

TABLE I.  
Comparison of Cells Derived from Normal and Irradiated Yeast Cells.

|                                                                           | Exp. 1   |            | Exp. 2   |            |
|---------------------------------------------------------------------------|----------|------------|----------|------------|
|                                                                           | Normal   | Irradiated | Normal   | Irradiated |
| Cells per cmm of culture (N)                                              | 101,300  | 46,400     | 153,000  | 69,000     |
| Fraction of culture volume occupied by packed cells (F)                   | 0.0108   | 0.0115     | 0.0147   | 0.0134     |
| Mean cell volume in cmm $\times 10^7$ (F/N)                               | 1.07     | 2.48       | 0.96     | 1.94       |
| Nitrogen content of cell suspension in $\mu\text{g}/\text{cmm}$ ( $Q_s$ ) | 0.318    | 0.323      | 0.382    | 0.384      |
| Nitrogen content of medium in $\mu\text{g}/\text{cmm}$ ( $Q_m$ )          | 0.094    | 0.109      | 0.091    | 0.135      |
| Nitrogen content of packed cells in $\mu\text{g}/\text{cmm}$ ( $Q_c$ )    | 20.7     | 18.6       | 19.8     | 18.6       |
| Specific gravity of suspension ( $\rho_s$ )                               | 1.000674 | 1.000632   | 1.000787 | 1.000648   |
| " " " " medium ( $\rho_m$ )                                               | 1.000010 | 1.000016   | 0.999886 | 0.999861   |
| Specific gravity of cells ( $\rho_c$ )                                    | 1.061    | 1.054      | 1.061    | 1.060      |

one may regard the data for the irradiated and control cultures to be substantially equal.

Accordingly it is to be concluded that, in the case of the yeast cell, the enlargement observed after irradiation is chiefly if not entirely due to a nonselective increase in water and nonaqueous constituents. This nonselective increase probably proceeds through normal mechanisms which are much more resistant to irradiation than is the mechanism of cell division.

So far as comparisons can be made, a similar state of affairs was produced in the protozoan *Bodo caudatus* by Robertson(2). However, the enlargement of certain types of cells, e.g. erythrocytes(5), after irradiation seems to be due almost entirely to uptake of water (swelling). Failla(4) has discussed this phenom-

enon and has offered a theory to explain it.

**Summary.** When yeast cells were exposed to 20,000 r of x-rays and cultured in liquid medium, they and their descendants attained a mean cell volume more than twice that of cells derived from nonirradiated controls. On the other hand, there were no corresponding differences in specific gravity and nitrogen content per unit of cell volume. It is accordingly concluded that enlargement of yeast cells after irradiation is largely a nonselective increase in cell constituents rather than a swelling due mostly to uptake of water.

5. Ting, T. P., and Zirkle, R. E., *J. Cell. and Comp. Physiol.*, 1940, v16, 189.

6. Doyle, W. L., and Omoto, J. H., *Analytical Chem.*, 1950, v22, 603.

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### Survival of *Trichomonas vaginalis* in Vaginal Discharge.\* (18038)

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The transmission of *Trichomonas vaginalis* is partially dependent upon the ability of the flagellates to survive in natural discharges. Vazquez-Colet and Tubanqui(1) reported that *Trichomonas vaginalis* would survive in

a semi-dry state for 6 hours and Swift(2) stated that the trichomonads could be cultured from vaginal discharge that had been dried for 3 hours. Kirby(3) reported that most trichomonads in vaginal discharge under paraffin-sealed cover-slip preparations kept at room temperature survived for 24 hours

\* Aided by a grant to the Microbiology Research Fund, by Protecto Products Co.

1. Vazquez-Colet, A., and Tubanqui, Med. J. Philippine Islands, M. A., 1936, v16, 231.

2. Swift, B. H., Med. J. Australia, 1937, v1, 123.

3. Kirby, H. J., J. Parasit., 1943, v29, 422.

and a few more were active after 5 days. Although these observations indicate that trichomonads may survive in drying or sealed vaginal discharges at room temperature for a considerable time, they do not establish the fact that they will multiply in serial cultures following such survival. It was considered that more exact information concerning the resistance of *Trichomonas vaginalis* under natural conditions of drying in the vaginal discharge and their reproduction in serial cultures following such drying would provide pertinent data concerning the possibility of their transmission by other than venereal methods.

**Materials and methods.** The discharge used in this study was collected from 50 untreated patients with *Trichomonas* vaginitis. A sample was taken from the interior surface of a sterile speculum immediately upon removal from the patient, and examined microscopically for the presence of trichomonads. (1) One-tenth cc was inoculated immediately into 8.0 cc of standard culture medium and served as a control. This was incubated at 37°C and examined after 48 hours, at which time transplants were made to fresh media for subcultures. (2) Five-tenths cc of the discharge was transferred for experimental study to an area within a limiting circle, 1.5 cc in diameter, drawn with a wax pencil on the enameled surface of a small wooden block. Each drop of discharge was examined at intervals of 10, 20, 30 and 45 minutes, and of 1, 2, 3, 4, 5, 6 and 7 hours respectively when a small portion of the material was removed and examined for active trichomonads. Simultaneously an equivalent amount was transferred to a culture tube of medium, incubated and observed after 48 hours for active flagellates. Transfers were subsequently made to other culture tubes and the fact established that the trichomonads were capable of growth in serial transfer. The drying droplet was sufficiently moist for the first 5 hours to permit the preparation of a routine smear, but the portion of the droplet remaining on the block, at both the 6 and 7 hour intervals, had dried to the point where it was first necessary to add saline to the sample in order to make a smear on a slide for microscopic examina-

tion. The culture medium used was a Casamino acid medium (Difco-Lash's Serum Medium) described by Lash(4,5). In this study 1.0 g of cysteine hydrochloride per 1000 cc was added, in an attempt to decrease through reduced oxygen tension the bacterial overgrowth, while establishing cultures(6-8). Penicillin and streptomycin were not used, since no attempt was being made to retain the trichomonads in bacterial free culture.

**Results.** Table I displays the results of studies on the discharges from 50 cases of vaginitis which were positive for *Trichomonas vaginalis* by the procedures outlined under *Materials and methods*.

It will be observed that the trichomonads remained active for a period of at least 45 minutes in all 50 of the drops of discharge studied. Also that growth occurred from all 50 of these samples when they were transferred to suitable culture media. After 6 hours, active trichomonads were found in material from only 2 of the 50 cases and only one of these survived in culture. The decrease in survival times of trichomonads between these two periods is displayed in Fig. 1.

Here it is noted that there is a gradual diminution in survival of trichomonads as the time increases. Little difference is observed between the number of samples in which active trichomonads occurred in direct smear and the number established in culture. One

TABLE I.  
Results of Direct Smear Examination and Culture of Droplets from Vaginal Discharge.

| Time       | Direct smear motility | 48-hr culture growth |
|------------|-----------------------|----------------------|
| 10-45 min. | 50                    | 50                   |
| ¼-1 hr     | 48                    | 49                   |
| 1-2 "      | 45                    | 46                   |
| 2-3 "      | 27                    | 29                   |
| 3-4 "      | 12                    | 13                   |
| 4-5 "      | 2                     | 3                    |
| 5-6 "      | 2                     | 1                    |
| 6-7 "      | 0                     | 0                    |

4. Lash, J. J., *Am. J. Trop. Med.*, 1948, v28, 111.
5. Lash, J. J., *Am. J. Trop. Med.*, Sept. 1950.
6. Trussell, R. E., *Trichomonas vaginalis and Trichomoniasis*, 1947, p. 24.
7. Wadsworth, A. B., *J. Inf. Diseases*, 1943, v73, 95.
8. Ward, T. G., *Proc. Soc. Exp. Biol. and Med.*, 1941, v48, 19.

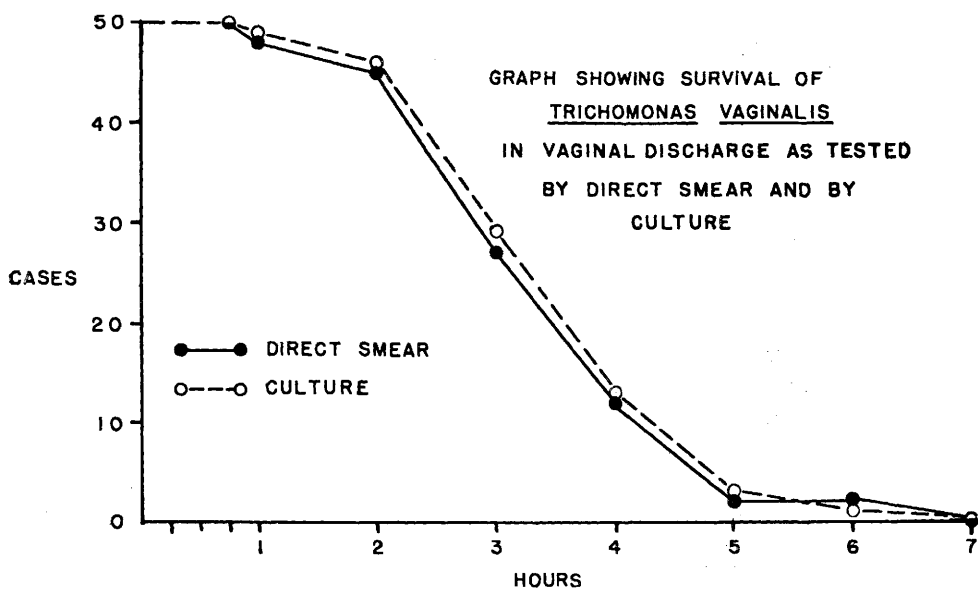


FIG. 1.

more sample at the one hour period yielded a positive culture than was demonstrated to show motility by direct mount. At the 6-hour period two cases still exhibited motility but only one of these was established in culture. No active trichomonads were observed and no positive cultures procured from any of the specimens examined at the 7-hour period.

It is thus concluded that the trichomonads which exhibited motility following the observed periods of standing within a drying drop of vaginal discharge, with one exception, were also capable of multiplying in culture.

**Discussion.** The distribution and transmission of *Trichomonas vaginalis* are discussed in detail in the excellent monograph by Trussell(6). He points out that one out of every 4 or 5 adult women harbors the parasite. He also accepts the conclusion that *Trichomonas vaginalis* is the only trichomonad associated with Trichomonas vaginitis. Since neither *T. hominis*(10,11) nor *T. elongata*(12) is capable of becoming established

in the vagina, there would appear to be no further need for considering the intestinal and oral trichomonads as potential etiologic agents of vaginal trichomoniasis.

Since *Trichomonas vaginalis* is frequently recovered from the genito-urinary tract of both males and females, sexual intercourse is generally accepted as an important method of transmission. However, this method does not appear to explain all cases of infection in adults, cases reported from infants and children(13-15) nor multiple infections in the same family, especially of mothers and children(15-17). It is accordingly quite natural to consider that certain fomites *e.g.* towels, wash cloths, toilets and bath tubs also may function in the transmission of this parasite.

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16. Stein, I., and Cope, E., *Am. J. Obst. and Gynec.*, 1933, v25, 819.

17. Katsunuma, S., *Bull. Soc. Path. Exot.*, 1924, v17, 216.

9. Berkman, S., *PROC. SOC. EXP. BIOL. AND MED.*, 1940, v44, 68.

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The current study was undertaken to confirm previous observations with reference to the length of time that *Trichomonas vaginalis* was capable of surviving in a droplet of vaginal discharge under natural conditions and also whether it was capable of multiplying in culture at the end of each test period. It is observed in Table I and Fig. 1 that trichomonads survived for 45 minutes in the discharges from all cases tested, that in 29 or 58% they survived for 3 hours and that survival was demonstrated in only 2 or 4% at the end of 6 hours.

Trussell(6) raises the question of the survival of trichomonads following desiccation. In the current study the drying droplets of discharge were sufficiently moist up to the 5-hour period to permit transferring by pipette to a microscope slide or culture tubes the amount of material required for testing. At the 6- and 7-hour intervals, however, the specimens had become so dry that it was necessary to add saline to the block before the material could be transferred. Since only one of the 50 specimens tested at 6 hours was positive by culture and none of the 7-hour specimens yielded either motile flagellates or positive cultures, it is concluded that trophozoites of *Trichomonas vaginalis* will not withstand complete desiccation.

The observations here presented, however, do indicate that trichomonads in droplets of vaginal discharge, deposited under natural conditions may survive sufficiently long to permit transfer to another individual where they may again be established. Although

actual experimental transfer of the recovered trichomonads has not been made to volunteers during this study, previous experiments by one of the authors(10) and also by other investigators(6,18) have demonstrated that *Trichomonas vaginalis* established in culture can be implanted and produce vaginal trichomoniasis. In view of these findings careful personal hygiene is indicated.

**Summary.** The survival of *Trichomonas vaginalis* was determined by studying discharge from 50 untreated patients with vaginal trichomoniasis. Droplets of the discharge were placed on the enamel surface of wooden blocks and tested under natural conditions of drying at room temperature.

Tests at intervals ranging from 10 minutes to 7 hours were made by comparing the activity of the flagellates in direct microscopic examination with their ability to multiply in serial cultures. A close correlation was observed between the two procedures. The trichomonads survived in 100% of the tests at 45 minutes, in 96% at 1 hour, in 56% at 3 hours and in 4% at 6 hours. No survival was demonstrated at 7 hours.

These data, together with experimental and epidemiologic reports of other workers indicate that fomites contaminated with *Trichomonas vaginalis* may, under natural conditions, act as agents in the transmission of vaginal trichomoniasis.

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### Relation of Cortisone Pretreatment to Mobilization of Lipids to Liver by Pituitary Extracts.\*† (18039)

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Since the initial report of Best and Camp-

bell(1), it has been shown in many labora-

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