by *T. vaginalis* appears to be also very rare in infancy and childhood.

### C. *Trichomonas* IN MEN

At first, *T. vaginalis* in the human male was considered a rarity and each case was published as an interesting report. Later recordings became more numerous, at first in connection with the so-called unspecific urethritis, later as some kind of latent infection without clinical symptoms. Today, trichomoniasis in the male is about as frequent as in the female, differing only in its course; in the male the infection is mostly latent or persisting in a subclinical form, while in the female the infection shows external symptoms—a discharge—in its acute and culminating stage. Since syphilis and gonorrhoea have been treated successfully with antibiotics, the subject of trichomoniasis in the male should be given more attention by venereologists and urologists. The number of papers published on this subject was about 5490 in the years 1894–1956 (Jíra, 1958), but these are only of historical value in view of the fact that trichomoniasis as a typical venereal infection is cosmopolitan in its distribution in the male and the female. In Germany trichomoniasis in the male was studied by Rodecurt (1929–1957), Bauer (1942–1966), Keutel (1955–1958) and others, who published numerous papers on this subject; in England by MacEntegart (1952–1959), Harkness (1933–1953), King (1959–1960), Lanceley (1953–1958), Whittington (1951–1957) and others; in Spain mainly by Bedoya (1957–1960); in Rumania by Perju (1951–1964); in Yugoslavia by Kostić (1954–1960); in Poland by Kozłowski (1951–1954); in Czechoslovakia by Kučera (1940–1957), Jíra (1954–1958) and others; in the USSR by Teocharov (1957–1958), Teras (1954–1966), Rožinski (1948) and others; in Italy by Nazzaro and Valenti (1953); in the USA by Coutts (1955–1958) and Feo (1944–1956).

Kostić for trichomoniasis in the male, has used the classification of the microbic pictures specified by Jírovec *et al.* (1947–1948). Jíra (1958) distinguished the following stages of these pictures: (a) primary acute stage, immediately following infection by coitus and mostly accompanied by strong urethral discharge; (b), primary subchronic stage with a slow onset and meagre discharge; (c), primary latent stage with a symptomless course throughout turning the infected male into a vector. All three stages change into the chronic stage especially if the infection has not been diagnosed correctly and treated accordingly. This stage may last for years; the discharge may disappear for some time only to exacerbate after being provoked by e.g. a cold, some wrong food, excessive sexual intercourse or alcohol. The standard treatment with antibiotics, sulphonamides and urinary antiseptics may have some effects on the accompanying bacteria but not on the trichomonads. According to Jíra (1958) the incubation period is 1–5 days in 20% of the cases, 6–10 days in about 40%, 11–15 days in 10%, 16–30 days in 20%, and 1–3 months in about 9%. Site of infection: *T. vaginalis* was recovered not only from the urethra, but also from

the preputial sac, the para-urethral glands, the seminal vesicle, Morgagni's lacunae and the prostate, less frequently from the epididymis or the testicles. Krupicz (1964) and others report on the occurrence of a purulent ulcerous or excoriative balanitis; there are also recordings of litreitis, cowperitis, vesiculitis, epididymitis (8–30% of cases), deferentitis, cystitis (Piringer and Piringer, 1957) and especially prostatitis (40–70%). Keutel (1955) found solitary trichomonads in an epididymis after epididectomy (in a frozen section and in phase contrast). In his opinion the prostate is the most common site for *T. vaginalis* in the male. In keeping with Holz (1953), Keutel considers the frequently occurring non-flagellate rounded *Trichomonas* in the male to be cysts in cultures of *T. vaginalis*, which had been kept under unsuitable conditions, e.g. changing temperature and pH, and considered them to be true cysts, even making a drawing of cyst partition! The author confessed to difficulties in confirming the cysts, respectively the round forms in the native preparation because of their similarity to inflammatory cells.

In the urethrogenital organs of the male *T. vaginalis* can either live as a commensal or change into a true pathogen, influenced by various factors. It is difficult to know whether this change is greater in younger men or whether more frequent exposure to infection through coitus is the decisive factor. According to Jíra (1958), the highest incidence of infection occurs at the age of 21–25 years (48%) and of 26–30 years (26%), decreasing with increasing age. Association with bacterial infection is of some importance, but Lanceley and MacEntegart (1953) confirmed in experiments with pure cultures of *T. vaginalis* on 5 volunteers that the ciliate has a primary pathogenicity. It was possible to produce urethritis five times and prostatitis twice; in three of the inoculated cases trichomonads were found in urethral discharge on the 6th–9th day and persisted for 44–94 days. According to Bauer (1960), 16% of urethral discharge in trichomoniasis in the male is microscopically sterile, 33% shows a moderate and 51% a high incidence of bacteria. Naturally, there are also mixed infections with gonococci. The bacteria in urethritis with or without trichomonads are practically the same not only in view of the species but also in their incidence (Feo *et al.*, 1956). Mostly they multiply secondarily in an environment inflamed by the trichomonads. Information on the change of resistance of the host's organism by an eventual activation of a simultaneous latent infection with certain bacteria or viruses, and also on the different pathogenicity of *trichomonas* strains, is very scanty. Hypospady, diverticule strictures and urethral stenosis seem to facilitate the infestations by *T. vaginalis* (Hancock, 1959; Shepard, 1959). Of 364 males whose wives or sexual partners had *T. vaginalis* infection Kostič has demonstrated the organisms in 39% in their urogenital tracts. The urethra was involved in all cases (39%), the prepuce in 1·38%, the prostate in 6·6%, the bladder in 25·4%, the renal pelvis in 1·7%.

The coital origin of male *Trichomonas* infection is generally recognized. Exogenous acquisition of the infection in the male—if this indeed exists—is extremely rare. According to various statistics, the percentage of *Trichomonas*-carriers by partners of infected females is estimated to range from 20–100% (e.g. after Bedoya *et al.*, 1958, it is at least 76%). These numbers are, in fact, greatly dependent on the methods of examination, their repetition, and especi-

ally on the experience of the investigators. A latent infection in both sexes may lead to a latent or evident disease in the partner (Keutel, 1958). The percentage of participations of trichomoniasis in the male infected with non-specific urethritis ranges, according to the various authors, from 3–68%; according to Jíra the average is about 30%. Keutel (1957) recorded a decrease of fructose content in the sperms, which in view of the shortened activity of the sperms may be considered one of the reasons for sterility. There is still some uncertainty about the length of time for which the trichomonads can persist in the urogenital organs of the male. Some consider the possibility of their spontaneous disappearance after 2–3 months, others assume the infection to persist for many years (Feo, 1956; Siboulet, 1957; Jíra, 1958; and others). Bedoya believes in a lifelong infection. During sexual intercourse the trichomonads from the prostates and from the walls of the urethra are carried with the ejaculate into the vagina.

#### IX. DIAGNOSIS OF *T. vaginalis* INFECTION IN THE HUMAN FEMALE AND MALE

Because the diagnosis of *T. vaginalis* infection cannot be based on clinical symptoms alone, especially if the infection is in its chronic or latent stage, microscopical examination has to be carried out. When this is negative, it must be completed by cultivation. Trichomonads are most abundant during the first 2 days following menstruation. Should no flagellates be found in the vagina it is necessary to examine the urethra, the paraurethral ducts and the greater vestibular gland. It is most difficult to demonstrate trichomonads in the cervix because even after a careful removal of the vaginal secretion with tampons, some trichomonads may still remain and simulate their presence in the cervix. There it can practically be proved only in operational material. For the practice it is sufficient if trichomonads are demonstrated in the vagina, the urethra and in the urinary sediment. Careful records are made of the clinical findings in the vagina, the cervix and the external genitals of the amount, colour, smell and appearance of the vaginal secretion.

In native preparations the flagellates retain their motility for several hours when kept at a temperature of 20°C under a coverslip sealed with paraffin. Temperatures exceeding 44°C kill the trichomonads in a short time and therefore the preparation should never be heated over a flame. The pressure of the coverslip sometimes causes the trichomonads to change their shape, and eventually to develop pseudopodia-like processes which serve neither for motion nor for the intake of food. Various mistakes may occur in a diagnosis made from a native preparation. In a weak infection the incidence of flagellates is so low that the preparation may be considered negative. On the other hand, leucocytes may be set into motion by viable spirochetes, flagellated bacteria, sperms or ciliated cells adhering to them (operation materia), thus simulating trichomonads. Coutts *et al.* (1959) recommend unstained dry smears observed in a dark field—the flagella are very clearly visible. Addition of 0·1% safranin after Starzyk *et al.* (1958) or brilliant cresyl blue after Holtorff (1957) facilitate

the finding of *T. vaginalis* in native preparations. Only dead flagellates are stained intensively red, resp. blue; the living remain unstained—in opposition to the stained leucocytes, epithelial cells etc., in the preparation.

The diagnosis from a native preparation should always be completed by a Giemsa-stained preparation. The original solution is diluted by 1:10 with freshly boiled or buffered distilled water (pH 7.2–7.6) and stained for one hour. The trichomonads can be easily recognized by the sky-blue colour of their plasma and the reddish-violet, oval to slightly pointed granulated nucleus. In a well stained preparation the locomotion apparatus with the axostyle stain carmine red; but frequently remain unstained. In such preparations it is even possible to determine the microbic vaginal picture given in the classification by Jírovec *et al.* (1947, 1948). Bacteria stain violet (including the genera *Vibrio*, *Leptotrichia*, *Fusiformis*, *Spirochaeta*), gonococci blueish, yeast cells blue with reddish granules in the plasma (Jírovec, 1960).

A Gram-preparation serves for completing the diagnosis: the gonococci are Gram-negative; the pyogenic staphylococci and streptococci, all yeast-like organisms and *Lactobacillus vaginalis* are Gram-positive. Trichomonads are Gram-negative but are difficult to recognize. Other staining methods (gentian, methylgreen-pyronin, methylene-blue, hematoxylin, Papanicolau's method) are unsuitable for an exact proof and although they may demonstrate a massive infection, a few disappeared trichomonads are always overlooked. The media used for cultivation are Johnson's CPLM medium, Vf-bouillon after Magara, Feinberg, etc. (see addendum). They increase the number of positive cases by about 10%, but there are instances where negative results are obtained in the culture even if the microscopical finding was positive. Therefore the use of both methods is recommended. Perju (1964) emphasized the need for examining also the mothers or all female persons who had been in close contact with *Trichomonas*-positive young girls or sucklings.

Also every male suffering from urethritis or other urological infection should be thoroughly examined for *T. vaginalis*, similarly his wife or female partner. Microscopical native preparation and Giemsa- and Gram-stained preparations have to be made from the urethral and prostatic secretion from the urinary sediment, and carefully examined for the presence of trichomonads. At the same time cultures should be made from the same material (Jíra, 1958; Kurnatowska, 1958; Hoffman *et al.*, 1961; Roigas and Rubanovitch, 1963; Témín, 1965, personal communication; and others).

#### X. CHEMOTHERAPY OF VAGINAL TRICHOMONIASIS

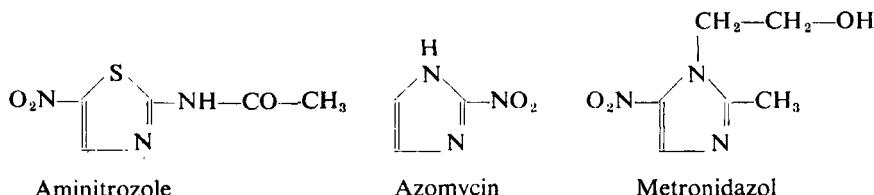
Chemotherapy of vaginal trichomoniasis is still a most difficult problem in spite of the use of various drugs in different combinations. Although drugs were found to remove *T. vaginalis* from the vagina, it was not possible to remove them from the urethra, the Skene's ducts and the greater vestibular gland, which caused relapses especially in patients with lowered defence mechanisms (through another infection, colds, etc.). Another drawback was the length of time which most of these therapeutic methods required. An even more difficult problem was the treatment of the male prostate; when this organ had been

infected with trichomonads, there was almost no hope for successful treatment. In addition to these difficulties it was almost impossible to persuade apparently healthy latent *Trichomonas*-carriers to undergo such long-lasting and personally most disagreeable treatment, and these remain a source of re-infection. The combination of trichomonadocidal, bacteriostatic and mycocidal substances after Peter and Jirovec (1946, 1947), effective on three pathogenic groups of organisms and serving as a basis for the production of effective preparations such as "Triflocid" and "Fluocid" (Spofa), "Anafluose" (Guillaumin), "Tricolpon" (Organon), "Viozol" (Ciba), and others, could in view of only local application in both sexes, not fully prevent the occurrence of relapses and re-infection. Also after the introduction of antibiotics given either by mouth or applied locally no permanent cure could be achieved. Treatment with e.g. tyrothrycine (Ibañez, 1951; Iger and Kupperman, 1955; Subert, 1957), terramycin (Greene, 1952; Kostič, 1954), aureomycin (Kostič, 1954; Bradburn, 1952) and others proved to be unsuccessful, although they were found to be highly inhibitive to growth or directly toxic in experiments *in vitro*; e.g. anisomycin 3–12 µg (Lynch *et al.*, 1955), actinomycin 200 γ/ml, chloramphenicol 125 γ/ml, oleandomycin 1000 γ/ml, iturin 250 γ/ml (Reginster *et al.*, 1958), tyrothricin and aureomycin 1:3000–30000, teramycine 1:10000–30000, chloramphenicol 1:25000–50000, tyrothricin 1:10000 (Lützenkirchen, 1952), thiolutin 0·2 µg/ml, neomycin 125–250 µg/ml (Seneca and Ides, 1953), sanazin 1:3500 to 10000 (Teras, 1959). No inhibitive effect was observed with penicillin and streptomycin (although both are used even for preparing bacteria-free cultures of *T. vaginalis*), bacitracin, griseofulvin, nystatin and triacetin (Barr and Brent, 1960; and others). The medium used in inhibition experiments should not contain too much serum, which greatly lowers inhibition. This fact was demonstrated in experiments by Körber and Fleckenstein (1954), who could not obtain inhibition with chloramphenicol 1:1000 after adding 50% of serum.

The findings by Magara *et al.* (1954) on the effect of oral application of the isolated antibiotic trichomycin, suggested the advantage of investigating further trichomonadocid substances which could be given by mouth. The first report by Magara *et al.* seemed most impressive: trichomycin 1:200000 completely inhibited *T. vaginalis* in the culture; 60000 IU of trichomycin given in daily doses to patients infected with trichomonads made the parasites disappear within 1–6 weeks, while after daily doses of 240000 IU the parasites disappeared after an average of 4 days. A total dose of 1 000 000 IU (100 000 IU daily) was supposed to cure trichomoniasis permanently. These experiments, repeated in Europe, did not confirm the successes achieved in oral treatment with trichomycin (Bauer, 1960; Bedoya and Fernandez Ortega, 1957; Caterall and Nicol, 1957; Durel, 1958; and others).

Recently reports became available on the highly trichomonadocidal effect of the antibiotic hamycin. According to some investigators the motility of trichomonads becomes arrested 10–30 min. after the application of a solution of 1–1·5 γ/ml; other writers report inhibition of growth in the Vf-bouillon with a dose of 0·5 γ/ml; a dose of 0·66 γ/ml kills the flagellates after 48 h. Hamycin seems to be five times as effective as trichomycin and Metronidazol.

The first systemic drug to be used in oral therapy was Aminitroazole (Tritheon = 2-acetylaminoo-5-nitrothiazol; Cuckler *et al.*, 1955), but in re-examinations by Caterall and Nicol (1957), Dunlop *et al.* (1958) and Thiery *et al.* (1960), disappointing results were obtained (toxic symptoms, successful cure in only 8–16%). This problem became definitively solved by the synthesis of Metronidazol (Cosar and Julou, 1959), developed from azomycin.



The first reports on a surprisingly successful oral therapy with Metronidazol—designated Flagyl were recorded by Durel *et al.* (1959) from France, and Sylvestre *et al.* (1959) from Canada. These results were confirmed by various authors (successful therapy in 90–100%). Today, Metronidazol is the best drug against *T. vaginalis*; its secondary effects are minimal (rarely vomiting, nausea, gastric complaints, sensation of dryness in the mouth, lingua pillosa, passing exanthema, foul taste) and never strong enough to discontinue the cure.

Metronidazol is a chemical drug (hydroxy-2'-ethyl-1-methyl-2-nitro-5-imidazol) produced by "Specia" under the name Flagyl, and also produced and sold by various firms under different names. Solubility in water is 1%, in ethanol, ether, chloroform 0·5%, pH of the saturated aqueous solution at 20°C is 5·8. Toxicity is very low: LD<sub>50</sub> for mice by mouth after a single dose is 4·3 g/kg (in comparison, Tritheon 0·63 g/kg). The maximum tolerable dose for mice (5 times daily) is 2·5 g/kg (compared with a daily oral dose of 0·2 g/kg Aminitroazole). Cultures of *T. vaginalis* are still killed with 1:400000 within 24 h (Bock, 1961). Tritheon becomes effective in doses of 1:75000, azomycin 1:25000. Inhibition of growth still occurs at a dilution of 1:1–13000000 (Durel and Roiron-Rattner, 1960; Watt and Jennison, 1960; and others). Daily treatment with 12·5 mg/kg by mouth prevents the formation of trichomonad abscesses after inoculation of *T. vaginalis* culture under the skin of mice, corresponding to about 1/200 of the LD<sub>50</sub> of the 5-daily doses. The urine remains trichomonadocid during the first 4 h after treatment even when diluted to 1:100–1000, the same applies for the blood serum 1:10. The urine contains 50–390 µg/ml, the blood 6–13 µg/ml, the saliva only 0·1–4 µg/ml.

Ten to 15% of the Metronidazol is eliminated by the urine in 48 h (Durel *et al.*, 1959). The dark urine of some patients receiving Flagyl is due to the presence of an azo-dye resulting from the condensation of two partially reduced Metronidazol molecules, probably following renal excretion of the metabolites (Manthei and Feo, 1964). Metronidazol has no bacterial activity and does not interfere with normal vaginal flora—*Lactobacillus vaginalis* develop also in the presence of 0·3% Metronidazol. It also has no antifungal activity, in contrast to trichomycin. Metronidazol also killed *Giardia lamblia*, *Trichomonas muris*, *Chilomastix mesnili*, *Entamoeba muris* and *Entamoeba histolytica*.

(Bock, 1961; Schneider, 1961; Mandoul *et al.*, 1961). Its action on *T. vaginalis* cultures is inhibited by purines (adenin, guanine, hypoxanthin, inosin, xanthin; Samuels, 1962).

Perju *et al.* (1963) studied the effect of Metronidazol on the ultrastructure of *T. vaginalis*. After 40 minutes of incubation in a 1:80000 solution the trichomonads showed the first alterations. Metronidazol penetrating the cell acts as a chemotoxic substance, the metabolic processes are paralysed, the plasma is lysed and disorganized, and the canalic membranes are altered. Perju and Strimbeanu (1964) showed that *in vivo* 99% of trichomonads are immobilized in 9–12 h, total destruction occurring in 13 h, while *in vitro* it occurs in 9 h without exception. Eight to 9 h after administration of Metronidazol round forms of *T. vaginalis*, condensed and contracted, appear; after 10–11 h important alterations occur on the cytoplasm, there is a loss of granules, an appearance of vacuoles, total cytosis at the periphery, pycnosis and caryorhexy of the nucleus; the end is a complete lysis of the parasite.

The number of leucocytes and of the pyogenic bacteria decreases rapidly within 24–48 h. After 4–5 days *Lactobacillus vaginalis* starts to multiply anew; 8–14 days later the leucocytes disappear almost completely and *Lactobacillus vaginalis* starts to reproduce heavily. After 6–10 days the colposcopic finding is normal. Only in about 22·6% of cases is there a partial reduction of leucocytes and a persistence of the mixed bacterial flora.

No essential changes either in the female or in the male blood proteins are brought about. Only in a few cases could a temporary decrease in the number of leucocytes be observed. Therefore it is necessary to examine the white blood count before Metronidazol treatment, and also in the course of it (Teras *et al.*, 1963b, c).

In treated patients pregnancy is normal and, up to present, no damaging effect of Metronidazol has been observed in newborn babies, although Metronidazol passes into the foetal circulation and is also present in relatively high doses in the milk of the mother (Bertrand, 1964; and others).

Rats treated with Metronidazol revealed no modification in fecundity, in duration of gestation, in number of rats per litter, in proportion of stillborn rats, in mortality during the first week of life, in malformations or changes of spermatogenesis. Only after 2 months and a daily dose of 1 g/kg did about half of the male rats show some lesions of limited extension, not inducing a decrease in fecundity (Gautier *et al.*, 1960).

The great and singular advantage of Metronidazol is its ability to kill *T. vaginalis* not only in the vagina but also in all extravaginal foci; the treatment is of short duration (250 mg 3 times daily for 7 days), highly effective, and the male partner can simultaneously be treated by mouth to forego possible re-infection right from the outset. There is some uncertainty about the possible resistance of *T. vaginalis* to Metronidazol. Jennison *et al.* (1961) could not observe any resistance *in vitro*, but de Carneri *et al.* (1963) described two therapeutic failures in 14 patients suffering from trichomonal vaginitis and treated both orally and locally with Metronidazol; this failure was attributed to natural resistance of the 2 strains of *T. vaginalis* to the drug. In experiments with mice a dose of 20 mg/kg, resp. 50 mg/kg was needed for both strains, in

comparison with only 5 mg/kg necessary for the other strains to avoid the formation of subcutaneous abscesses.

It is still uncertain whether Metronidazol may provoke vaginal mycosis (Rom *et al.*, 1961). In numerous cases treated with Flagyl, the occurrence of yeast-like organisms has been observed. Metronidazol is known not to effect pyogenic bacteria, and therefore the use of some bacteriostatic drug (e.g. sulphonamide intravaginally) is recommended. In keeping with the findings of Teras *et al.* (1963c) an intravaginal treatment with Metronidazol seems superfluous. The healing process is accelerated by an intravaginal application of combined drugs (e.g. Triflocid or Fluocid), at the same time preventing the occurrence of *Candidae* by the contents of boracic acid in the drugs. No definite decision has been made as yet on the necessity of implanting Döderlein *Lactobacilli* during Metronidazol treatment. In practice it would be difficult to ascertain that the "living" cultures are still viable at the time of implantation.

The oral therapy with Metronidazol has greatly eased treatment of trichomoniasis in the male and has therefore become most successful. There is no need to make urethro-vesicle irrigations using a variety of disinfectant on therapeutic agents. Metronidazol effects the urethra, the prostate and all other sites in the urino-genital system. We confirmed the 100% success of Metronidazol treatment on our own material. In every case it is necessary to treat the other sexual partner to prevent re-infection ("ping-pong infection"). Since the introduction of Metronidazol the use of all other trichomonadocidal agents has become superfluous. Further research may develop even more suitable oral substances which are less toxic.

## XI. EPIDEMIOLOGY OF TRICHOMONIASIS

The epidemiology of trichomoniasis is the object of two extensive reports by Kučera at Reims (1957) and Chappaz at Montreal (1960b), both convinced about the venereal transmission of *Trichomonas* infection. Concerning some publications from recent years we can summarize our knowledge as follows: The average frequency of *T. vaginalis* infection is about 10% in normal population (2–15% after Kučera, 1957). The percentage depends on the age—the maximum being achieved during the highest sexual activity (Mascall, 1954; and others); on the methods employed for detection; and on the experience of the authors. As an example of *T. vaginalis* distribution in central Europe we give the results of Petrú (1964): of 123 000 gynaecologically examined women in Bohemia and Moravia, 27.3% were found to be infected (material from gynaecological and obstetric departments). In the districts of Prague the percentage of the infected women was, in the years 1954–1956, 24.2%, and 10 years later 25.8%; the maximum number of cases occurring in 30–40 year-old patients. The transmission by sexual intercourse becomes evident when estimating the social situation. In married women coming to the gynaecological clinics the percentage of positive cases was 23.7%, in divorced women 48.2%! The authors assumed more sexual promiscuity in the divorced women. In female clerks the positivity was 28.1%, in female factory workers 28.9%, in unemployed women only 20.9%. The normal infection rate was demonstrated

TABLE IX  
*Distribution of Trichomonas vaginalis in healthy female population*

Age groups	England		USA				Poland		Czechoslovakia			
	Whittington (1951a)		Feo (1956)				Stroczyńska <i>et al.</i> (1961)		Jirovec <i>et al.</i> (1942)		Vojtěchovská and Petrů (1968)	
	Number positive	%	Number	%	Number	%	Number	%	Number	%	Number	%
15-19	13	7.7	—	—	—	—	1	—	93	5.3	44	11.4
20-29	321	3.1	38	2.6	38	10.5	52	22.6	69	17.3	107	16.9
30-39	1981	8.6	54	3.7	33	9.1	65	15.4	16	37.5	185	13.0
40-49	26	7.7	52	3.8	78	19.2	47	21.3	3	—	193	14.1
50 and over	5	0	295	2.8	152	10	34	17.7	—	—	147	7.5
Total	562		169		301		199		181		676	

in examinations of about 700 female workers of a factory in Central Bohemia—the positivity was 12·6% (Vojtěchovská and Petrů, in press). Petrů *et al.* (1956) performed *Trichomonas* examinations on 116 female patients suffering from psychosis. The positivity of 25% is in approximate keeping with the general gynaecological praxis. There was no striking coincidence between a positive toxoplasmine test and the incidence of *Trichomonas* infection.

TABLE X

*Distribution of Trichomonas vaginalis in women visiting gynaecological ambulatories, etc. (sick population)*

Age groups	Jírovec <i>et al.</i> (1942) ČSSR		Kučera and Král (1944) ČSSR		Ašmera and Linhart (1962) ČSSR		Engelbrecht and Müller (1962) DDR	
	Number positive	%	Number	%	Number	%	Number	%
15–19	15	20	33	42	144	25	—	13·1
20–29	18	35·5	336	31·8	278	47·2	—	16·5
30–39	72	41·6	185	42·8	273	34·4	—	19
40–49	54	22·2	35	68·6	122	36·8	—	21·8
Over 50	31	13	99	44·0	43	30	148	13·3
Total	252		498		860		820	

Coloured women showed an increase in positivity (Feo, 1953), due to bad hygienic conditions, more sexual promiscuity and the lack of contraceptives (Gray, 1961). After the menopause in negroes over 60 years about 10% were infected, in white people only 1%. Burch *et al.* (1959) showed in negroes a frequency nearly eight times higher than in white people, with highest prevalence in women of both races between the age of 30–50 years. Lambillon *et al.* (1954) found highly virulent infection in 20% of the white female population. In negro women the positivity was 40–50%, mostly without clinical symptoms. Mandoul and Fleurette (1950) noted the same infection as in Europe or the USA in Mohammedan women in North Africa (24%); here too the maximum incidence occurred during the sexual period of life.

The frequency of infection does not seem to change with the various seasons—in spite of the persistence of infections for many years. Only Glebski (1964), observing the frequency of *T. vaginalis* in the urine sediment of 40 000 males and females (1:1) for 5 consecutive years, found a positivity in 7–14% with a minimum infection rate from December to February and a maximum from August to November. His results should be confirmed by other methods, because the observation of urine sediment alone is not sufficient for such research.

The recent study of Gaudefroy and Vernier concerns 19 680 cases detected; and in the light of statistical determinations and a security limit of 95%, the

frequency diagrams between 1959 and 1961 have evidenced a maximal frequency in March, in July and in October–November. On the other hand, from 1962 to 1965 the seasonal variations disappear in a progressive manner. No plausible explanation may be advanced; however, if the venereal nature of this infestation is admitted, it may be noted that there exists a relation between the seasonal variations of Trichomoniasis and the frequency of marriages; this relationship having been encountered on the national scale and even abroad. Furthermore, these variations appear to have been influenced by the systemic trichomonicides, the use of which dates from 1960. In all cases, the frequency diagrams passed from 25–29% in 1960 to 11–13% in 1965.

Buxton (1958) found 221 female prisoners 70% positive; from 715 patients from a psychotherapeutic clinic only 15% were positive. Of 157 subordinated members of the staff of a girls' college none were positive. Herbst *et al.* (1960) examined 450 female prison inmates and found *T. vaginalis* in 62%. In older women and in those condemned many years earlier the infection was very rare; women older than 60 years were all negative.

Bogusz-Rożkowska and Zablotniak (1966) investigated prisoners (478 men and 111 women) confined for 1–24 months. Of the men 8·8% were infected with *T. vaginalis*, of the women 47·7%. In persons deprived of sexual intercourse the trichomonads may persist in their genito-urinary tract for at least 2 years.

Trichomoniasis genito-urinaria is a typical venereal infection, as almost unanimously accepted at the conferences at Reims (1957) and Montréal (1959) and also earlier (Bauer, 1943; Bedoya, 1957; Caterall and Nicol, 1960; Jírovec, 1957, 1960; Jíra, 1958; Dellepiane, 1957; Kean, 1955; Keutel, 1955–1957; Kostić, 1954; Kučera, 1957; Ottolenghi-Preti, 1957; Peter, 1945, 1957b; Teras *et al.*, 1961–1966; and others). Teras *et al.* (1961–1966), tracing the sexual contacts of numerous females and males, proved 61 infestation chains transmitting the infection by sexual intercourse. Of the 1135 examined women about 70% were positive, of the 998 men 36·6%. In the opinion of these authors the same measures as for any other venereal disease should be applied in the control of trichomoniasis. The further spread of *T. vaginalis* can only be prevented by regarding it as a venereal disease falling under the act of compulsory registration. Kaarma and Koplus (1963) determined that "conflicts in family are often caused by the fact that the wife does not recover from trichomoniasis in the course of several years, the consequences being dispareunia and other psychic disorders which often make family life impossible".

Extragenital contamination has not yet been proved in any of the cases, although it is possible. Experiments of Peter (1945) and Jírovec and Peter (1948a) showed, contrary to the claims of others (Rodecuret, etc.), that *T. vaginalis* is not resistant to freezing, temperatures above 44°C, drying and direct sunlight. Tests with *T. vaginalis* revealed that in urine at 20°C, inoculated with vaginal secretion, the majority of trichomonads remained viable after 9 h, whereas after 20 h only very few were still alive. In vaginal secretion applied to wood, brass, toilet paper, towels or bathing sponges the flagellates lived for 1–2 h, rarely 5–6 h. Contact with water killed them within 35–40 min (Fig. 16). Transmission of *T. vaginalis* takes place mainly through sexual intercourse,

exceptionally by indirect contact at toilets with towels etc. These findings were confirmed by many authors: Kessel and Thompson (1950) found that *T. vaginalis* persisted only a few hours, when droplets of the discharge were placed on the enamel surface of wooden blocks and tested under natural conditions of drying at room temperature. No survival was demonstrated after 7 h. Whittington (1958) demonstrated that *T. vaginalis* in vaginal discharge remained alive for only up to 45 min on polished surfaces of lavatory seats. Only 4 out of 30 patients with *T. vaginalis* left infective material on a toilet seat after they had used it. Burch *et al.* (1959) showed the possibility of transmission by means of communal use of fomites, by culturing *T. vaginalis* on pieces of wash cloth keeping them at room temperature for up to 23 h after being used for cleansing the external genitalia of infected women. Chappaz *et al.* (1961)

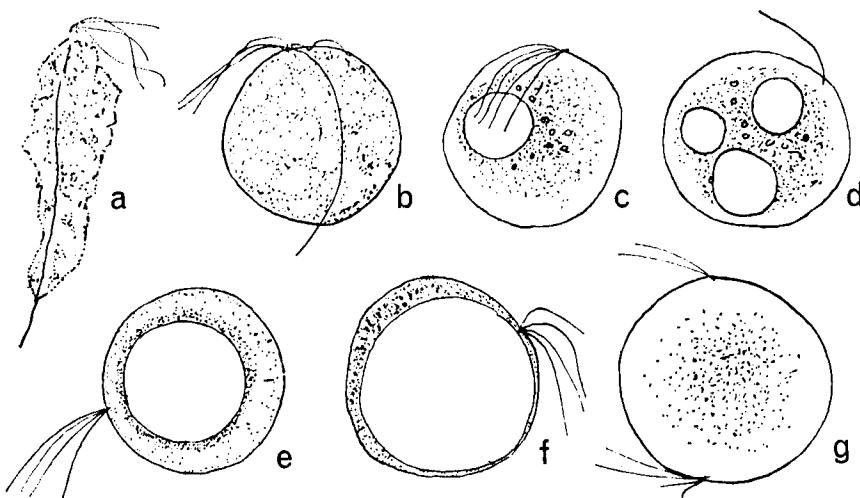


FIG. 16. *T. vaginalis*. *a*: in hypertonic solution, *b-g*: in hypotonic solution—vacuolization and total degeneration. (After Jírovec and Peter, 1948a.)

showed in 30 observations, that the survival time of *T. vaginalis* on clothes does not exceed 4 h providing there is desiccation. The authors have been brought almost to the point of denying the possibility of transmission of *T. vaginalis* from one woman to another by clothes, underclothes or sanitary towels. In another paper Chappaz *et al.* (1961) emphasize that extragenital infection has not yet been proved in any case. Paralleling the opinion of Whittington, the authors denied emphatically any possibility of contamination by toilet seats.

Also the short survival time of *T. vaginalis* in fresh water, described by Weiler (1938), Wu (1938), Morenas (1945), Peter (1945) and Jírovec and Peter (1948a), was confirmed in new publications by Teras *et al.* (1961). The rapidity of the destruction of *T. vaginalis* depends on the concentration of mineral salts in the

water. In the water supply in Tallinn the lysis of *T. vaginalis* occurred within 10 min, in water of swimming pools the trichomonads perished in 10–30 min, in soap-solution of 0·15–0·3% they die immediately. Infection by *T. vaginalis* in water is impossible and the authors see no reason to have any doubts on the venereal origin of this infection. Also Sarosiek (1967) could not prove the presence of *T. vaginalis* in culturing water from artificial and natural swimming-pools in Szczecin—such water cannot be regarded as harbouring trichomonads. The failure to obtain *T. vaginalis* cultures may be caused by its rapid spread in water, its short life and by chlorine disinfection of artificial water-ponds.

Čatár *et al.* (1967) admitted the possibility of transmission by the thermal waters of a spa used for the treatment of gynaecological affections (Lúčky, near Ružemberok). On admission to the spa there were 15·6% *trichomonas*-positive women. After the treatment for the entire period investigated, a total of 22·7% of females were positive. Three of the 16 examined strains of *T. vaginalis* survived in mineral waters for as long as 22–72 h with well preserved motility and multiplication capacity. Four of the examined strains lived for only 35–55 min in the thermal water. Recovery isolation of *T. vaginalis* was successful at the end of 15 and 45 min from the beginning of thermal water treatment. (One factor was forgotten by the authors—the possibility of sexual promiscuity in the spa and fresh infection, or reactivation of an old, until now latent, infection with *T. vaginalis*.)

The opinion of Depoorter (1959) that the toxicity of the urine for *T. vaginalis* explains the spontaneous cure of urethritis, is certainly wrong. Not only is diluted urine an excellent medium for maintaining *T. vaginalis* for some time, but also the infection of the urinary tract persists for many years. In diluted urine the viability of *T. vaginalis* is retained for a longer time than in water (Jirovec and Peter, 1948a).

In spite of the low resistance of *T. vaginalis* to external factors (low osmotic pressure, temperature up to 25°C, sunshine, drying etc.) and the absence of true cysts, the transmission by other routes than sexual intercourse must be extremely rare and the principal pathway of the infection in both sexes is the sexual one.

## APPENDIX

### MEDIA FOR CULTIVATION OF *T. vaginalis*

#### A. CONTAINING SERUM

##### 1. Johnson CPLM medium

Bactopeptone 32 g, bactoagar 1·6 g, L-cysteinchloride 2·4 g, maltose 1·6 g, liver infusion (Disco, made as directed on bottle) 320 ml, Ringer solution (NaCl 0·6%, NaHCO<sub>3</sub>, KCl, CaCl<sub>2</sub> each 0·01%) 960 ml, NaOH N/1 10–13 ml are boiled 10 min to melt agar; filter through coarse paper. Add 0·7 ml of 0·5% aqueous methylene blue. Adjust to pH 5·8–6. Tube in 8 ml amounts in medium size tubes, plug tubes and autoclave 15 min. After cooling add 1 ml

of sterile (filtered) human serum. Incubate at least 4 days. This medium is stored at room temperature and discharged when the blue zone of unreduced methylene blue extends past the middle of the column of fluid, usually after 2-3 weeks.

Petrú (1961) developed a method for storing this medium to be used in routine diagnosis of *T. vaginalis* infection: instead of tubes, transfusion flasks of 250-500 ml are used for storage of the ready-made medium CPLM. The rubber stoppers are pierced with a thick injection needle and the flasks are steam-sterilized at 100°C. Then the injection needle is removed from the hot flasks to prevent non-sterile air from entering the flasks during cooling. Sterilization in steam (1 h) is repeated for 2 consecutive days without replacing the injection needles. After cooling, 10% of human or sheep serum is added (by injection needle) to the sterile medium, and the flasks can then be kept at +2°C for at least 3 months. Shortly before use, 1 000 IU of crystallized penicillin and 1 mg streptomycin are added to 1 ml of medium, and Hall tubes are filled with this mixture to about 6 cm height. Fresh media are yellowish-green in colour; when the colour of the medium turns bluish-green, they are no longer fit for use. No differences have been found between the use of a fresh and a 3-month-old medium.

#### 2. *Trypticase medium after Sprince and Kupferberg (1947)*

Dissolve 1 g maltose and 1 g bactoagar by boiling in 400 ml distilled water and filter through filter paper. Then dissolve 20 g trypticase BBL, 1.5 g cysteinchloride and 0.48 ml of 0.5% methylene blue and 600 ml of Ringer solution. After correcting the pH to 6 with N/1 NaOH, dispense the medium (9.5 ml) into sterile tubes and autoclave. Before use add 0.5 ml sterile serum to each tube.

#### 3. *Medium after Feinberg (1953), sterilized by filtration*

Proteolysed liver Panmede 25 g, NaCl 6.5 g, dextrose 5 g, inactivated horse serum 80 ml, dist. water 1 000 ml, penicillin 1 million IU, streptomycin 0.5 g, pH adjusted to 6.4 by adding approximately 9 ml N/1 NaOH per litre. The mixture is sterilized by Seitz-filter and stored in screw-capped bottles in a refrigerator. The medium can also be dried or freeze-dried. After 3 months' storage at +4 to 5°C no deterioration of the antibacterial power is evident. For diagnosis 7 ml of this medium are filled in glass tubes and examined 4-5 days after inoculation. If only few trichomonads are present, it is desirable to centrifuge the cultures before examination.

#### 4. *Senton's modification of the Feinberg's medium*

2 g ascorbic acid, 1.2 g L-cysteinchloride, 5 g glucose, 0.5 g Oxoid-liver dry extract are dissolved in 920 ml Hartley digest broth, pH corrected to 6. The mixture is autoclaved. Before use add 80 ml horse serum, 0.5 g streptomycin and 500 000 units penicillin-G and pour into tubes.

5. *Vf-bouillon after Magara et al. (1953)*

Hacked beef liver 50 g, 10% HCl 10 ml, bactopepsin 0.5 g, dist. water 1000 ml are heated after mixing for 24 h at 48°C, stirring occasionally. Next day the solution is heated for 10 min at 80°C, filtered through filter paper and heated once more for 15 min at 100°C. After correcting the pH to 5.4 with 10% NaOH, the solution is heated for 25 min to 100°C, and after filtering it is poured into Hall's culture tubes, sterilized by heating for 30 min at 100°C for 3 consecutive days and then stored at +2°C. Glucose as a 3% sterile solution is added to make the glucose content 0.5%. Sterile serum is added to the extent of 10%. Penicillin (1000 IU) and streptomycin (1 mg/ml) could be added together with the glucose.

6. *Medium after Diamond (1957)*

20 g trypticase BBI, 10 g yeast-extract, 5 g maltose, 1 g L-cysteinhydrochloride, 0.2 g ascorbic acid, 0.8 g K<sub>2</sub>HPO<sub>4</sub> are dissolved in 900 ml dist. water, pH corrected to 6, 0.5 g bactoagar added and the mixture autoclaved. After cooling to 48°C add 100 ml serum, 1 million IU penicillin-G and 1 g streptomycin-sulphate, fill in sterile tubes (5 ml). Can be stored for 3–4 weeks in refrigerator. Before use heat tubes in water-bath to 35–37°C.

7. *Medium after Roiron-Ratner (1957/8)*

20 g peptone, 1 g asparagine, 5 g glucose, 10 ml liver extract, 490 ml meat extract, 2 g cryst. Na<sub>2</sub>HPO<sub>4</sub>, 2.5 g NaCl, 1 g ascorbic acid, 500 ml dist. water are poured into tubes (5 ml), autoclaved and before use mixed with 1 ml inactivated horse serum and the standard dose of penicillin and streptomycin. pH = 6. Supposed to be most suitable for cultivation of *T. vaginalis* from the genitals of the male.

8. *Medium after Pavlova (1938)*

Is used in some East-European countries: 8.5 g NaCl, 1.469 g Na<sub>2</sub>HPO<sub>4</sub>, 12 H<sub>2</sub>O, 0.45 g KH<sub>2</sub>PO<sub>4</sub>, 1000 ml dist. water are dissolved and filled in tubes (5 ml) and autoclaved. 1 ml horse serum and 2 loops of sterile rice starch are added to each tube. Suitable especially for primary culture. Trichomonads grow together with bacteria.

## B. WITHOUT SERUM

9. *Medium after Jirovec and Peter (1948)*

Used Johnson CPLM-medium without serum but added a small piece of cooked rabbit or guinea-pig liver (2 × 0.5 × 0.3 cm) to each tube, pH = 6–6.2. Sterilization 3 times for 1 h at 100°C. Penicillin and streptomycin are added before use.

10. *Medium after Sorel (1954)*

Used a pepton-bouillon (pH = 6.2) with a small piece of meat (1 × 0.5 × 0.5 cm) in each tube, sterilized by autoclaving 20 min at 120°C. Antibiotics added before use. Microscopical examination 3 days after inoculation, transmission after 7 days.

### 11. Medium after Samuels and Bell (1962)

They devised a new serum-free medium C-6 containing cream: 28 g trypicase BBL, 3 g dry yeast extract, 0·075 g  $\text{KH}_2\text{PO}_4$ , 1·5 g glucose, 0·08 g citric acid, 0·4 g ascorbic acid, 0·2 g thiomalic acid, 25 ml 10% liver-powder infusion, 0·1 ml light-cream (table or coffee cream), 0·08 g cholesterol, dissolved in 4 ml ethylalcohol. The dry ingredients are added to 800 ml dist. water, then the liver extract and cream, and finally cholesterol are added to the medium heated at 50°C. N/1 KOH is added to reach pH 6·8 and the volume brought up to 1000 ml. After standard autoclaving this medium retains nutritional value for at least 3 weeks, when stored at room temperature in the dark. Approximately  $5 \times 10^4$  organisms are transferred weekly to 10 ml of medium in screw-capped tubes. *T. vaginalis* attains a population density of  $10 \times 10^4$  organisms/ml and above.

### 12. Medium after Barbarowski (1966)

Used for primary cultures of *T. vaginalis*: 3·5 ml Ringer solution (1000 ml dist. water, 8·6 g NaCl, 0·3 g KCl, 1 g  $\text{CaCl}_2$ ), 1 ml 10% glucose, 0·5 ml 10% ascorbic acid. The growth is sometimes very rich, subcultures are usually not possible.

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