THE SURVIVAL OF *TRICHOMONAS VAGINALIS* AT TEMPERATURES BELOW +37° C.

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CONTENTS

I. INTRODUCTION

Although cultures of *Trichomonas vaginalis* Donné cease to multiply below +25° C. (Johnson & Trussell, 1944), there is evidence that they survive and can regain their normal reproductive capacity when the temperature is raised to a suitable level. If the parasites are to survive outside the host's body, the most obvious change with which they will have to contend will be that from a constant temperature of +37° C. to lower and fluctuating temperatures. Very little is known about their reactions to these conditions. Moisture is essential for the survival of the flagellates, which die instantly if dried, and it is important to know if they can tolerate dilution with water. The accounts in the literature concerning these factors are conflicting. Before summarizing them briefly, I must explain that I am concerned, not with the effect of controlled experimental conditions on *T. vaginalis*, but first with its capacity to survive at the fluctuating 'room temperatures' which it would encounter if exposed in houses and public conveniences in this country, and secondly with its reaction to dilution with water at similarly varying temperatures.

(i) *The survival of* Trichomonas vaginalis *at or near* 0° *C.*

Rodecurt's (1934) claim that his cultures withstood repeated freezing and thawing cannot be taken seriously; his descriptions and figures suggest he was dealing with a yeast. Mohr (1937) and Jírovec & Peter (1948) are the only other authors who claim that *T. vaginalis* can withstand freezing. The former obtained subcultures

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from tubes which had been kept at unknown room temperatures for 8 days and then frozen for 24 hr., while the latter authors state that trichomonads in vaginal material remained viable after freezing for 5 min. at about −20° C., but died after 10 min. at this temperature. Fukushima (1934) kept his cultures alive in an ice-box of unknown temperature for 3 days, and Matsuda (1935) found that flagellates in cultures exposed to 4–2° C. (it is not clear whether these temperatures were above or below zero) were still viable after 3 days. Fischer (1935) kept cultures alive for 21 days at temperatures of +3 to +5° C., and Jírovec (1941–4) for 2–3 weeks at +6 to +10° C.; both obtained successful subcultures thereafter, though Jírovec reports that only a few trichomonads survived. Johnson & Trussell's (1944) bacteria-free strain lived for 4–6 days at a temperature of +1 to +4° C. but was killed by exposure for 1 min. to a freezing mixture at −72° C., though it apparently survived at this temperature for shorter periods.

(ii) *The survival of* Trichomonas vaginalis *at room temperature*

Fukushima (1934) and Mohr (1937) report the survival of *T. vaginalis* at unspecified room temperatures for 7 and 8 days respectively. Cultures of bacteria-free *T. vaginalis* survived for 13 days at room temperature (Trussell, 1947). On the other hand, Weiler (1938) failed to obtain subcultures from organisms kept at +18 to +20° C. for 36–48 hr., and Jírovec (1941–4) found that all his parasites died after 2–3 days at +20° C.

Clinically these last results are most important, as they refer to a temperature range closely resembling the uncontrolled conditions with which the exposed parasites might have to contend in nature. Unfortunately, it is in this temperature range that the evidence is most conflicting and vague. We do not know to what extent the type of culture medium used, the accompanying bacterial flora, and variations in sensitivity among the strains themselves affect the survival of *T. vaginalis*, and are responsible for inconsistent results.

(iii) *The survival of* Trichomonas vaginalis *in water*

Records of the effect of water on the survival of *T. vaginalis* at room temperatures suggest that this parasite can endure such conditions for a limited time only. Fukushima (1934) reports that the flagellates can survive for 9 hr. in distilled water, although Matsuda (1935) found that death occurred in less than 6 hr. in this medium. Ying Wu, in 1938, investigated the survival times of *T. vaginalis* in river water and discovered that trichomonads from cultures and in vaginal secretion lived for 6–7½ hr. in stationary tubes of water. Jírovec, Breindl, Kučera & Šebek (1942) found that *T. vaginalis* can survive in tap water at +20° C. for 12–16 hr. at the most, while Morenas, in the same year, states that the flagellates are dead after 5 hr. in a similar medium at +18° C. According to Jírovec & Peter (1948) all the trichomonads in vaginal material were dead after 35–40 min. contact with tap water.

I have been unable to find any records of the behaviour of *T. vaginalis* in water at lower temperatures.

The following account records the survival of trichomonads in freshly isolated

and in well-established cultures, and in vaginal material, undiluted or mixed with tap water, when stored in a cellar whose temperature varied between +3·3 and +15·5° C. The survival times of a few strains of *T. vaginalis* in vaginal exudate at temperatures near freezing-point have also been investigated.

II. MATERIALS AND METHODS

The strains of *T. vaginalis* were obtained from patients attending the Exeter Women's Welfare Clinic. Samples of exudate were removed from the vaginal walls with small plastic spoons (Jackson, Malleson, Stallworthy & Walker, 1948), and inoculated into tubes of culture medium which were incubated at temperatures ranging from +28 to +37° C. It was necessary to subculture the strains every 3–7 days.

(i) *Culture medium*

The culture medium used was a modification of Dobell & Laidlaw's 'HSre' medium (1926), dextrose being added as recommended by Boeck & Drbohlav (1925). I have found the most satisfactory concentration of dextrose to be 0·5%. This modified 'HSre' medium, which will be referred to as 'HSre+*d*', had a pH range of 6·85–8·8. Strains of *T. vaginalis* have been maintained in excellent condition in this medium for more than 12 months. As, in every case, an accident caused the death of the culture, there is good reason to believe that strains can be continued for an indefinite length of time in this medium.

The types of vaginal bacterial flora were divided into the three grades commonly used by gynaecologists (Schröder, 1921; Cruickshank & Sharman, 1934; Liston & Liston, 1939; *inter alia*). A Grade I bacterial flora consists almost entirely of Döderlein's bacilli—the normal inhabitants of a healthy vagina; Grade II consists of a mixture of Döderlein's bacilli and other types of bacteria; and Grade III comprises various kinds of bacteria with no, or only very few, Döderlein's bacilli.

(ii) *Strains of* Trichomonas vaginalis *used*

Strain S was isolated on 5 March 1948 from an unmarried woman with a troublesome vaginal discharge. The exudate contained numerous active trichomonads accompanied by a Grade III bacterial flora. This strain always grew well in culture and was maintained for 17 months and 25 days through 161 transplants.

Strain Ho was isolated from a married woman on 1 November 1948. The vaginal flora in this case was also Grade III. This strain was maintained in culture for 6 months and 19 days, through fifty-six subcultures.

Strain K. The patient from whom this strain was isolated had been suffering from a persistent and troublesome discharge, which had resisted all treatment for some years. Samples of her vaginal fluid were obtained on many occasions. The trichomonads always grew well in culture, and the accompanying flora, which contained numerous Döderlein's bacilli, was generally Grade II and sometimes even Grade I. A culture from vaginal material obtained on 19 April 1949 was maintained for 12 months through eighty subcultures at a temperature of +37° C. The occasional addition of sterile rice starch to the culture medium was necessary

to keep this strain in a healthy condition. In addition to the culture, fresh vaginal material from this patient was available for experimental purposes.

The woman from whom *Strain F* was isolated had no symptoms of vaginal disorder. A Grade III flora accompanied the numerous active trichomonads in the vaginal material.

Strain X was isolated from a patient with a vaginal discharge. The infection in this case was not a heavy one. The bacterial flora comprised Grade III organisms.

The strains used in Exps. 4, 5 and 6 consisted of trichomonads in vaginal material from twenty-two different patients, and are listed in Tables 1–3. The heaviness of the infection and the accompanying bacterial flora in each case are recorded in these tables.

(iii) *Experimental methods*

In the experiments described in §III(i), trichomonads from actively growing 2- or 3-day-old subcultures, or from vaginal exudate containing the parasites, were inoculated into tubes of 'HSre+*d*' medium at room temperature. These tubes were placed immediately in the cellar, the maximum and minimum temperatures of which were recorded daily. Other tubes were inoculated from the same sources and placed in the incubator to serve as controls. At daily intervals, a little of the material from the bottoms of the experimental tubes was withdrawn with a Pasteur pipette, a drop examined microscopically, and the rest inoculated into tubes of fresh medium previously warmed to +32° C. (strains S, Ho, F and X) or +37° C. (strain K), incubated at those temperatures, and examined on three successive occasions during the following 6–8 days. The parent culture tubes stored in the cellar were transferred to the incubator in groups of three on successive days (Exps. 1 and 2), or all together at the end of the cooling period(Exp. 3), and were examined microscopically at intervals during the succeeding week for living flagellates.

After the control tubes had been inoculated in the experiments described in §III(ii), the spoons of vaginal material were placed in test-tubes plugged with damp cotton-wool to prevent the exudate from drying, transferred immediately to the cellar or refrigerator and the temperatures recorded. At daily intervals, a small quantity of the vaginal exudate was examined microscopically in Ringer's solution at cellar temperature. A tube of culture medium, previously warmed to +37° C., was inoculated with a loopful of the exudate, incubated at +37° C. and examined three times during the succeeding week for living trichomonads.

III. EXPERIMENTS AND RESULTS

(i) *The effect of temperatures in the range* +5·9 *to* +15·5° C. *on the survival of* Trichomonas vaginalis *in culture medium*

The following three experiments were designed to determine for how long well-established and freshly isolated cultures of *T. vaginalis* remained viable at natural, i.e. fluctuating and uncontrolled, temperatures ranging from +5·9 to +15·5° C.

Exp. 1. Twelve tubes of medium were inoculated with trichomonads from the 73rd subculture of strain S, which was 8 months and 10 days old. Three of the tubes

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were incubated as controls, and the remaining nine tubes placed in the cellar. During the following 3 days, which constituted the experimental period, the cellar temperature fluctuated between +7·2 and +13·0° C., a range of 5·8° C.

After 1 day in the cellar, living but sluggish flagellates were observed microscopically in eight of the nine tubes. Subcultures were made from three of the tubes, including the one in which no living flagellates could be seen, and the subcultures and parent tubes were incubated at +32° C. (±4° C.). During the succeeding week, living flagellates were found in the three subcultures and in the three parent tubes.

After 2 days in the cellar, microscopic examination of the six remaining experimental tubes showed living flagellates in only one of them. Subcultures were made from three of the tubes in which the organisms were apparently dead, and these subcultures and parent tubes were also incubated at +32° C. During the succeeding week, living flagellates were recovered from all three parent tubes and from two of the subcultures.

After three days in the cellar, no living flagellates could be seen in the three remaining experimental tubes. Again subcultures were made, and the subcultures and parent tubes incubated at +32° C. After 5 days' incubation, living trichomonads were observed in two of the three parent tubes, but none of the subcultures was positive for *T. vaginalis*.

Throughout the experimental period, flagellates were present in the incubated control tubes, and subcultures made from them on the third day were positive for trichomonads.

Exp. 2. Exp. 1 was repeated using a different strain of *T. vaginalis* and prolonging the experiment for 5 days.

Eighteen tubes of medium were inoculated with the 30th subcultures of strain K which had been growing in artificial medium for 6 months and 11 days. Three of the tubes were incubated as controls, the remaining fifteen being placed in the cellar. At daily intervals, a different set of three of these cooled tubes was examined, subcultures were made from them, and the subcultures and parent tubes were incubated at +37° C. The cellar temperature varied between +10·5 and +15·5° C.—a range of 5·0° C.—during this 5-day experimental period.

After 1 day in the cellar, although only a few inactive trichomonads were seen in one of the three experimental tubes examined, living flagellates were recovered from all three of these tubes after they had been incubated for 3 days at +37° C.

After 2 days in the cellar no living trichomonads could be demonstrated in the next set of three experimental tubes, but living flagellates appeared in one of the tubes after 3 days' incubation.

Similarly, after 3 days, living trichomonads could not be seen in the third set of cooled tubes; but one of these tubes, on subsequent incubation, was found to contain living flagellates.

After 4 and 5 days in the cellar, no living trichomonads could be found in the remaining sets of experimental tubes, either before removal from the cellar or after incubation for 1 week.

None of the subcultures made from any of the cooled tubes was positive for

T. vaginalis, although numerous active flagellates were present in all the fifteen subcultures inoculated from the three control tubes during these 5 days. This failure to obtain subcultures was probably due to the small size of the inoculum. It is generally found that a fairly substantial inoculum is necessary to initiate cultures of *T. vaginalis*, especially when the number of living trichomonads in the inoculum is not great.

In this experiment, strain K survived at temperatures between +12·0 and +15·5° C. (a range of 3·5° C.) for 3 days.

Exp. 3. An attempt was made to compare the survival time of four different culture strains of *T. vaginalis*, two of which were well established, and two freshly isolated.

The strains used were:

Strain S (used in Exp. 1) now 13 months old. 116th subculture.

Strain Ho, which had been cultured for 4 months and 11 days. 36th subculture.

Strains F and X, which were isolated from the patients 4 hr. before the experiment was started. (See §II(ii) for details of these strains.)

Each strain of *T. vaginalis* was inoculated into three tubes of culture medium; one tube was incubated at +32° C. (±4° C.) as a control, and the other two were placed in the cellar, giving totals of four controls and eight experimental tubes. The contents of every tube were examined microscopically and subcultures made from them on each of the 4 succeeding days; at the end of this experimental period the cooled tubes were transferred to the incubator. The cellar temperature during this 4-day period ranged from +5·9 to +7·0° C.—a variation of 1·1° C.

After 1 day in the cellar, a few feebly moving flagellates were recovered from both of the strain S tubes (cf. Exp. 1). Though the organisms in all the other cooled tubes appeared to be dead, living trichomonads were found, after incubation, in the subcultures from both of the strain S and from one of the strain X parent tubes.

After 2 days in the cellar, microscopic examination of the experimental tubes failed to reveal any living trichomonads, and only one subculture, that from one of the strain S parent tubes, was positive for *T. vaginalis*.

The findings after 3 days at cellar temperatures were similar to those obtaining after 2 days. No living trichomonads could be demonstrated microscopically in any of the parent tubes, and again living flagellates subsequently appeared only in the subculture made from the strain S parent tube whose transplant, made after 2 days, yielded living trichomonads.

After 4 days in the cellar, all the trichomonads in the experimental tubes appeared to be dead, and no living flagellates were recovered from the parent tubes or from subcultures made from them on this day and then incubated for 1 week.

Living trichomonads were not found in any of the parent tubes after these had been removed from the cellar and incubated at +32° C. (±4° C.) for 1 week.

All the subcultures made from the control tubes on each of the 4 days contained active trichomonads except the first-day transplant from strain F in which no living flagellates could be found.

In this experiment strain S survived for 3 days, and strain X for 1 day, at temperatures between +5·9 and +7·0° C., a range of 1·1° C.

(ii) *The effect of temperatures in the range* −4·0 *to* +14·0° C. *on the survival of* Trichomonas vaginalis *in vaginal exudate*

The previous experiments show that certain strains of *T. vaginalis* can withstand fluctuating low temperatures for as long as 3 days if they are in a culture medium particularly favourable to their continued survival. In order to simulate more closely the conditions with which the trichomonads would have to contend if exposed in nature, the effect of low temperatures between −4·0 and +14·0° C. on the survival of flagellates in vaginal exudate, A, undiluted, and B, mixed with an equal quantity of cold tap water, was investigated.

A. *The survival of* Trichomonas vaginalis *in undiluted vaginal exudate*

(*a*) *At temperatures between* +5·0 *and* +14·0° C. *Exp.* 4. Table 1 shows the results which were obtained using the methods described on pp. 402, 403 when eleven

Table 1

Vaginal exudate containing *T. vaginalis*—undiluted

Strain	Flora Grade	Before cooling: Degree of *T. vaginalis* infection	Before cooling: Cult.	After cooling for 1 day: Micr. exam.	1 day: Cult.	2 days: Micr. exam.	2 days: Cult.	3 days: Micr. exam.	3 days: Cult.	Cellar temperature range in ° C.
Hw	III	Heavy	+	⊙	−	⊙	−	.	.	+10·5 to +14·0
L	III	Light	−	⊙	−	⊙	−	.	.	+ 9·5 to +11·7
K	II	Heavy	+	⊙	−	⊙	−	−	−	+ 9·0 to +11·0
	II	Light	+	⊙	−	⊙	−	⊙	−	+ 6·6 to + 9·5
Bn	II	Light	+	⊙	−	⊙	−	.	.	+ 5·0 to + 8·3
Mr	III	Heavy	+	⊙	−	⊙	−	.	.	+ 8·3 to +11·1
W	III	Medium	+	⊙	−	⊙	−	.	.	
R	III	Heavy	+	⊙	+	⊙	−	.	.	+ 9·4 to +10·6
Pa	III	Medium	+	±	+	⊙	+	.	.	
Pe	III	Light	+	⊙	−	⊙	−	.	.	
C	III	Heavy	+	⊙	−	⊙	−	.	.	+10·0 to +12·8

In this table and the two following:
+ denotes active living flagellates present,
± denotes a few sluggishly moving trichomonads present,
⊙ denotes all the trichomonads apparently dead, and
− denotes no trichomonads present.

A heavy infection showed several flagellates in a single high-power microscope field (×420 magnification).

A medium infection showed about one flagellate in 1–5 high-power fields.

A light infection showed about one flagellate in 10–15 high-power fields.

Micr. exam. = microscopic examination.

Cult. = culture.

spoons containing vaginal material from ten different patients were kept in the cellar for 2–3 days. Trichomonads in one sample lived for 1 day, and in another for 2 days, at temperatures between +9·4 and +10·6° C.

(*b*) *Survival at temperatures between* −4·0 *and* +12·2° C. *Exp.* 5. Spoons of vaginal exudate placed in a refrigerator enabled the effect of temperatures near freezing-point on the survival of *T. vaginalis* to be estimated. Out of six samples,

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one survived for 2 days between −4·0 and 0·0° C., and one lived for 2 days between +2·2 and +8·9° C. (see Table 2).

B. *The survival of* Trichomonas vaginalis *in vaginal exudate diluted with tap water at temperatures between* +3·3 *and* +10·6° C.

Exp. 6. The survival time of trichomonads in vaginal material mixed with an equal quantity of cold tap water and placed in the cellar is shown in Table 3. One

Table 2

Vaginal exudate containing *T. vaginalis*—in refrigerator

		Before cooling		After cooling for 1 day		After cooling for 2 days		After cooling for 3 days		
Strain	Flora Grade	Degree of *T. vaginalis* infection	Cult.	Micr. exam.	Cult.	Micr. exam.	Cult.	Micr. exam.	Cult.	Refrigerator temperature range in ° C.
Mk	III	Heavy	+	±	+	⊙	+	.	.	−4·0 to 0·0
N	III	Light	+	⊙	−	−	−	.	.	+0·5 to + 4·0
Mc	III	Heavy	+	⊙	+	⊙	+	⊙	−	+2·2 to + 8·9
Hr	III	Light	−	⊙	−	⊙	−	.	.	+1·7 to +12·2
	III	Heavy	+	⊙	−	⊙	−	.	.	+3·0 to + 3·5
Bw	II	Heavy	+	⊙	−	⊙	−	⊙	−	

Table 3

Vaginal exudate containing *T. vaginalis*—mixed with cold tap water

		Before cooling		After cooling for 1 day		After cooling for 2 days		After cooling for 3 days		
Strain	Flora Grade	Degree of *T. vaginalis* infection	Cult.	Micr. exam.	Cult.	Micr. exam.	Cult.	Micr. exam.	Cult.	Cellar temperature range in ° C.
Hd	III	Medium	+	± (1 trich. seen)	−	⊙	−	⊙	−	+7·8 to +10·0
K	II	Heavy	+	⊙	−	⊙	−	.	.	+3·3 to + 6·1
Mo	III	Medium	+	⊙	−	⊙	−	−	−	+5·0 to + 7·8
Mm	III	Medium	+	⊙	−	⊙	−	.	.	
J	III	Heavy	−	⊙	−	⊙	−	.	.	+5·0 to + 8·3
C	III	Heavy	+	±	+	⊙	+	⊙	−	+4·4 to + 6·7
D	III	Heavy	+	⊙	−	⊙	−	.	.	+8·9 to +10·6
Bk	II	Medium	+	−	−	−	−	.	.	
Hw	III	Heavy	−	⊙	−	⊙	−	.	.	+8·3 to +10·0
Br	II	Heavy	−	⊙	−	⊙	−	.	.	+8·9 to +10·0

out of the ten strains employed remained viable for 2 days at temperatures between +4·4 and +6·7° C. Although one feebly moving flagellate was seen on microscopic examination of strain Hd after 1 day at cellar temperatures, a positive culture of *T. vaginalis* was not obtained.

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IV. DISCUSSION

The foregoing experiments show that, though different strains of *T. vaginalis* vary in their resistance to temperatures below +37° C., some strains can survive in a culture medium for 3 days at fluctuating low temperatures between +5·9 and +15·5° C. (Exps. 1, 2 and 3), while trichomonads in undiluted vaginal exudate can live for 2 days between −4·0 and +10·6° C. (Exps. 4 and 5). The flagellates can remain viable for 2 days in vaginal secretion mixed with tap water and stored at temperatures between +4·4 and +6·7° C. (Exp. 6).

The recovery of viable cultures from material in which all the trichomonads appeared to be dead on microscopic examination is significant, as such material could presumably be infective. Plainly, an assessment of infectivity based on microscopic examination alone is not reliable.

In recent years, attention has been drawn increasingly to the presence of *T. vaginalis* in the male urino-genital system (Pattyson, 1937; Liston & Lees, 1940; Feo, 1944; Roth, 1944; Freed, 1948, *inter alia*). These discoveries emphasize the venereal character of the disease; yet while it is probable that many *T. vaginalis* infections in women have been acquired during coitus, there remain instances, notably among young girls and women whose denial of intercourse seems trustworthy, which require a different explanation. Jírovec & Peter (1948) found that some of the trichomonads in vaginal secretion applied to wood, brass, toilet paper, towels or bathing sponges remained viable for 4–6 hr., and my own experiments show that *T. vaginalis* is sometimes able to survive adverse conditions of temperature and dilution with water for periods long enough to enable transference from one woman to another to occur. The evidence indicates that infection by contact other than sexual, though never actually proven, is at least a possibility.

V. SUMMARY

When *Trichomonas vaginalis* in culture medium was exposed in three successive experiments to temperatures fluctuating between +7·2 and +13·0° C., between +10·5 and +15·5° C. and between +5·9 and +7·0° C., two strains survived for 3 days but no longer. One other strain used in these experiments lived for 1 day only, and two other strains did not survive for a single day.

One strain, kept in undiluted vaginal exudate, survived exposure to temperatures between +9·4 and +10·6° C. for 2 days but no longer, and one other strain lived for 2 days at temperatures between +2·2 and +8·9° C. Most strains do not live for as long as a day under these conditions.

One strain, kept in vaginal exudate diluted with an equal volume of tap water, survived exposure to temperatures between +4·4 and +6·7° C. for 2 days.

One strain in undiluted vaginal exudate survived freezing between −4·0 and 0·0° C. for 2 days.

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