TRICHOMONAS VAGINALIS*

I. SURVIVAL IN SOLID STUART'S MEDIUM

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Stuart's medium was originally prepared for use as a transport medium for gonococci (Stuart, 1946; Stuart, Toshach, and Patsula, 1954), but has now gained wide employment as a suitable transport medium for many micro-organisms (Stuart, 1956; Cooper, 1957; Gästrin, Kallings and Marcetic, 1968) including *Trichomonas vaginalis* (Stuart, 1956; Whittington, 1957; Ødegaard, 1962).

The original Stuart's medium is a semi-solid, non-nutrient medium, which prevents oxidation and dessication of the micro-organisms transported. Since a solid medium is easier to handle, the original medium has been modified by increasing the agar concentration (Reyn, Korner, and Bentzon, 1960; Ringertz, 1960).

This modification, together with charcoal-impregnated applicators (Moffett, Young, and Stuart, 1948), has been used since 1960 in this Department, for the transport of freshly-obtained material to be cultured for gonococci. Since 1962 the same equipment has been used for transport of *T. vaginalis* (Nielsen, 1965).

Apart from the investigation by Ødegaard (1962), no detailed information is available concerning the survival time of *T. vaginalis* in Stuart's medium. Since knowledge on this point is of importance for laboratories using the medium for transport purposes, this problem became the subject of the present investigation.

Material and Methods

Medium For "transport" of *T. vaginalis* on charcoal-impregnated applicators, the modified solid Stuart's medium (as described by Reyn, 1965) was used. The fluid medium described by Diamond (1957), with the omission of agar was used for culture. The medium was dispensed in amounts of about 10 ml. in screw-capped tubes.

Strains Twenty freshly-isolated strains of *T. vaginalis*, taken at random from routine specimens, formed the material of this study. Before use, the trichomonads were grown in Diamond's medium at 36°C. Subcultures were made by inoculation of 1 ml. of 48-hr-old cultures into 10 ml. modified Diamond's medium. After 48 hrs' incubation at 36°C. abundant growth was usually achieved.

Estimation of Culture Density Fifty to sixty fields per preparation were examined, using a Zeiss Standard microscope fitted with phase contrast equipment and with a magnification of 320×. Before microscopy the cultures were dispersed thoroughly by a Vortex mixer (Model K 500-J, Scientific Industries Inc.). The density of the cultures was expressed by arbitrary symbols (+, ++, +++, +++++), on the basis of the average number of living trichomonads per sight field. The number of organisms/ml. corresponding to the symbols used was ascertained by counting them in a Coulter counter (Model F, aperture 100 µm., manometric vol. 0.5 ml.).

Method (Preparation of "transport" specimens) A 48-hr-old culture of each strain of *T. vaginalis* in modified Diamond's medium was centrifuged for 5 min. at approximately 600 G in a Stock cup centrifuge. The sediment was resuspended in the supernatant to achieve a density of +++, corresponding to 5–10 trichomonads per sight field (about 1.5×10^6 organisms/ml.). Of this suspension 2 ml. were dispensed into each of eighteen small tubes. A charcoal-treated applicator was dipped into each of these tubes and left there for 2 min. before being placed in Stuart's medium and stored at 4°, 20°, and 36°C., with six tubes at each temperature. From the first to the sixth day of storage, a swab from each strain was examined daily for living trichomonads. Simultaneously, the swabs were inoculated into modified Diamond's medium, incubated at 36°C., and examined every other day for trichomonads. If no growth was obtained after 8 days' incubation, the tubes were discarded.

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Results

The number of applicators from which trichomonads were recovered can be seen from the Table. After 24 hrs, all strains were alive, irrespective of the temperature at which they were kept. Extension of storage time resulted in the loss of an increasing number of strains. After 6 days the strains kept at 20°C. were dead, while trichomonads could be recovered from five and four applicators kept at 4° and 36°C., respectively. These nine applicators represented growth from seven strains, three after storage at 4°C. only, two after storage at both 4° and 36°C., and two after storage at 36°C. only.

TABLE

RECOVERY FROM TWENTY STRAINS OF *T. VAGINALIS* STORED IN SOLID STUART'S MEDIUM ON CHARCOAL-IMPREGNATED APPLICATORS

No. of Days of Storage	Number of Applicators from which Trichomonads were recovered after Storage at:		
	4°C.	20°C.	36°C.
1	20 (1)	20	20 (1)
2	18 (4)	16 (4)	19 (3)
3	12 (2)	12 (3)	16 (5)
4	7	5	12 (3)
5	5 (1)	2	4
6	5	0	4

Note: Numbers in brackets are number of applicators from which living *T. vaginalis* were observed by direct microscopy but where no growth was obtained after inoculation into Diamond's medium.

In order to evaluate the best temperatures for storing *T. vaginalis*, the survival times of each strain kept at two different temperatures were compared. In Figs 1, 2, and 3, the survival time in days at one temperature was plotted against that of the same strain at another temperature. In Fig. 1 the survival times of the twenty strains at 20°C. and at 4°C. are compared. For five strains the survival time was the same at 4° and 20°C., for three strains 20°C. was

better, and for twelve strains 4°C. was better. Of these twelve strains, six had days with no growth before the last day when growth was obtained. In Fig. 2 the survival times of trichomonads kept at 36°C. and at 4°C. are compared. Of the four strains with the same survival time at both temperatures, two had days with no growth before the day of survival registered. Two of the eight strains that kept better at 36°C. and four of the eight strains that kept better at 4°C. had days with no growth before the last day of survival registered. Comparison of the survival times at 36° and 20°C. (Fig. 3) showed that four strains survived equally well at both temperatures. 36°C. was better for fourteen strains, of which four had days with no growth before the last survival day registered, while 20°C. was better for only two strains.

Discussion

Several micro-organisms can survive for a long time in Stuart's medium. Cooper (1957) found that various respiratory and enteric pathogens survived for 8 to 12 weeks at room temperature and Korner (1962) observed that large inocula of gonococci could be preserved for 6 weeks at 4°C. in freshly-prepared Stuart's tubes. It is obvious from the results of the present study that Stuart's medium is not suitable for storing *T. vaginalis* for long periods of time. However, solid Stuart's medium is very suitable as a transport medium, particularly if the transportation time does not exceed 24 hours (which, in fact, is seldom the case with the specimens received in our laboratory).

For the first 24 hours, the influence of temperatures from 4° to 36°C. on the recovery of trichomonads kept on charcoal-impregnated applicators in Stuart's medium, is of no importance. However,

Figs 1, 2, and 3 Comparison between days of survival of twenty strains of *T. vaginalis* stored for 6 days in solid Stuart's medium at 20° and 4°C. (Fig. 1), at 36° and 4°C. (Fig. 2), and at 36° and 20°C. (Fig. 3).

○ indicates strains from which no growth was obtained for one or more days before the last day on which growth of trichomonads was obtained.

with 6 days of storage the highest number of strains of *T. vaginalis* survive at 36°C. and the recovery of strains stored at 4°C. is better than that of those stored at 20°C.

These results are supported by the author's previous, unpublished investigations. Fifty strains of *T. vaginalis* were examined, but only one applicator from each strain was kept in Stuart's medium at 4°, 20° and 36°C. A wet preparation from the same applicator was examined daily until no living trichomonads could be observed. The swab was then inoculated into Diamond's medium in order to check the negative results obtained by microscopy.

The organisms survived for the longest time at 36°C., judged by the number of days on which there was microscopical evidence of living trichomonads. However, by culture from microscopically "negative" swabs, it was found that 48 of the strains stored at 4°C., 41 of the strains stored at 20°C., and 46 of the strains stored at 36°C. were still alive. This indicated that 20°C. was the least favourable temperature for storing *T. vaginalis*.

Ødegaard (1962) has shown previously that about 37°C. is the best temperature for storing *T. vaginalis* in Stuart's medium. He stored seventeen strains of *T. vaginalis* in the refrigerator, at room temperature and at 37°C., and found that the trichomonads kept best at 37°C., room temperature being slightly better than refrigerator temperature. The latter result has not been confirmed by the present investigation.

For "true" diagnostic specimens, it has been the author's general experience that after inoculation of positive swabs into Diamond's medium, growth of *T. vaginalis* was obtained not later than after 4 days' incubation at 36°C. The present investigation (Table I) revealed that after 8 days' incubation no growth was obtained from 27 swabs that were found positive by microscopy.

An explanation for this observation might be sought in the quality of the medium used for culture or in the size of the inoculum, which would depend on the amount of cotton on the applicators used for preparing the artificial specimens.

During the experimental period, 150 positive specimens from the routine work were inoculated into Diamond's medium, all except two having been transported for less than 48 hours. Growth of *T. vaginalis* was obtained from them all after 3 days' incubation. The medium employed was from the same batch as that used in the experiment proper. A check on the size of the applicators used in the present experiment showed that, on the basis of the amount of cotton, the applicators could be divided

into three groups: large, medium, and small. The cotton layer of the small applicators was so thin that the wooden stick could be seen through it. Out of the total number of 360 applicators used, 13 per cent. were large, 71.4 per cent. were medium-sized, and 15.6 per cent. were small. Of the 27 applicators from which no growth was obtained although trichomonads were found by microscopy of wet preparations, 4.3 per cent. were large, 74.1 per cent. were medium-sized, and 18.5 per cent. were small. It is thus unlikely that the quality of the culture medium or the size of the applicators *i.e.* the size of the inoculum, might be responsible for the lack of growth from the swabs which were found "positive" by microscopy of wet preparations. The fact that the artificial specimens were prepared by resuspending the sediment of trichomonads in the supernatant and not in fresh medium might also contribute to the lack of growth from several "positive" swabs.

Another, and probably more reliable, explanation of this discrepancy in growth from positive artificial and routine specimens would be that trichomonads survive longer in their natural environment and will therefore survive longer on swabs if they are surrounded by some vaginal discharge rather than by culture medium. The fact that the viscosity of the vaginal discharge is greater than that of the culture medium is probably also of significance. Thus, the vaginal discharge may adhere to the applicators, while the culture medium may spread out into the Stuart's medium, thus leaving the trichomonads without anything on which to live, except dead trichomonads. The conclusion to be drawn from the results of the present investigation is that solid Stuart's medium is very suitable as transport medium for *T. vaginalis*. After 24 hours' transportation at temperatures between 4° and 36°C. growth or positive microscopical findings were obtained from all strains, but if the storage of *T. vaginalis* in Stuart's medium is to last longer than 24 hours, 36°C. is preferable to 4°C., and 4°C. is preferable to 20°C.

Stuart's medium is not recommended for the storage of *T. vaginalis* for a longer period of time.

Summary

Twenty strains of *T. vaginalis* were stored on charcoal-impregnated applicators in solid Stuart's medium at 4°, 20°, and 36°C. for 6 days. For the first 24 hours the temperature had no influence on the recovery of *T. vaginalis*, but an increasing number of strains died when the storage time exceeded 24 hours. During 6 days' storage the highest number of strains survived at 36°C., and the recovery of strains stored at 4°C. was better than

that of those stored at 20° C.

The investigation revealed that Stuart's medium is very suitable for the transport of *T. vaginalis*, but cannot be used for storing trichomonads more than 24 hours.

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Trichomonas vaginalis

I. Survie du *Trichomonas vaginalis* dans le milieu de Stuart solide

SOMMAIRE

Vingt souches de *Trichomonas vaginalis* recueillies sur des écouvillons imprégnés de charbon furent conservées en milieu de Stuart solide pendant 6 jours à 4°, 20°, et 36° C. Pendant les premières 24 heures, la température n'eut aucune influence sur la récupération de *T. vaginalis* mais un nombre croissant de souches moururent lorsque le temps de conservation dépassa 24 heures. Pour 6 jours, ce furent les souches conservées à 36°, qui eurent la meilleure survie et les souches conservées à 4° C. furent mieux récupérées que celles conservées à 20° C.

Cette recherche montre que le milieu de Stuart est très approprié pour le transport de *T. vaginalis*, mais qu'il ne peut pas être choisi pour conserver les trichomonas plus de 24 heures.