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Effect of Different Species of Trichomonads on Monkey Kidney Cell Cultures

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Abstract. The effect of axenic cultures of *Trichomonas vaginalis*, *T. gallinae*, *T. tenax*, *Tetratrichomonas gallinarum*, *Pentatrichomonas hominis*, *Tritrichomonas foetus* and *T. suis*, and of trichomonad-free filtrates and supernatants from these cultures upon the monolayers of the monkey kidney cells was studied. The accomplished observations revealed that the species: *T. vaginalis*, *T. gallinae*, *T. foetus* and *T. suis* multiply within the cell cultures, causing there degenerative changes resulting in deterioration of the monolayer and a subsequent cell destruction. *T. gallinarum* multiplies in the cell cultures without producing any ill effect on the cells. *P. hominis* and *T. tenax* are neither exhibiting an apparent unfavourable influence upon the cell cultures, nor do they multiply within them. *T. vaginalis* and *T. gallinae* display an affinity to the cell layer. The presence of the parasite cells is a precondition of the monolayer destruction; a direct adherence to the cell layer exhibited by trichomonads multiplying in the intercellular spaces, is most probably of essential importance in this process. *T. foetus* and *T. suis* do not display any affinity to the monolayer until it starts falling off the glass. Thus most of the changes occurring in the monolayer take place without an immediate contact with the cells of the parasite. The presence of an exotoxin could not be proved. A monolayer injury, resulting in a final cell culture destruction and caused by ultrafiltrates or supernatants from rich trichomonas cultures, was not observed in any of the studied species. The specificity of cytologic changes, manifested during a later period after inoculation is questionable. In strains of the same trichomonad species, showing statistically evident differences in virulence according to the "mouse assay", no significant difference in their effect upon the monkey kidney cells was noted.

The results presented were obtained during a larger study of trichomonas virulence, including the "mouse assay" experiments (KULDA in press). The original aim of this investigation was to check the possibility of testing the virulence of trichomonads on a medium more simple and defined than represented by mice. For this purpose monkey kidney cell cultures were chosen, because they represent a simple cell culture, relatively resistent to external influence. The method of their cultivation had been exactly worked out and standard suspensions of these cells are easily available as commercial products. The experiments were carried out so as to enable a comparison of 7 species of trichomonads, i.e.: *Trichomonas vaginalis* and *T. gallinae*, recently studied by some authors using other types of tissue

Table 1. Survey of the strains of trichomonads used

Species	Designation of strain	Host	Isolated by	Date and place of isolation	Medium	Length of cultivation before use	Notes
Trichomonas vaginalis	101	*Homo sapiens*	Petrů	February 27, 1964 Prague, Czechoslovakia	TYM	5 months	
Trichomonas vaginalis	108	*Homo sapiens*	Petrů	February 27, 1964 Prague, Czechoslovakia	TYM	5 months	
Trichomonas gallinae	DP 3	*Columba livia, dom.*	Diamond	May 1959 Washington, USA	TYM	5 years	
Trichomonas gallinae	H 4a	*Columba livia, dom.*	Jírovcová	October 1961 Prague, Czechoslovakia	TYM	3 years	Isolated on CPLM Clone isolation September 1962
Trichomonas tenax	HS 2 : NIH	*Homo sapiens*	Diamond	1954 U.S.A.	TTY	10 years	Full history of the strain see Diamond 1962
Tetratrichomonas gallinarum	KR 109	*Meleagris gallopavo*	Kulda	March 1963 Ustrašice Czechoslovakia	TYM	20 months	
Tetratrichomonas gallinarum	A 3	*Anas platyrhynchos, dom.*	Kulda	February 2, 1964 Libuš, Czechoslovakia	TYM	5 months	
Pentatrichomonas hominis	TH/A	*Homo sapiens*	Stoklasová	February 11, 1963 Český Brod, Czechoslovakia	TYM	17 months	Isolated and maintained agnobiotically for several weeks in a medium of coagul. serum
Tritrichomonas suis	PC 6	*Sus scrofa, dom.* (coecum)	Kulda	January 28, 1964 Prague, Czechoslovakia	TYM	6 months	
Tritrichomonas foetus	KV 1	*Bos taurus, dom.*	Lípová	1962 Žalmanová Czechoslovakia	TYM	2 years	

cultures (HOGUE 1947, KOTCHER, HOOGASIAN 1957, HONIGBERG, MCLURE 1960, HONIGBERG, BECKER, LIVINGSTON, MCLURE 1964), *Tritrichomonas suis* and *T. foetus* on which there are only incomplete data in this respect (HOGUE 1938a, b, SWITZER 1959, ALLSOP, HUCK, JOYNER, MILLAR 1961), *Trichomonas tenax*, *Pentatrichomonas hominis* and *Tetratrichomonas gallinarum*. The effect of axenic cultures of the last three mentioned species upon the tissue cultures has not been studied till now.

MATERIAL AND METHODS

a) Trichomonads

Table 1 surveys the strains of trichomonads used in this study and the main data on them. In all cases axenic cultures were used, maintained on TYM (*T. tenax* on TTY) medium (DIAMOND 1957, 1962), with 10 % horse serum and antibiotics (penicillin G, potassium salt 1,000 i.u. per ml., streptomycin—sulphate 1,000 gamma per ml.). The pH of media were adjusted to 6.0 for *T. vaginalis*, to 7.0 for other trichomonads. All cultures were incubated at 37 °C and transferred every third day.

The virulence of trichomonads was tested on CBA mice by the method of HONIGBERG (HONIGBERG 1961). On Fig. 1 the virulence values are indicated by means of volumes of subcutaneous abscesses measured on the 5th day after inoculation. The values given in cmm represent a mean of 30 measurements. Statistical evidence of the differences ascertained in the virulence of trichomonads is illustrated on the same figure. For this purpose a comparison of the confidence intervals of the mean values on a 5 % significance level was used under premise of a logarithmically normal distribution.

b) Cell cultures

In all experiments the first passage of monkey kidney cells was used. The monolayer cultures were kept in 10×1.5 cm SIAL test tubes each with an inserted cover-slide (No. 0: 20×10 mm) and closed with a nontoxic rubber stopper. The cells were maintained on a TC 199 medium modified by SLONIM and MICHL (1960), with 2 % calf serum and 1 % delipidized fraction of dried Lactino milk (KOZA, MOTEJLOVÁ 1964). The pH of medium was adjusted with mixture of 5 % CO_2 in air to about 7.0. The tubes were incubated in a slanting position at 37 °C.

c) Preparation of inoculum

Cultures of trichomonads in the logarithmical phase of growth were pooled in a 50 ml Erlenmayer flask. Using a haemocytometer of Thoma the concentration of trichomonads was ascertained and adjusted by dilution with fresh TYM medium (TTY in *T. tenax*) to about 2×10^6 organisms per 1 ml, i.e. approx. 4×10^5 trichomonads in 0.2 ml of inoculum.

Trichomonad-free filtrates were prepared from rich cultures by a vacuum filtration method, using Seitz's filter with an EK asbestos inset properly washed with saline.

Trichomonad-free supernatants were prepared by centrifugation of the cultures under sterile precautions in closed thickwalled 100 ml vessels with round bottom, at 2 000 rpm for 10 minutes. Immediately after centrifugation a part of the supernatant designed for the experiment was transferred into a 50 ml Erlenmayer flask. All the inocula were prepared immediately before use.

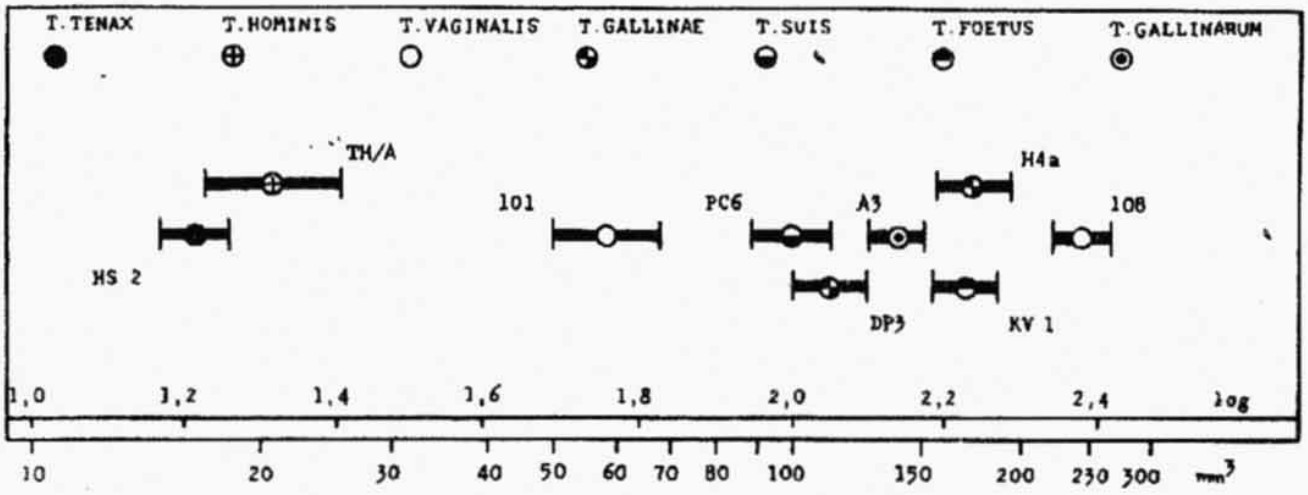


Fig. 1. Comparison of the results of virulence tests and their confidence intervals on a 5 % significance level in the used strains of trichomonads.

d) Controls

Each experimental series was completed by control experiments in which the inoculum was replaced by a fresh TYM medium (or TTY in experiments with *T. tenax*) in the same quantity (0.2 ml) and by control cell cultures, containing no foreign ingredients.

A standard inoculum of all used strains of trichomonads was inoculated into 2 ml of tissue culture medium, to verify the multiplication ability of trichomonads in this medium without the presence of host cells.

e) Inoculation of cell cultures and reading of results

After a 48-hour incubation at 37 °C, during which a coherent monolayer was usually formed, the old medium was cautiously removed from the cell cultures. Afterwards 2 ml of fresh cell culture medium and 0.2 ml of the respective inoculum were added into each test tube. The incubation at 37 °C followed.

The experimental as well as control cell cultures were examined at 4 hours' intervals for a period of 36 hours, later on after 48 and 60 hours. The experiments were carried on simultaneously in so many test tubes as to enable collection of 5 samples from experimental cultures and 2—4 samples from controls, at each examination. The cover slides with attached monolayer were then taken out of the test tubes. 4 of them were fixed in Bouin-Hollande's solution, one was mounted

as fresh preparation and after being framed in white vaseline, observed by means of a phase contrast and photographed. Cursory observations were made by examining the monolayer adhering to the tube walls (at a low power magnification, approx. × 100).

Table 2. Frequency of multinuclear cells in monkey kidney cell cultures at various intervals after inoculation with *Trichomonas gallinae* H4a, *Tritrichomonas foetus* KV1, trichomonad-free supernatants from rich cultures of these strains and *Trichomonas tenax*, compared with controls. Frequency determined on 100 fields at a magnification approx. ×200 (Zeiss, Lumipan, obj. 20).

Inoculum	Number of nuclei	Number of multinuclear cells per 100 fields	Time after inoculation in hours							
			8	12	16	20	24	28	32	60
Trichomonas	2		18	25	24	44	39	33	36	—
gallinae	3 a. more		2	4	7	4	8	9	8	—
T. gallinae	2		24	—	42	51	61	—	48	84
supernatant	3 a. more		4	—	2	9	14	—	12	16
Tritrichomonas	2		37	65	62	—	68	—	—	—
foetus	3 a. more		6	14	14	—	10	—	—	—
T. foetus	2		42	—	—	52	—	—	63	—
supernatant	3 a. more		8	—	—	6	—	—	8	—
Trichomonas	2		39	30	37	—	42	63	35	—
tenax	3 a. more		2	2	1	—	6	8	4	—
TYM	2		17	15	13	16	18	—	30	42
medium	3 a. more		2	1	3	5	2	—	4	6
Controls without	2		12	15	10	20	16	20	29	35
ingredients	3 a. more		1	3	0	2	0	4	4	7

After thorough shaking, samples were taken from each test tube and trichomonads counted in a haemocytometer of Thoma. The growth of trichomonads or their survival on a tissue culture medium without host cells was usually examined every 4 hours, exceptionally every 8 and 12 hours. All growth curves (Figs. 2—5) show the mean values obtained from 5 samples.

Fixed preparations were stained with Harris's or Weigert's haematoxylin and Protargol according to standard methods. The frequency of mitoses and other cytological changes (Figs. 6—10, Tab. 2) in the respective preparations was recorded from 100 or 50 random fields at a magnification approx. × 200 (Zeiss, Lumipan, obj. 20).

RESULTS

A. Behaviour of different species of trichomonads in the monkey kidney cell cultures

Trichomonas vaginalis, *T. gallinae*, *Tritrichomonas suis* and *T. foetus* multiply within the monkey kidney cell cultures (peak populations 0.5—3.6 × 10^6 organisms per 1 ml, Figs. 2, 3), causing degenerative changes resulting in deterioration of the monolayer and a subsequent gradual destruction of cells.

Trichomonas gallinae, *Tritrichomonas suis* and *T. foetus* multiply even when inoculated into a tissue culture medium free from host cells (Figs. 2, 3), but under such conditions, their maximal growth values are lower. (The only exception is the strain *T. foetus* KV 1.) When further transferred in this medium, all the cultures die in the 5th passage at the latest. *Trichomonas vaginalis* multiplies only when host cells are present. In a pure tissue culture medium it survives only for 36 hours (Fig. 5).

The strains of *Tetratrichomonas gallinarum* grow in cell cultures (maximal growth values 0.5—2.0 × 10^6 organisms per 1 ml, Figs. 2,4), as well as in a tissue culture medium alone, but they exhibit no distinct ill effects on the cells (Plate I, Fig. 1).

Trichomonas vaginalis and *T. gallinae* show a distinct affinity to the monolayer soon after the inoculation. *Tritrichomonas suis*, *T. foetus* and *Tetratrichomonas gallinarum* multiply within the entire volume of the medium, without a distinct concentration on the monolayer. As to *T. suis* and *T. foetus*, only in the final stages of the cell layer destruction, a greater concentration of trichomonads can be observed in its vicinity.

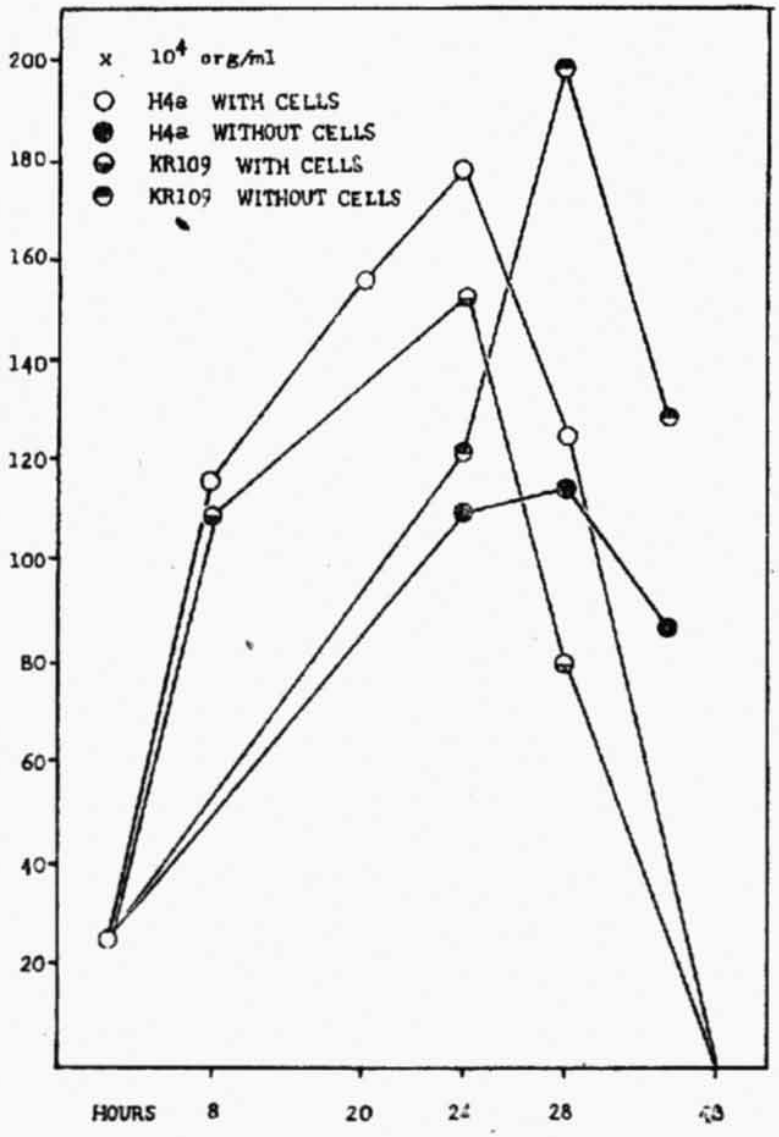


Fig. 2. Growth of the strains *Trichomonas gallinae* H 4a and *Tetratrichomonas gallinarum* KR 109 in monkey kidney cell cultures and in a tissue culture medium without host cells.

During their development in the cell cultures, none of the mentioned species display signs of degeneration. Their morphology as well as the process of division show normal features (Plate II, Figs. 3, 4; Plate IV, Fig. 4).

The strains of *Trichomonas tenax* and *Pentatrichomonas hominis* used in this study do not exercise any apparent ill effect on monkey kidney cell cultures and do not multiply either in them or in a pure tissue culture medium. If cells are present in the medium, they survive for 32 up to 48 hours, in a pure medium they die within 28 hours (Figs. 4, 5).

B. Effect of the species *Trichomonas vaginalis* and *T. gallinae* on monkey kidney cell cultures

Most of the trichomonads inoculated into the cell cultures are adhering to the monolayer between the 4th and 8th hour after inoculation. Trichomonads concentrate especially in the intercellular spaces respectively in the interspaces occurring here and there in the otherwise compact cell cover (Plate I, Fig. 3, Plate II, Figs. 1—4). As the number of trichomonads increases, cell injuries begin to appear from the 12th hour following inoculation. These are especially: a) a considerable decrease in the division rate of cells of the infected cultures—when compared with controls (Fig. 6), b) increasing number of bi-nuclear and multinuclear cells (Tab. 2), c) changes in the cell cytoplasm, especially an increased vacuolisation and granulation (Fig. 7), d) changes in the nuclei of cells, especially condensation of the nuclear substance in form of row granules (Fig. 8), e) changes in the cell form; the cells are getting wider and shorter, losing thus their fibroblast-like character (Plate III, Fig. 2). As shown in Figs. 6—8 and Tab. 2, frequency of these changes is increasing with time.

Growing wider and shorter, the cells provoke dilatation of the intercellular spaces which are soon filled with intensively dividing trichomonads. Most probably a direct mechanical effect of trichomonads contributes to a further dilatation of these spaces. After 20 hours they are considerably expanded (Plate I, Fig. 4, Plate II, Fig. 5) and a gradual tearing up of the cell layer takes place. After 24 hours cells are pealing off the glass on large areas. Trichomonads are concentrated on the perifery of these areas in close contact with the injured cells (Plate II, Fig. 6, Plate III, Fig. 1). Destruction of the cell layer culminates after 28 to 32 hours. After 48 to 60 hours only its withered rests are found on the glass (Plate III, Fig. 3).

Up to the 8th hour after inoculatin, an intracellular localization of trichomonads is observed (2 to 4 trichomonads per 100 fields, magnif. approx. × 200). Most of the invaded cells are badly damaged. A possible secondary occurrence of trichomonads in the injured cells cannot be excluded.

No essential difference was observed in the effect exercised on monkey kidney cells by *Trichomonas vaginalis* and *T. gallinae*. Only in the more advanced stages of the monolayer destruction (24 to 28 hours) penetration of T. vaginalis deeper among the cells on the periphery of the peeled-off areas is noted. Contrary to this,

specimens of *T. gallinae* are usually only rimming the periphery of the lesions.

No difference was found as to the effect of the H4a and DP3 strains of *T. gallinae* on the cell cultures, though the difference in their virulence, determined by Honigberg's method, is of statistical evidence (Fig. 1).

No essential difference was furthermore found in the final effect of *Trichomonas vaginalis* strains 101 and 108 (Fig. 1) displaying various degrees of virulence. However, in the strain with a lower virulence the destruction of the cell layer runs a slower course. In the strain 108 the destruction is actually completed after 48 hours, in the strain 101 only after 60 hours or later.

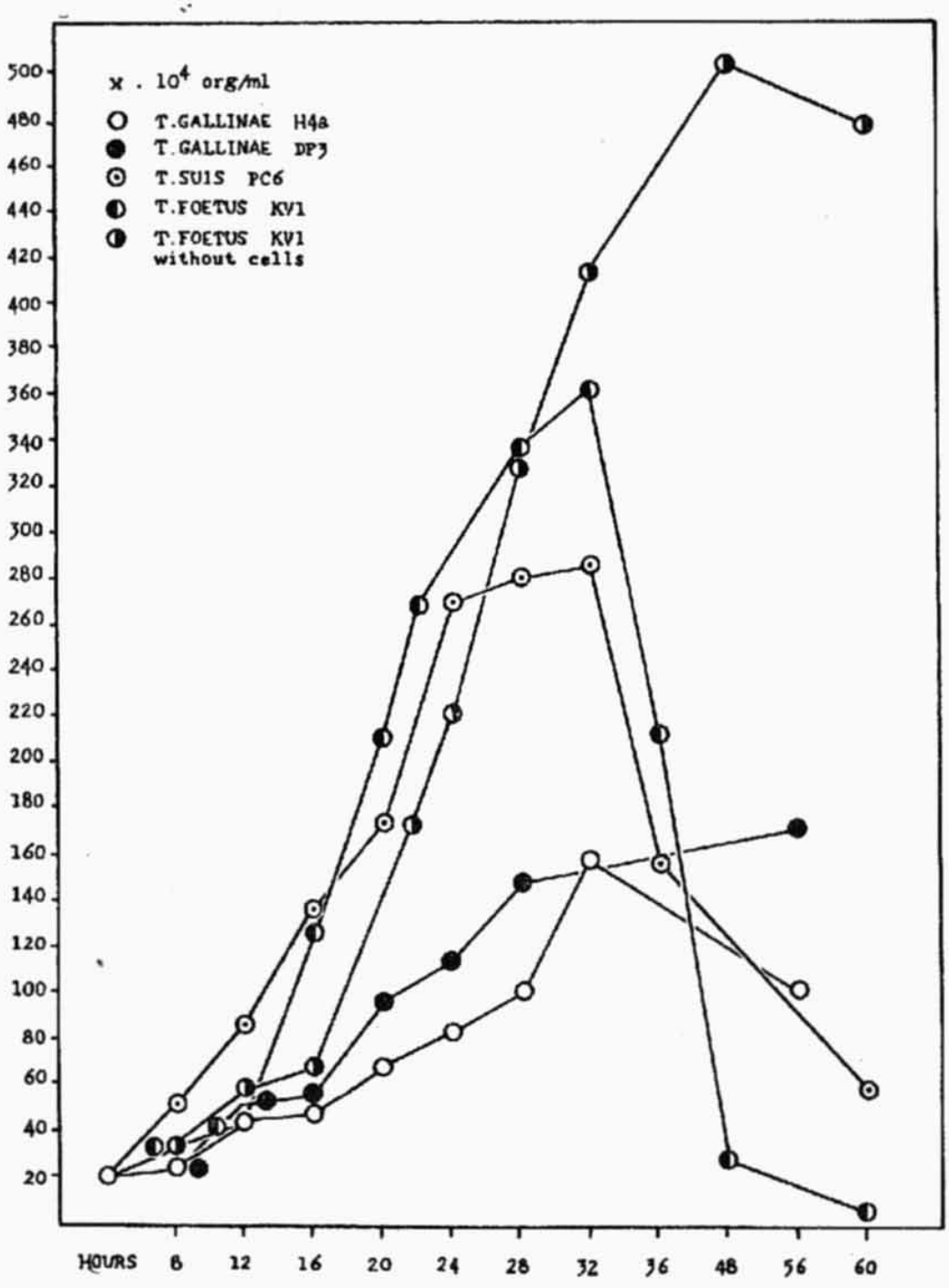


Fig. 3. Growth of the strains *Trichomonas gallinae* H 4a and DP 3, *Tritrichomonas suis* PC 6 and *T. foetus* KV 1 in monkey kidney cell cultures and of *T. foetus* KV 1 on a tissue culture medium without host cells.

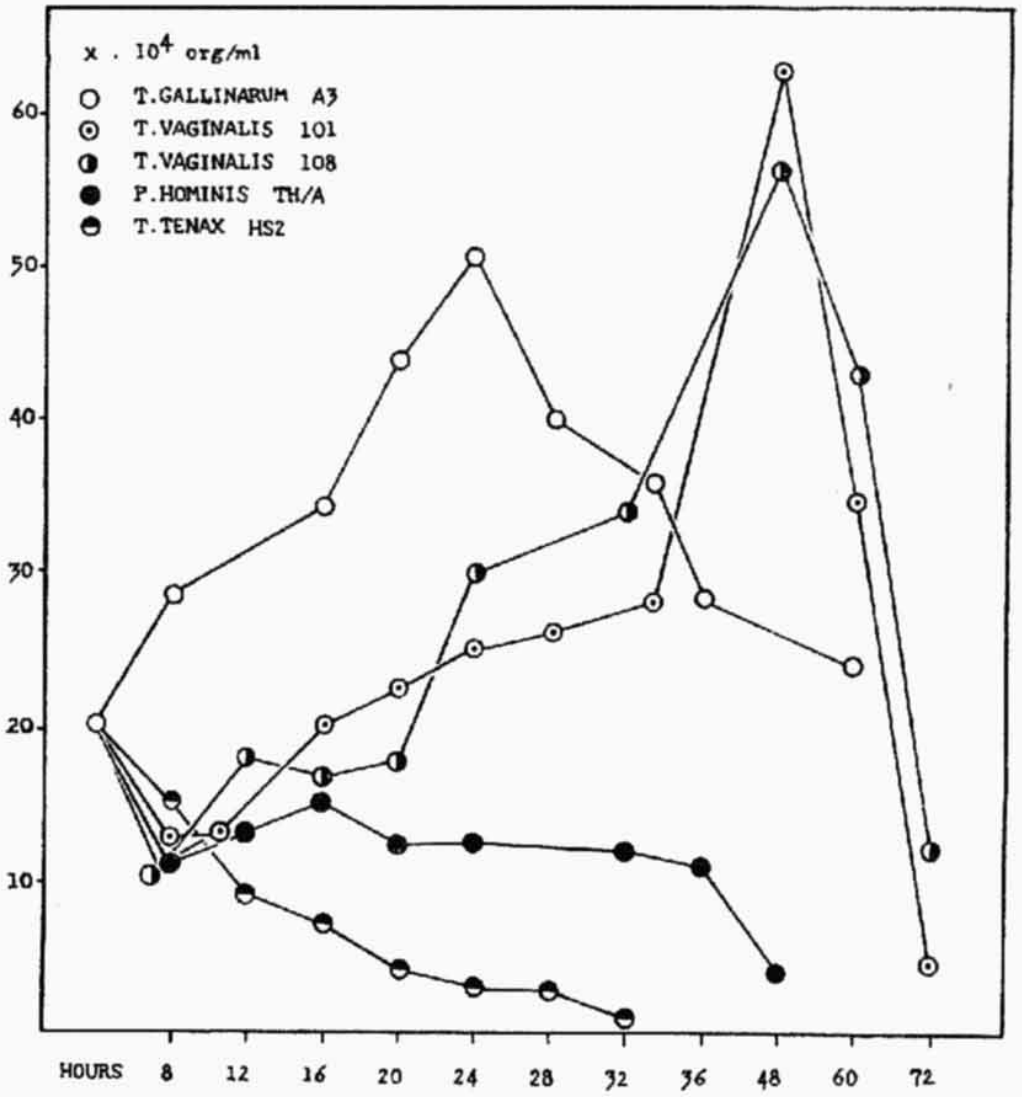


Fig. 4. Growth of the strains *Trichomonas vaginalis* 101 and 108, *Tetratrichomonas gallinarum* A 3 and survival of the strains *Trichomonas tenax* HS2 : NIH and *Pentatrichomonas hominis* TH/A in monkey kidney cell cultures.

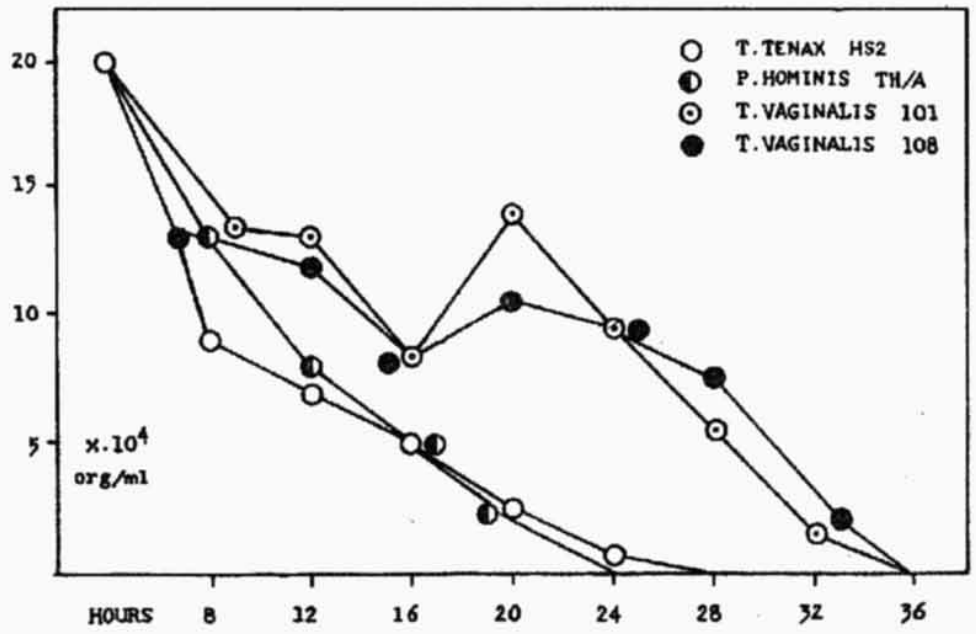


Fig. 5. Survival of the strains *Trichomonas vaginalis* 101 and 108, *T. tenax* HS2 : NIH and *Pentatrichomonas hominis* TH/A on a tissue culture medium without host cells.

C. Effect of the species *Tritrichomonas suis* and *T. foetus* on monkey kidney cell cultures

During the first 20 hours after inoculation, the strains of *T. suis* and *T. foetus* used in our study do not display any special affinity to cells. The increasing number of trichomonads found on the cells is only the result of a rapid multiplication of the parasite within the medium. Trichomonads are spread quite evenly, do not show any tendency to concentrate in intercellular spaces or on certain areas of the cell layer.

From the 16th hour onwards, a significant frequency of vacuolised cells (Plate III, Fig. 4), of cells with abnormal nuclei and multinuclear cells can be observed. In

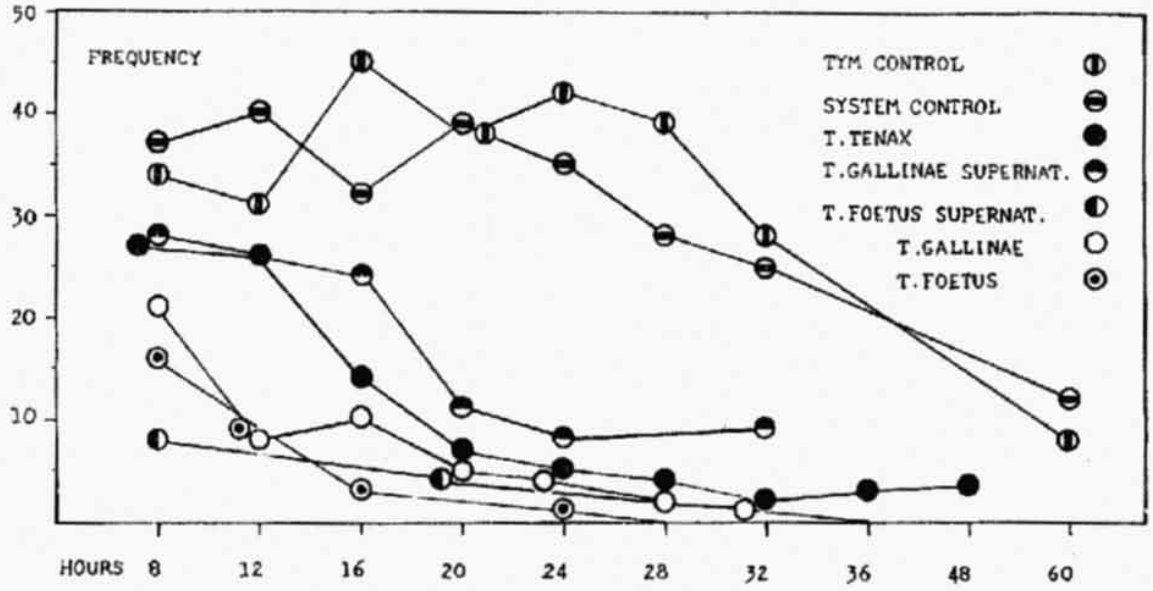


Fig. 6. Frequency of mitoses in monkey kidney cell cultures at various intervals after inoculation with *Trichomonas gallinae* H 4a, *Tritrichomonas foetus* KV 1, trichomonad-free supernatants from rich cultures of these strains and *Trichomonas tenax* HS2 : NIII compared with controls, with the addition of TYM medium (TYM control) and without any ingredient (system control). Figures on the vertical axis denote frequency of mitoses per 100 fields at a magnification of approx. × 200 (Zeiss, Lumipan, obj. 20).

comparison with the controls, division of cells is strongly suppressed (Fig. 6). After 24 hours the cells are getting wider and shorter and the cell layer starts pealing off. The frequency of the mentioned changes is markedly increasing (Figs. 7—10). After 28 hours, in fact no cells of a normal appearance can be found within the cultures (Plate IV, Fig. 1). Tearing-up of the monolayer continues. Only now a concentration of trichomonads on the damaged areas of the cell layer takes place. The destruction process culminates after 32 hours. By this time we find masses of trichomonads on the stripes of the degenerated monolayer coming off the glass (Plate IV, Figs. 2—4). A complete destruction of the monolayer occurs within a very short time. After 36 hours only withered rests are found on the glass.

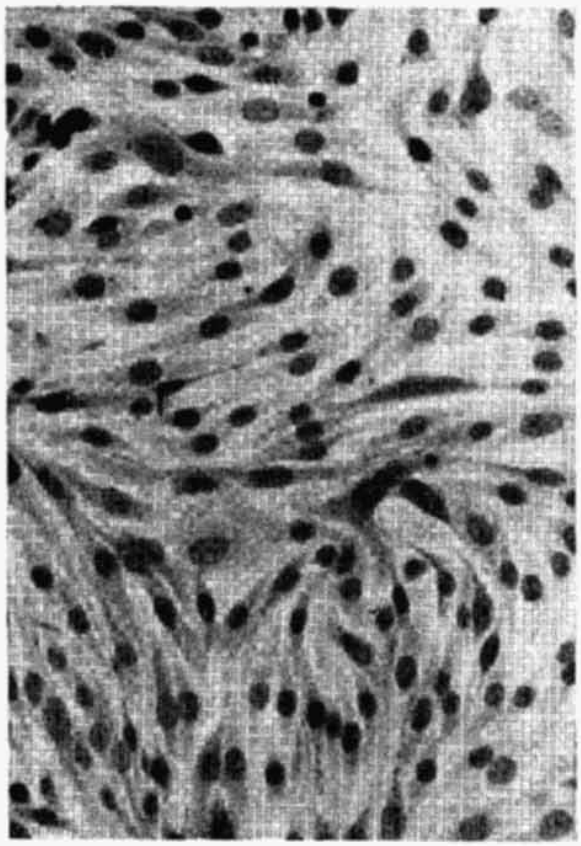


Fig. 1. Monkey kidney cells, 32 hours following inoculation with *Tetratrichomonas gallinarum*. Protargol, 150 ×.

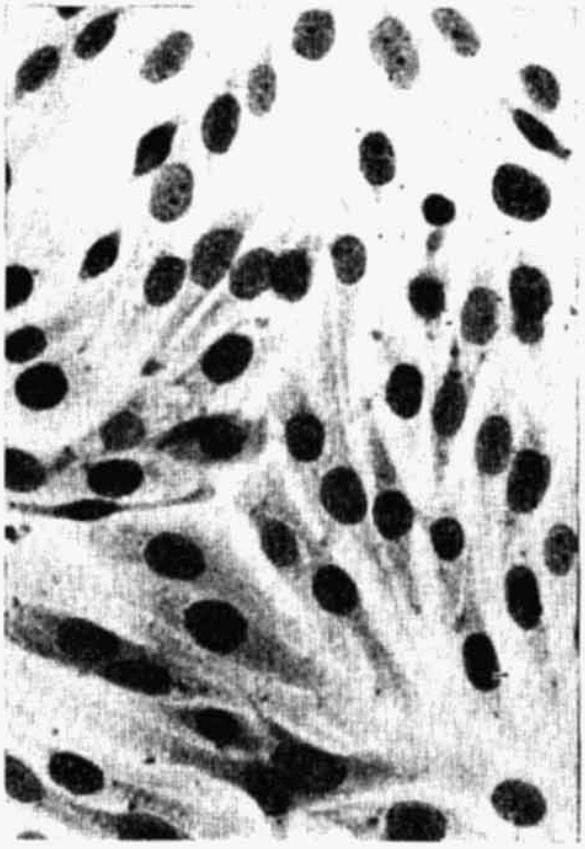
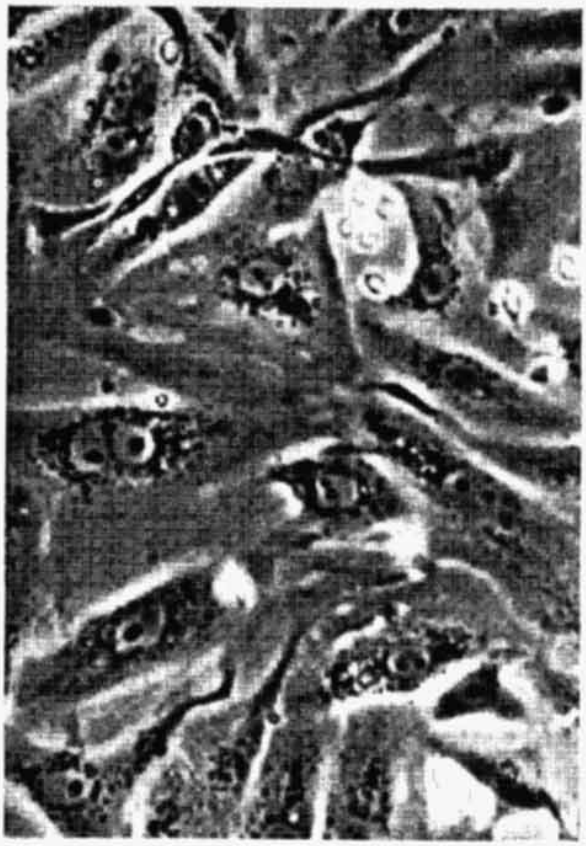


Fig. 2. Control examination, 48 hours after addition of TYM medium. Protargol, 300 ×.



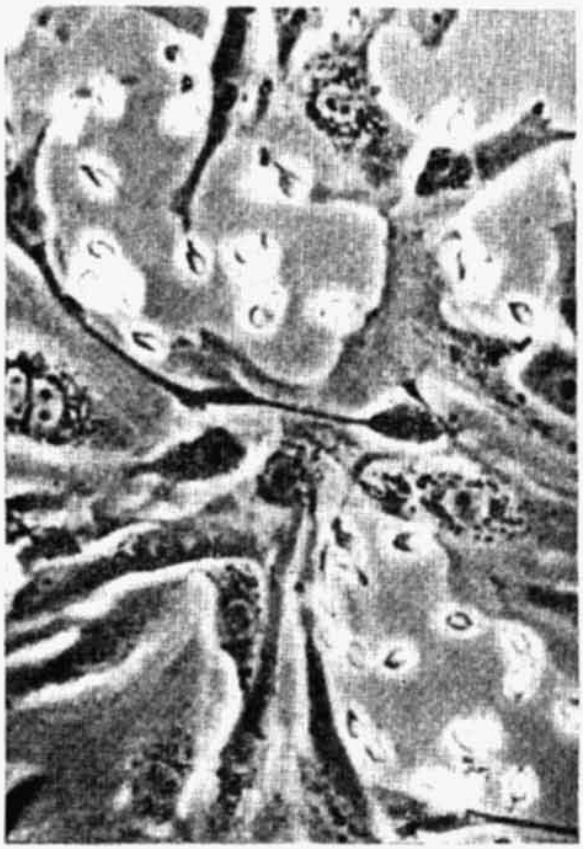


Fig. 3 and **Fig. 4.** *Trichomonas gallinae* in monkey kidney cell culture, 12 hours (Fig. 3) and 20 hours (Fig. 4) after inoculation. Fresh mount, phase contrast, 300 ×.

Fig. 1

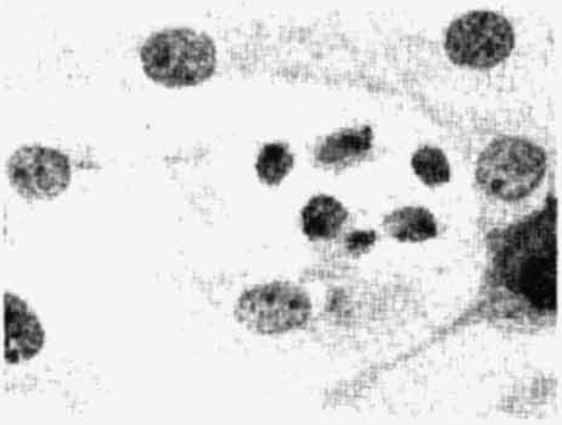


Fig. 2

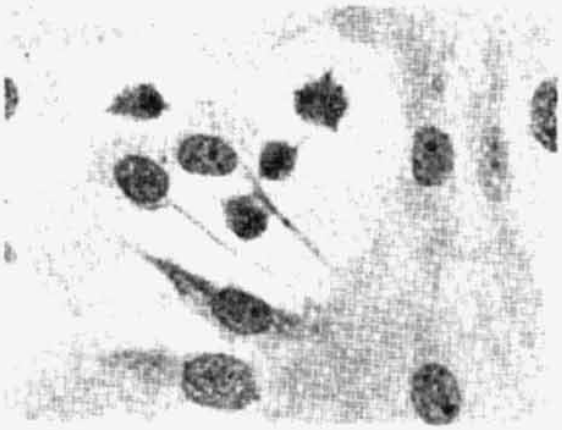


Fig. 3

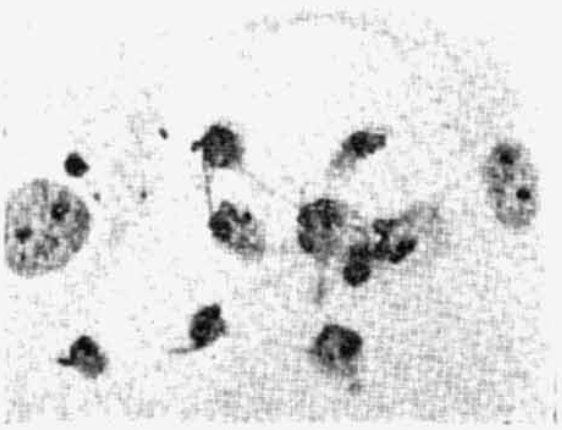


Fig. 4

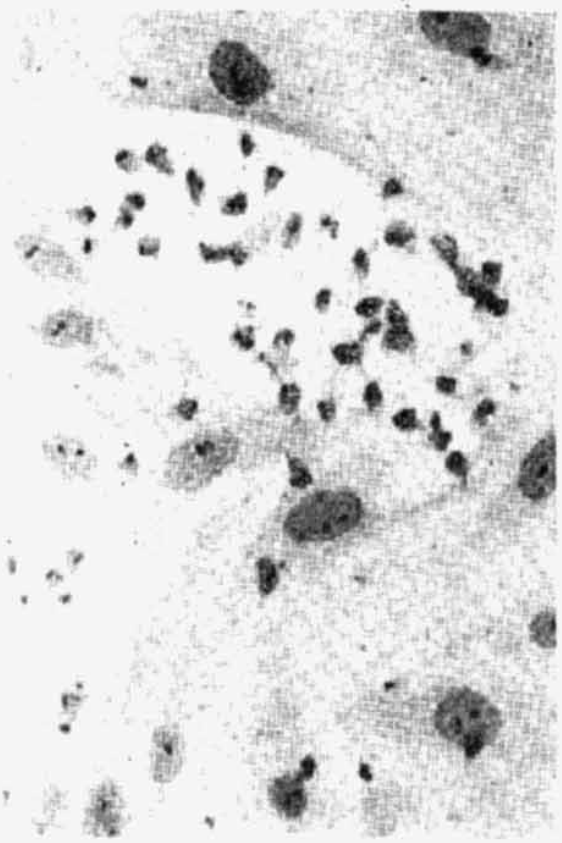


Fig. 5

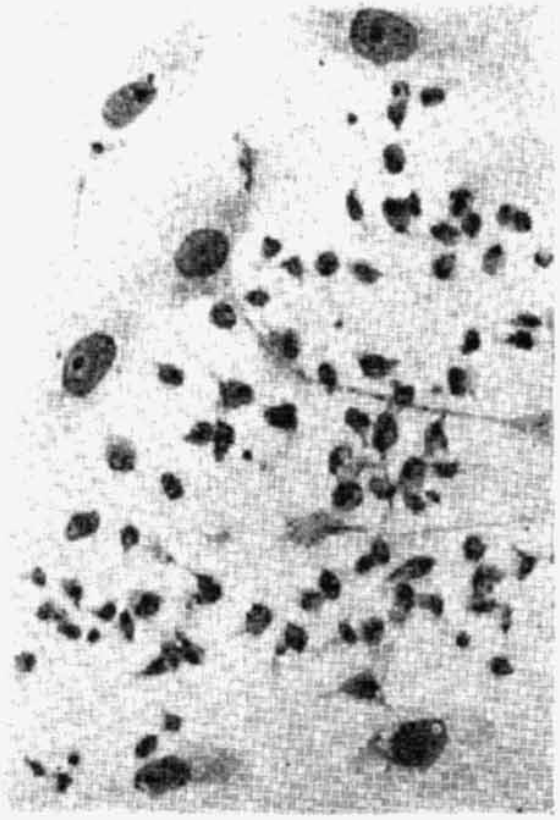


Fig. 6

Figs. 1—4. *Trichomonas vaginalis* in monkey kidney cell culture 12 hours after inoculation. **Fig. 5** and **Fig. 6.** *Trichomonas gallinae* in monkey kidney cell culture, 20 hours (Fig. 5) and 24 hours (Fig. 6.) after inoculation. Protargol. Figs. 1—3: 450×, Fig. 4: 600×, Figs. 5, 6: 300×.

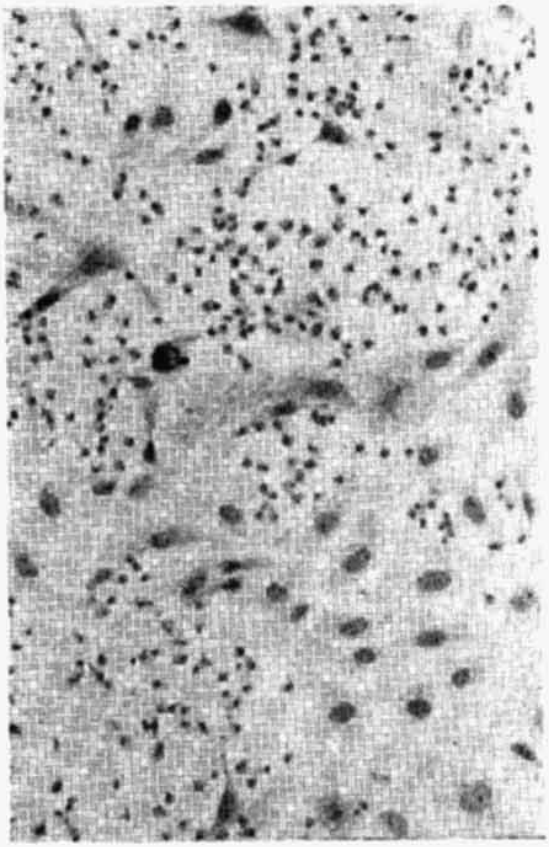


Fig. 1

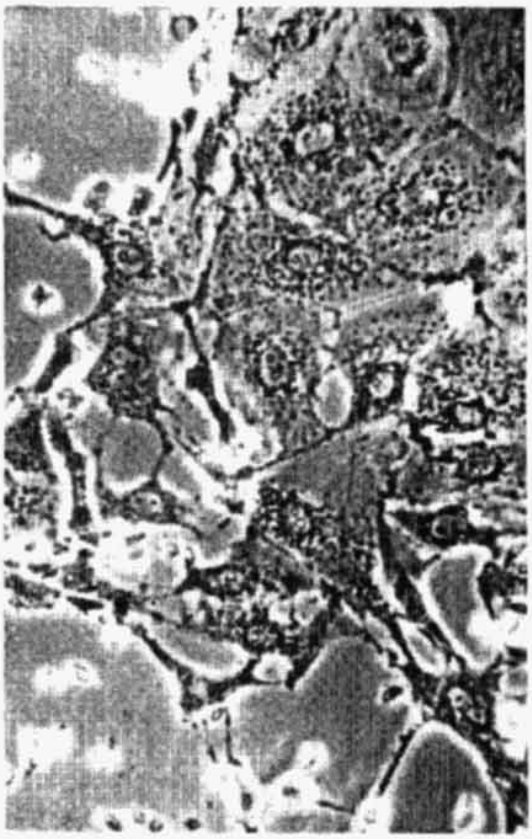


Fig. 2

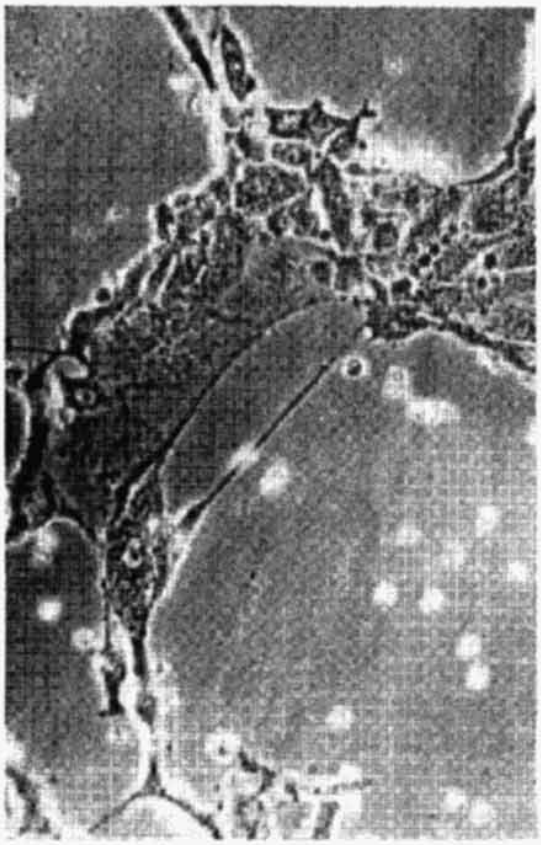


Fig. 3

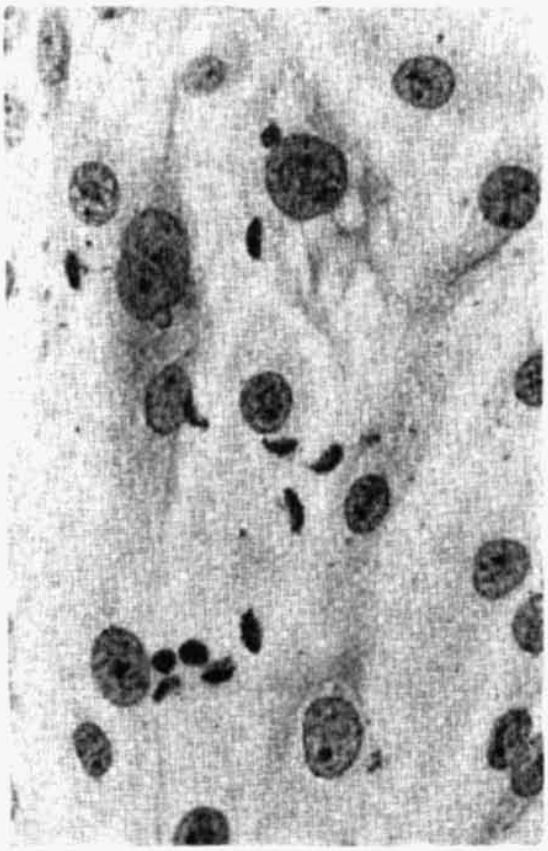


Fig. 4

Figs. 1–3. Monkey kidney cell culture 24 hours (Fig. 1, protargol, 100×), 36 hours (Fig. 2, fresh mount, phase contrast, 200×), and 48 hours (Fig. 3, ditto) after inoculation with *Trichomonas gallinae*.

Fig. 4. *Triurichomonas foetus* in monkey kidney cell culture, 16 hours after inoculation. Protargol, 400×.

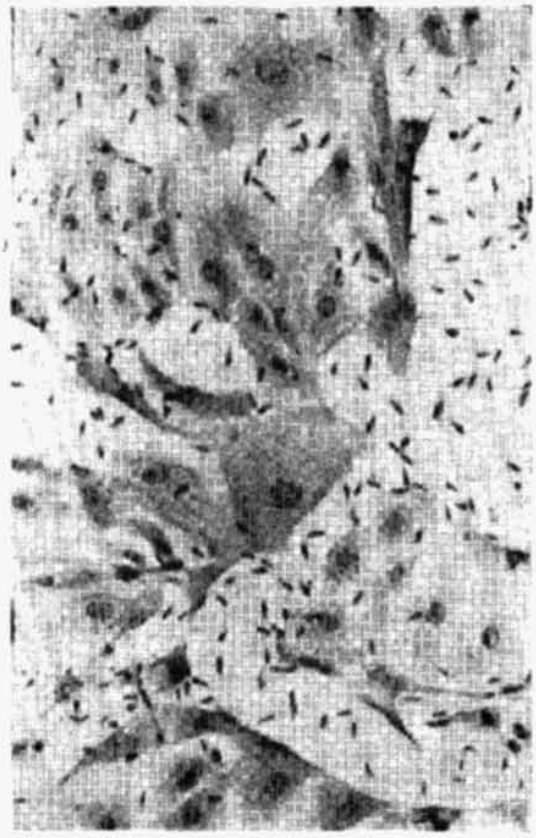


Fig. 1

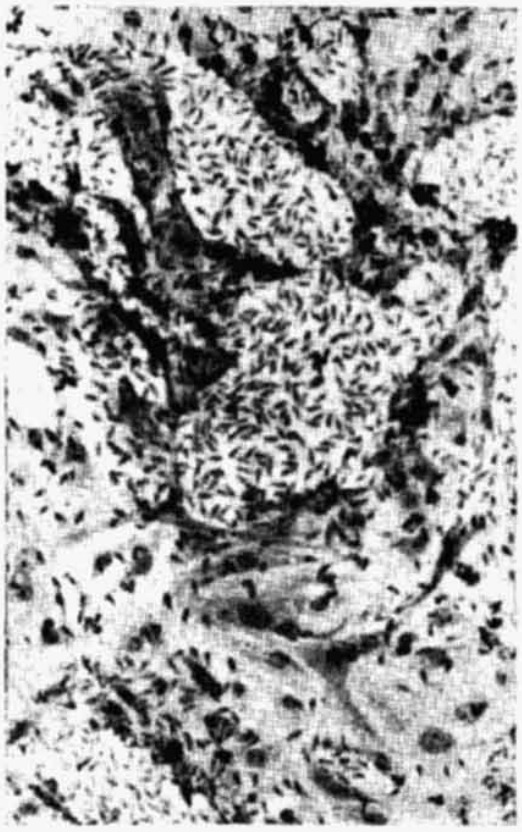


Fig. 2

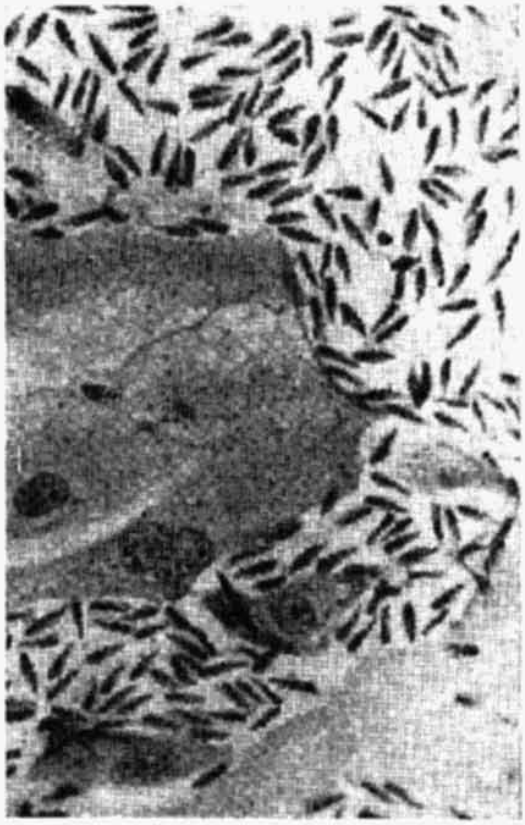


Fig. 3

Fig. 4

Figs. 1—4. *Tritrichomonas foetus* in monkey kidney cell culture, 28 (Fig. 1) and 32 (Figs. 2—4) hours after inoculation. Protargol, Figs. 1, 2: 100×, Fig. 3: 300×, Fig. 4: 900×.

Intracellular occurrence of either *Tritrichomonas suis* or *T. foetus* has never been observed in experimental cell cultures.

No differences were also found in the behaviour of the used strains of *T. suis* and *T. foetus* within the cell cultures and in their effect upon the cells.

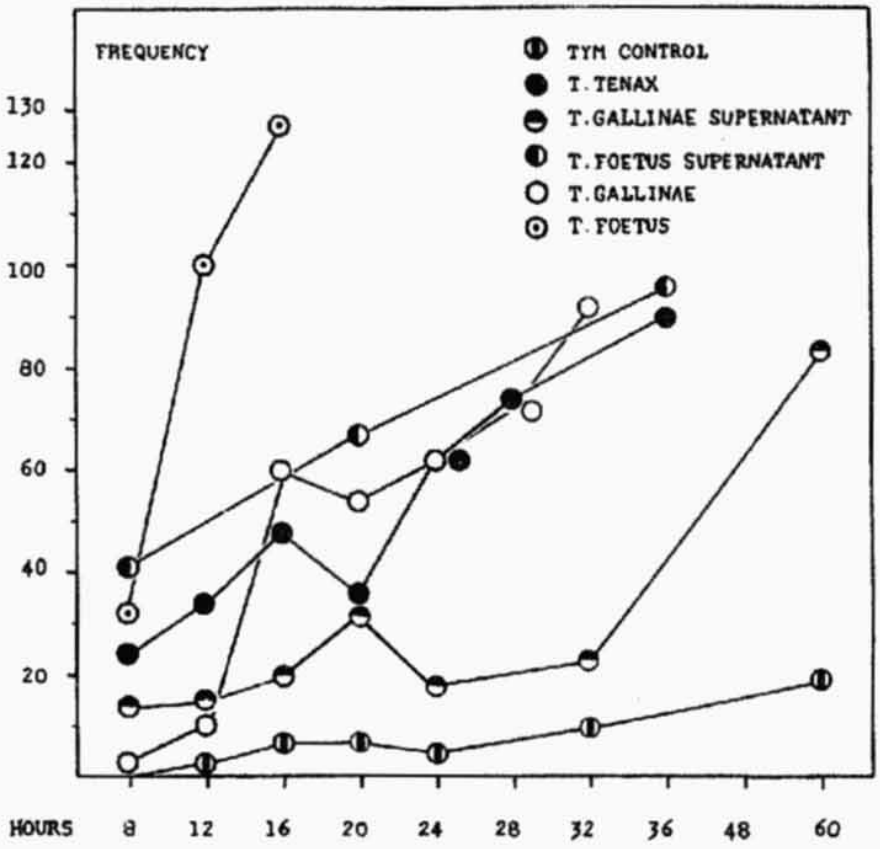


Fig. 7. Frequency of vacuolized cells in monkey kidney cell cultures at various intervals after the inoculation with *Trichomonas gallinae* H 4a, *Tritrichomonas foetus* KV 1, trichomonad-free supernatants from cultures of these strains, with *Trichomonas tenax* and TYM medium (frequency ascertained on 50 random fields, microscope Lumipan, Zeiss, obj. 20).

D. The effect of ultrafiltrates and centrifugates from rich cultures of trichomonads on monkey kidney cell cultures

In none of the trichomonad species studied, the ultrafiltrates and supernatants from rich cultures exhibited any remarkable effect on the cells which would result in a rapid cell degeneration and a subsequent cell layer destruction, proving thus the presence of a higly effective exotoxin.

A more detailed morphological comparison of the cells inoculated with a supernatant from the culture of the strains *Trichomonas gallinae* H4a and *Tritrichomonas foetus* KV1 with controls revealed, however, a higher intensity and frequency of the changes taking place in the aging cell cultures. These changes include especially a decrease of the division rate, occurrence of multinuclear cells and cells with deformed nuclei, raw granulation of nuclear substance and vacuolisation of the cytoplasm (Tab. 2, Figs. 6—10). They were more significant in cultures exposed to supernatants from *T. foetus* culture.

DISCUSSION

The published papers dealing with the effect of *Trichomonas vaginalis* on various types of tissue cultures, are differing as regards the factors causing injuries of the cell cultures. The standpoint of HOGUE (HOGUE 1942a, b, c, 1943, 1947) ascribing the pathogenic effect of trichomonads exclusively to the influence of an exotoxin,

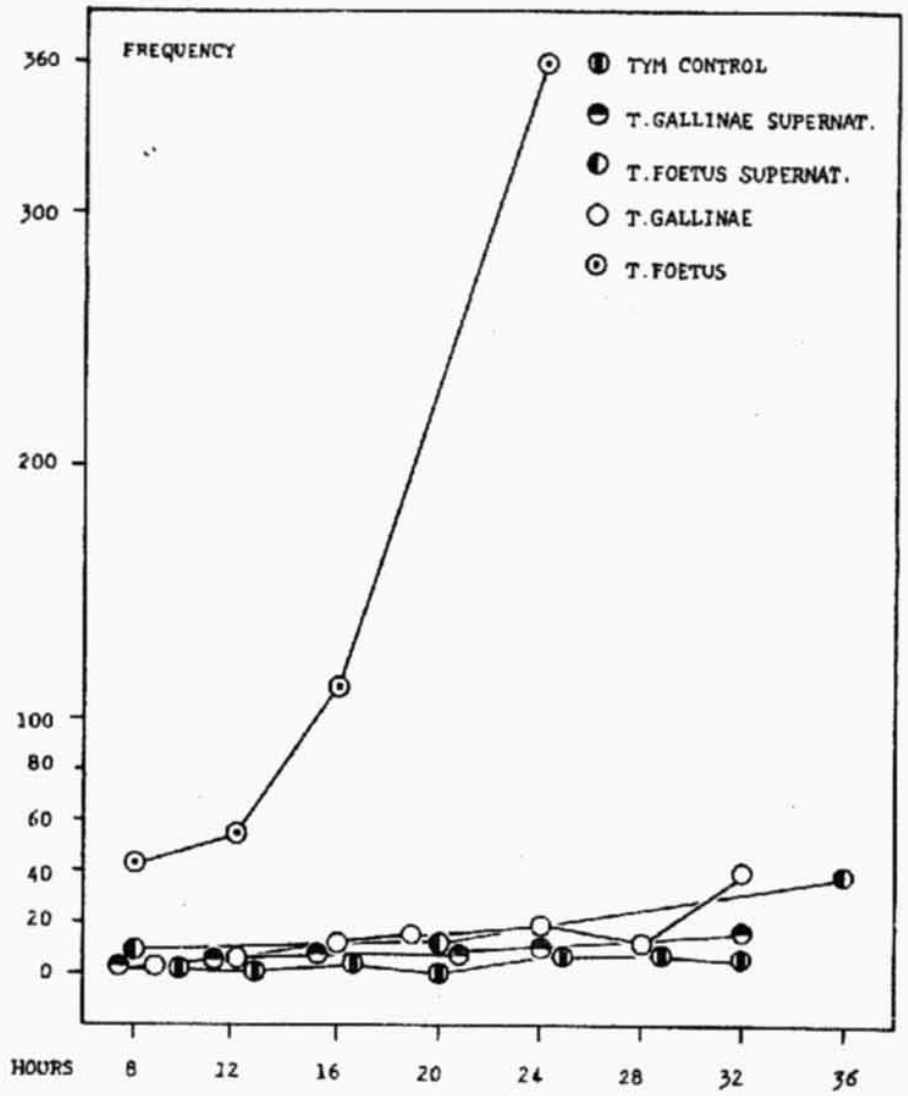


Fig. 8. Frequency of rawly granulated nuclei in monkey kidney cell cultures at various intervals after inoculation with *Trichomonas gallinae* H 4a, *Tritrichomonas foetus* KV 1, trichomonad-free supernatants of these strains and the TYM medium (frequency ascertained on 50 random fields, Zeiss, Lumipan, obj. 20).

was opposed by some authors denying the presence of exotoxic substances (KOTCHER, HOOGASIAN 1957, CHRISTIAN, MILLER, LUDOVICI, RILEY 1963). HONIGBERG and EWALT (1963) do not exclude a possible influence of toxic substances produced by the parasite, but they do not specify them as exotoxin. They take the resulting effect for a combination of the direct and indirect effect of the parasite upon the tissue culture.

HONIGBERG and MCLURE 1960 state that in *Trichomonas gallinae* "there seems to be no evidence of the presence of true exotoxin". In a more detailed paper by HONIGBERG et al. 1964, dealing with the effect of *T. gallinae* on chick liver cell cultures, an essential importance in the cell injury is ascribed to the "direct" effect of trichomonads. Nevertheless, these authors presume also the occurrence of labile toxic substances.

From the results presented in this paper it is obvious that a destruction of the cell layer takes place only if trichomonads are present. Ultrafiltrates and supernatants from rich cultures do not exhibit such effects. The behaviour of the parasite within the cell culture as well as the development of the cell layer destruction give evidence that a mechanical effect of trichomonads, respectively the influence of chemical factors bound to the parasite cell, are of essential importance.

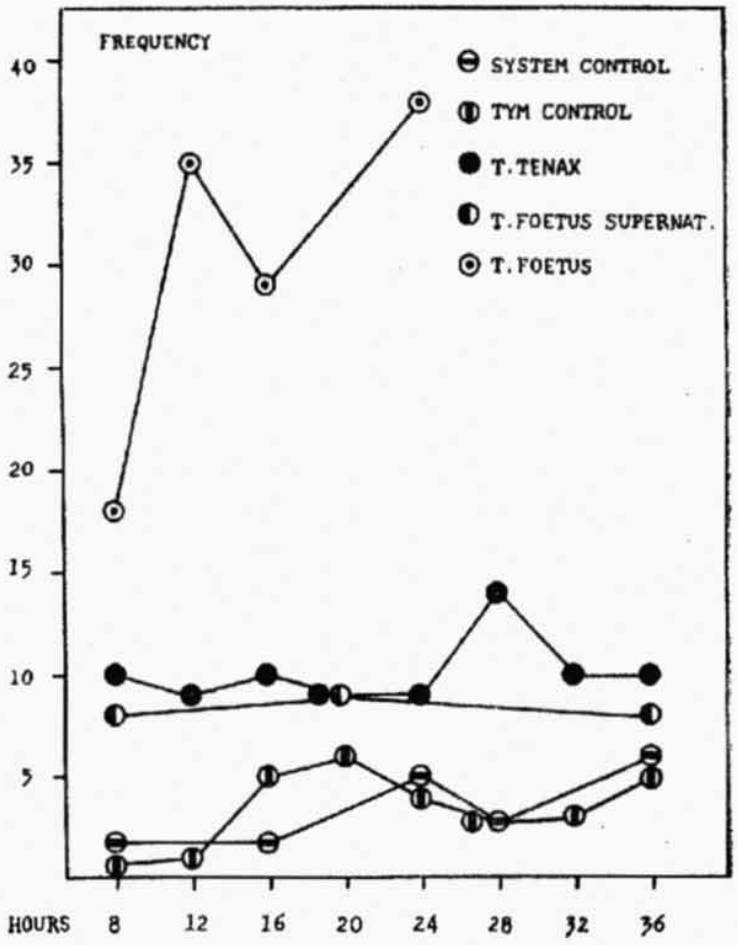


Fig. 9. Frequency of pyknotic nuclei in the monkey kidney cell cultures at various intervals after inoculation with *Tritrichomonas foetus* KV 1, trichomonad-free supernatant from a culture of this strain and *Trichomonas tenax* compared with controls. Frequency determined per 50 random fields (Zeiss, Lumipan, obj. 20).

The results obtained with *Tetratrichomonas gallinarum* strains proved that a multiplication of trichomonads within a cell culture by itself must not be responsible for the cell culture injury. A comparison of Figs. 2 and 5 reveals that the maximal population density in the strain *T. gallinarum* KR 109, displaying no distinct effect on the cells, surpasses that found in *Trichomonas vaginalis* strains 101 and 108. (In *T. gallinarum* it amounts to 1.53×10^6 organisms per 1 ml, in *T. vaginalis* to $0.56—0.63 \times 10^6$ organisms per 1 ml.) Evidently the effect of trichomonads upon the cells cannot be explained merely by the unfavourable effect of a foreign milieu; in *T. gallinae* and *T. vaginalis* the existence of a specific complex of qualities, resulting in the mentioned manifestations, can be presumed.

According to HONIGBERG and EWALT 1963, suppression of the cell culture growth and changes in the nucleus as well as in the cell cytoplasm, evoked by addition of trichomonad-free filtrates, is in connection with the pathogenic effect of trichomonads. The fact that all these changes in the aging cell cultures appear sponta-

neously and the addition of filtrates or supernatants only increases their frequency, and the fact that the frequency of these changes is increasing even when nonpathogenic trichomonads are inoculated (see curves for *T. tenax* on Figs. 6, 7, 9, 10), throw doubt on the specificity of this effect.

When attention is paid to the process of destruction taking place in the monolayer of the monkey kidney cell culture, the interpretation of HOGUE (1938), presupposing the influence of an exotoxin, seems to be acceptable. Trichomonads do not display any affinity to cells and their mechanical effect can be considered only in the last stages of the cell culture destruction, when the cell layer had been damaged by the influence of other factors. Experiments with ultrafiltrates and supernatants revealed that small changes within the cultures, especially inhibition of the cell division and cytoplasmic vacuolisation, are more expressive in these trichomonads than in supernatants from cultures of other used species (Figs. 6—10). This can however hardly be taken for a sufficient proof of the existence of an exotoxin. It is possible to presuppose that in the period of the maximal growth of trichomonads the concentration of toxic substances in the cell culture reaches considerably higher levels compared with those found after addition of 0.2 ml of a filtrate or supernatant. On the other hand, it is questionable whether the growth of *Tetratrichomonas gallinarum*, showing no distinct effect on the cells, may be considered as a sufficient control of the specificity of the effect of the used *T. suis* and *T. foetus* strains. The maximal population density of these two strains within a cell culture surpasses namely by

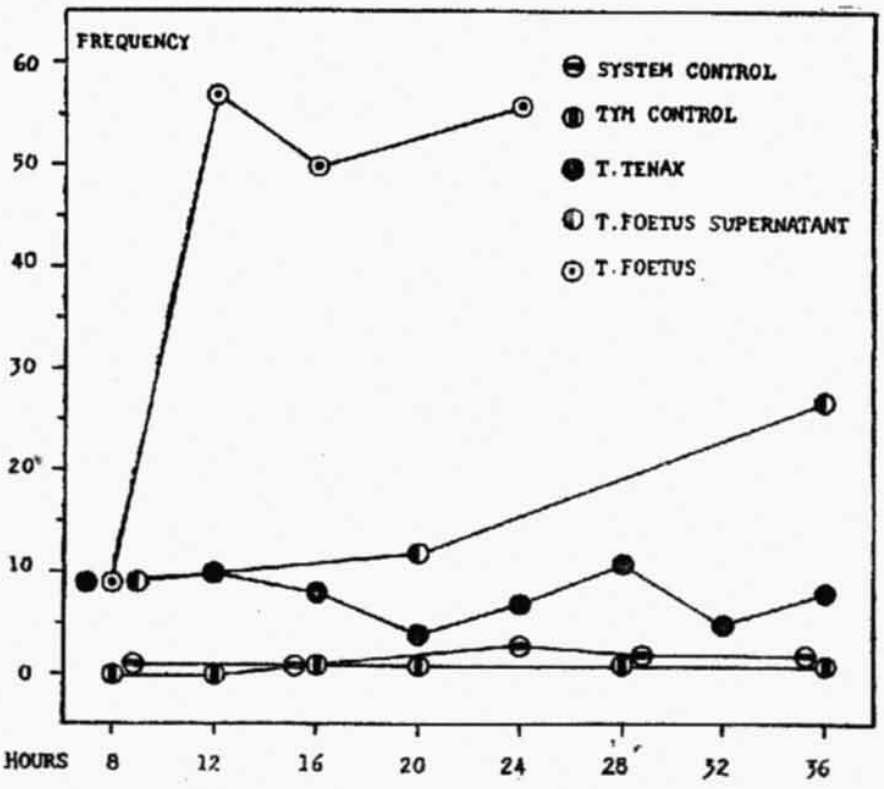


Fig. 10. Comparison of the frequency of lobular nuclei in monkey kidney cell cultures at various intervals after inoculation with *Tritrichomonas foetus* KV 1, trichomonad-free supernatant from a culture of this strain, *Trichomonas tenax* and in controls.

more than 2 times the value found in the best growing strain of *T. gallinarum* (Figs. 2,3). This is why we cannot exclude the possibility that *T. suis* and *T. foetus* species are manifesting themselves only as competitive factors within a tissue culture; the destruction of the tissue culture may therefore not express a specific feature of their pathogenity. As stated by SHORB 1964, a similar conclusion was made by SWITZER 1959 in his study of *T. suis* in the swine kidney cell cultures. For elucidation of these problems a more detailed study would be necessary.

Using the monkey kidney cell cultures, this study did not succeed in proving sufficient differences in the virulence of various strains of the same trichomonad species. HONIGBERG, EWALT 1963, HONIGBERG, MCLURE 1960 and HONIGBERG et al. 1964 achieved better results by using another cell system. Nevertheless, even their results demonstrate that it will be hardly possible to use tissue cultures for the elaboration of a simple virulence test. There is, however, no doubt that cell cultures can be used as a suitable model in the study of the host-parasite relationship. Recent studies dealing with cytochemical changes in exposed cells (ABRAHAM, HONIGBERG 1965, SHARMA, HONIGBERG 1966) are pointing to a new path.

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