Proc. Estonian Acad. Sci. Biol., 1990, 39, N 4, 242—258
https://doi.org/10.3176/biol.1990.4.02
УДК 576.858+576.89

Jüri TERAS and Leida KESA

THE RELATIONSHIPS BETWEEN PROTOZOA AND VIRUSES

4. PROTOZOA AS HOSTS OF MAMMALIAN VIRUSES

The recent years have witnessed the revival of interest in protozoa as possible reservoirs and transmitters of mammalian viruses. This is by and large due to great progress in the investigation of protozoan viruses, virus-like particles and cytopathogenic agent in protozoa and protozoa as inactivators of viruses which have been dealt with in the previous three parts of the present paper (Teras, Kesa, 1988 a, b; 1989).

Research into this problem started about 50 years ago when Swedish virologist Kling and his colleagues, studying sewage water during and after one of poliomyelitis epidemics in Stockholm in the autumn of 1939 put forward the hypothesis of the role of protozoa as hosts of poliovirus. Their investigations started after American virologists Paul et al. (1939) reported on findings of poliovirus in sewage water of three towns of the USA during an outburst of poliomyelitis in the summer of 1939.

For their first experiments Kling et al. (1942 a, b) used 10 litres of sewage water collected from sewage in a large district of Stockholm. After two months of preservation of the material at 4 °C the authors removed the subsediment and injected 60 ml of it to monkeys intraperitoneally and 1.0 ml into nerves. By the 18th day of the experiment clinical symptoms, characteristic of poliomyelitis, appeared. Dissection revealed extensive and manifest typical pathohistological changes. Identical pathology was observed in the monkey who had been intraperitoneally and intraneurally infected with the material taken from the first animal, and also in the monkey infected, in turn, with the material taken from the second animal.

Being now sure that sewage water of Stockholm contained poliovirus during the epidemic of poliomyelitis, Kling (1940) reported on it to the Medical Academy in Paris. Stressing in this report of his mainly the finding of poliovirus and its remaining infections in sewage water of Stockholm, Kling later paid more attention to the reasons of discovering poliovirus outside human organism on the basis of special experiments. Kling et al. (1942 a, b) ascertained that poliovirus rapidly lost its infectiousness in the phaeces of patients suffering from poliomyelitis. Besides, they discovered poliovirus also in the sewage water taken from the central sewage pipe of Stockholm three months after the last registered case of poliomyelitis. Consequently, the poliovirus could not remain in sewage for such a long time, having got there only from the phaeces of patients suffering from poliomyelitis. Especially as in that district of the city where the material for the first investigation had been collected, i. e. during the epidemic, only 10 cases of poliomyelitis were registered within a year, whereas four of them were diagnosed even six months before the pandemia started. In that district ca 20 million litres of sewage ran through the pipe daily. According to Kling et al. such water should contain 3 million infectious doses of poliovirus, since injection of 60 ml already caused in monkeys changes, characteristic of poliomyelitis. On the basis of the obtained data the authors suggested two possible variants. According to these either the poliovirus replicated most rapidly in phaeces of patients suffering from poliomyelitis (whereas replication had to be repeated up to 30 000 times), or all the 100 000 citizens residing in the

district from where the material for investigations had been taken during the epidemic, must have been carriers of poliovirus. Since both these variants were groundless, Kling et al. came to the conclusion that poliovirus could have replicated only in sewage pipes, which also explains why it was found even after the epidemic.

Rodents and insects, due to the closed sewage system of Stockholm, could not be regarded as reservoirs of poliovirus. Having studied the biological composition of sewage water, Kling et al. chose protozoa as the most likely reservoirs of poliovirus. It appeared that 80% of the examined samples of sewage water contained protozoa of *Bodo* and *Monas* genera of *Protomonadina* order. They were detected both in the phaeces of poliomyelitis patients and healthy people. Taking into account more frequent presence of protozoa of *Bodo* than other genera, including *Monas*, in the phaeces of poliomyelitis patients (in 73% of the patients), the researchers supposed that namely these flagellates, being able to multiply both in the human organism and water, might be possible reservoirs of poliovirus.

It should be mentioned here that yeast cells had also been regarded as possible reservoirs of poliovirus in sewage water earlier, but as there were no data in literature on the spreading of any infection through yeast, the authors paid no special attention to these protista.

Although Kling et al. could neither prove their hypothesis nor establish the role of protozoa in the preservation and replication of poliovirus, their hypothesis evoked a keen response as the problem of poliomyelitis was very urgent at that time. Scientists began to seek for poliovirus not only in sewage water but also tried to establish experimentally the protozoa in which this virus might persist and replicate.

The first experimental investigations were carried out by Evans and Osterud (1946), who, in their first series of experiments during one of the outbursts of poliomyelitis in the USA in autumn, 1944 tried to elucidate the possibility of adaptation of poliovirus in protozoa as well as various other microorganisms inhabiting rivers, lakes and sewage water.

For such experiments the authors used 12 virulent strains of poliovirus that had been passaged only a few times through monkeys. From each poliovirus strain 10% suspension of monkey's spinal cord was made to infect the protista. Adding 15 ml of that suspension to 135 ml of water which contained protozoa as well as bacteria in natural quantity and composition, the authors got a conditional dilution of the virus 1 : 100. Seven days later 15 ml of this material was, in turn, added to 135 ml of water, recently collected for investigation. Similar passages were repeated every seven days till the dilution of poliovirus in water reached 1 : 100 000. A week after the last passage samples of each series were treated with ether and injected to monkeys. Analogical experiments were carried out with the Armstrong strain of poliovirus adapted to mice, as well as with two strains of *Theiler* virus (blue tongue virus). The results of all these experiments proved to be negative, i. e. in none of the animals symptoms of poliomyelitis appeared.

Later on the authors carried out investigations with cultures of protozoa grown in a special medium, consisting of 10 ml of wheat-water, 25 ml of agar, and mineral and organic substances. Mainly species of *Bodo* genus were used for research, for they predominated in the investigated sewage water of Minneapolis and Saint-Paul. Seven strains of these flagellates were studied simultaneously, one strain of the genus *Monas*, one strain of the genus *Oikomonas* and 3 mixed cultures, mainly containing protozoa of *Tetrahymena*, *Uronema*, *Monas* and *Pleuromonas* genera.

For infecting the cultures of protozoa the authors used six strains of

poliovirus, one of which was able to replicate in the organism of rodents. The virus was added to the cultures of protozoa in 0.2 ml portions of 10% suspension of monkey's spinal cord, infected with poliovirus, whereas the initial dilution of the virus in protozoan cultures was 1 : 400. Depending on the intensiveness of multiplication, every 5—14 days the protozoa were inoculated anew into a fresh medium in 0.1 ml portions until the virus dilution reached $4 \cdot 10^{-6}$. The material of the second subculture was treated with ether, purified from associating bacteria by centrifuging, and then intracerebrally infected to monkeys. As protozoa multiplied intensively in the cultures, infected with the virus, the authors did not concentrate the virus in their first experiments, but later they increased the supposed concentration 40 times.

All the results of these experiments were negative and the authors decided that the replication of poliovirus in microorganisms, including protozoa inhabiting lakes and rivers as well as sewage water, is very doubtful, and therefore the data on finding poliovirus in quantities in protozoa in sewage water pipes do not seem convincing.

Brutsaert et al. (1946) expressed a more cautious opinion of the possible replication of poliovirus in protozoa. They induced a highly virulent strain of poliovirus, adapted to white mice, to mixed intestinal bacterial flora of man and to xenic cultures of *Bacterium coli communis*, *Leptospira biflexa*, *Entamoeba coli*, *Trichomonas hominis*, *Chilomastix mesnili*, *Tetrahymena geleii*, *Leishmania donovani* and *Trypanosoma gambiense*. In all the experiments the authors used a 10% suspension of mice's brain, infected with poliovirus, which was mixed with the culture of microorganisms under study so that the final concentration of the virus were 1 : 100. After two or seven days part of these cultures was inoculated into a fresh medium, and the virus concentration was diluted in this as well as every following passage ten times. The material from control test-tubes, initially containing a 10% suspension of brain and sterile medium, was analogically reinoculated. After each passage the vitality and amount of the microorganisms were studied with a microscope, and presence of the virus — by injecting the culture to white mice. The cultures of enterobacteria were, after being filtered by Seitz, intracerebrally induced to experimental animals in 0.3 ml portions. The cultures of *Entamoeba coli*, *Chilomastix mesnili* and *Trichomonas hominis* were induced intraperitoneally in 0.1 ml portions without previous filtering.

The infected mice were observed until characteristic symptoms of poliomyelitis occurred, whereas the suspension, prepared from tissues of experimental animals, which had died without apparent paralysis, were once more induced to mice. As a matter of fact, all the protozoa under investigation, except *T. hominis*, as well as enterobacteria caused paralysis in white mice not later than in control experiments with mediums containing the virus only.

In xenic cultures of *T. hominis* induced with poliovirus the virus was detected even after the 4th successive passage (the concentration of the initial virus-containing material was 10^{-5}), while from the control medium poliovirus disappeared already after the first passage. Therefore the authors decided to carry out a more thorough study of this protozoon. But the earlier hypothesis of the replication of poliovirus in trichomonads was refuted, as typical changes of poliomyelitis appeared also in those test animals that were intraperitoneally infected with trichomonads to which poliovirus had been added only after their heat-killing. Despite the fact that in these experiments poliovirus lost its infectiousness by one passage earlier than in the experiments with trichomonads, the authors drew the following conclusion: poliovirus did not replicate in the organism of *T. hominis*, but it remained infectious in cultures of both killed and live

trichomonads longer than in a pure medium, merely as a result of more favourable conditions.

Although Brutsaert et al. could establish the replication of poliovirus neither in the studied protozoa nor in bacteria, they did not deny that, using other species of protozoa, such experiments might prove more successful.

Two years later Toomey et al. (1948) tried to find out whether *Amoeba proteus*, which, as a rule, lives in clean water, could be infected with poliovirus, and for how long it might remain a carrier of this virus. The authors isolated a strain of *A. proteus* from a natural spring and started cultivating it in a medium of distilled and wheat-water. Multiplication of the amoebas was especially intensive a month later and that provided the necessary populations for the planned experiments. Protozoa of these cultures were first washed with distilled water in a centrifuge for five minutes, then some distilled water was added to the sediment, and poured into test-tubes in 3 ml. To every portion 0.3 ml of penicillinum and streptomycinum solution, containing respectively 1000 and 100 units of the antibiotic per 1 ml was added.

After that the amoebas were induced with Lansing strain of poliovirus adapted to mice. It was always added to protozoa in the same amount of 10% suspension of mice's brain, which had been injected with this virus. After a 24-hr contact the authors injected these amoebas to CFW type of mice weighing 10 to 15 g, whereas each test animal was intracerebrally injected 0.03 ml of the material. All in all 21 series of experiments were carried out, 24 mice in each. For infecting test animals the authors used unwashed amoebas and those washed to 11 times with distilled water after a 24-hr contact with poliovirus, as well as supernatants obtained at the washing of amoebas, a 10% suspension of mice's brain who had died due to poliovirus infection, and the same material incubed for 24 hr in distilled water at room temperature.

Taking into account that sometimes amoebas themselves proved to be toxic for test animals, the authors regarded the infection as a cause of death of mice only when it was preceeded by paralysis.

The majority of the controlled mice (17 out of 24) infected with virus-containing suspension of mice's brain and also most of the mice (18 out of 24) intraperitoneally infected with amoebas contacting poliovirus for 24 hr, manifested changes typical of poliomyelitis. A great number of mice (23 out of 48), infected with amoebas that had been 10—11 times washed with distilled water after a 24-hr contact with poliovirus in order to remove viruses, which could absorb on protozoa, died showing signs of paralysis. That made the authors think that *A. proteus* is capable of not only absorbing poliovirus on its surface but also phagocyting it.

This conclusion is supported by a remarkable fact that the supernatants obtained during the first washings of the amoebas induced with poliovirus, did not contain any virus. The latter appeared in the supernatants only after the 11th washing of this population of protozoa, which was explained by destruction of protozoan cell membrane as a result of their repeated washing in hypotonic medium, thus releasing the virus. While interpreting the above-described phenomenon the authors noticed that *A. proteus*, known to be very sensitive to environment, became after a contact with poliovirus more resistant to factors causing disintegration than the individuals of normal populations. The authors could not explain this and, while summing up the results of their experiments, only stressed that due to the contact with poliovirus and resistance to the environment acquired through this, *A. proteus* may become a transitory carrier of this virus, which is able not only to absorb on their surface, but also penetrate into their organism.

Thus, Toomey et al. were the first to establish more or less positively that viruses, having penetrated into protozoan organism remain, though for a short period, infectious. Nevertheless, these data were insufficient to prove the hypotheses of Kling et al., the more so since already a year after Toomey's article appeared, another work by Young et al. (1949) was published, which confirmed neither replication nor persistence of viruses in a protozoan organism. These authors also studied poliovirus, using *Entamoeba histolytica* as a model, which was cultivated together with *Escherichia coli* and gram-positive bacteria, similar to *Bacillus subtilis*, in biphase egg-serum Locke medium, the liquid phase of which contained antibiotics to restrain the growth of the bacteria.

The virus was added to 24-hr-old culture of amoebas in the form of a 5% suspension of mice's brain, who had died of poliomyelitis. After their incubation for 10 min, 6, 24 and 48 hr, these cultures were added 500 units of penicyllinum and streptomycinum leaving them then for an hour at room temperature. Thereafter the cultures were centrifuged for 15 min at 1000 turns per min. After separating supernatants from sediments containing amoebas, 2 ml of ether was added to both components, the material was thoroughly mixed and then ether was removed by a pipette. The rest of the sediment was suspended in 2 ml of 0.85% solution of sodium chloride, and 0.03 ml of the obtained suspension was intracerebrally injected to each test animal.

Using the same methods the material necessary for control tests, consisting of poliovirus and bacteria as well as poliovirus alone, was incubated in the same medium for the same period of time.

For the above-mentioned investigation all in all 1584 test animals were used. The research showed that beginning with a 6-hour contact time the highest virus concentration was in the system *Entamoeba histolytica*+poliovirus. Thus, the mice, intracerebrally infected with the material extracted, after separating the supernatants from the amoebas, which had been in contact with poliovirus for 6 hr, died on the average in 3.9 ± 5 days, those which had had a 24-hour contact — in 5.4 ± 8 days, and those with a 48-hour contact — in 6.4 ± 0.8 days. Since the test animals infected with poliovirus incubated for 6, 24 and 48 hr in Locke medium containing no amoebas or any other bacteria or containing bacteria only, died considerably later (resp. in 5.6 ± 5; 12.1 ± 1.1; 11.6 ± 1.4 or 5.2 ± 0.8; 6.8 ± 0.9 and 10.6 ± 1.1 days after infection), the authors carried out some additional experiments. They tried to elucidate whether poliovirus in the system with *E. histolytica* remained more infectious due to replication of the virus in the culture of these protozoa or due to their absorption on the surface of the amoebas. For this purpose Young et al. (1949) prepared a series of dilutions of amoebial cultures not separated from supernatants and associating bacteria, grown together with poliovirus, and each dilution was intracerebrally infected to 6 mice. Since LD_{50} of the dilutions of these two systems did not differ relevantly, remaining practically identical to the dilutions made of poliovirus incubated without protozoa and bacteria in Locke medium, the authors decided that the virus in the system *E. histolytica*+poliovirus did not replicate and that the heightened concentration of the poliovirus there was caused by its absorption on the surface of protozoa.

Either the results obtained by Young et al. proved so convincing that further investigation of Kling's hupotheses seemed pointless or virologists were preoccupied with other important problems at the time of rapid progress of virology in the 50s, but within the next 15 years no data appeared in literature supporting or refuting the ability of poliovirus or any other virus of man or animals to replicate or persist in protozoa.

The problems evoked interest again in 1966, when a Canadian scientist

Kovács (1966) reported at the VI Annual Meeting of American Cell Biology Society that by adding RNA of poliovirus to axenic cultures of *Tetrahymena pyriformis* he had succeeded in reisolating infectious poliovirus from the homogenate of these ciliates already in 24 hr and then identified it by neutralization reaction. The author was sure that absorption and penetration of the virus into protozoa had different mechanisms not yet quite known, and that phagocytosis had an important role in it, especially in *T. pyriformis*.

The same year Kovács et al. (1966) published their first more detailed article on the results of their investigation of the interaction between protista and viruses. In this article they discuss the results of interaction between the complete encephalomyocarditis virus (EMC-virus) and *T. pyriformis* as well as yeast *Saccharomyces cerevisiae*. The method they used was direct immunofluorescence.

The authors took for their experiments a strain of EMC-virus, adapted to and passaged on mice and marked with acridine orange, and axenic cultures of GL strain of *T. pyriformis*, grown in Scherbaum-Zeuthen medium. The ciliates in their stationary phase of growth were washed in conical flasks 5—10 times with physiological solution by centrifuging at 3600 turns per min, and thereafter portioned out into flasks by 1.0 ml. All in all 5 series of experiments were carried out. The flasks of the first series contained only washed ciliates; those of the second one — washed ciliates and acridine-orange; of the third — washed ciliates and marked EMC-virus, grown after isolation from mice on cell cultures; of the fourth — washed ciliates and marked EMC-virus isolated directly from the brain of mice; and of the fifth — washed ciliates and marked virus isolated directly from the tissue, not brain, of mice.

15—60 min after adding virus, up to 4.5 ml of Zeuthen medium was put into each flask. The flasks were placed on a roller regulated at 70 turns per min. Material for investigation was taken from the flasks at certain intervals, the longest period of incubating at 28 °C being 36 hr. The ciliates of all the samples were precipitated by centrifuging and tenfold washing: twice with distilled water, twice with PBC, twice with 0.15 M solution of NaCl, twice with 0.3 M solution of saccharose and water, and again twice with distilled water. After the last centrifuging the ciliates were spread on a stage, fixed with acetone for two min and washed with buffered glycerole. Such preparates were kept in darkness at 4 °C those not fixed — at −20 °C. The preparates were studied by an all-purpose Zeiss microscope with a fluorometre and spectrophotometre for measuring the quantity of fluorescence.

The same scheme and methods were used also for investigating *Saccharomyces cerevisiae*, induced with EMC-virus and cultivated in a semi-defined medium.

The obtained data revealed that EMC-virus marked with acridine orange appeared after incubation with *T. pyriformis* and yeast in the cells of both protista. This was evidenced by fluorescence in the cytoplasm of the protista, whereas neither the ciliates nor yeast that had been in contact with the virus had it.

The authors regarded the appearance of fluorescence as a proof of not only penetration into, but even replication of viruses in the organism of *T. pyriformis* and yeast cells. The real data, though, allow us, at least as much as *T. pyriformis* is concerned, to speak only of detecting the virus in the cytoplasm, since we cannot exclude the possibility that the virus appeared there only as a result of phagocytosis.

A year later Kovács and Bucz (1967) presented more valid data both on the penetration and replication of the virus in these protista. In their work the authors described the experiments carried out on *T. pyriformis*

and *S. cerevisiae* with complete EMC-virus and infective RNA of an attenuated strain of poliovirus.

The methods for these experiments, worked out in the course of numerous preliminary tests, were much more complicated and precise than those applied in previous investigations. For establishing virus in ciliates and yeast cell cultures and for determining the titer of the virus in these protista — the reaction of hemagglutination with guinea pigs' erythrocytes, twice washed and then suspended in 0.15 M solution of NaCl was used.

In the first test series the authors used comparatively small doses of complete EMC-virus for induction of *T. pyriformis* and *S. cerevisiae*, and ascertained the titer of the virus by hemagglutination after 48-hr incubation of the protista with the virus. The virus titer in yeast cultures grew from the initial 0.005 hemagglutination units per cell to 0.065 units in the homogenate of yeast cells, and even up to 0.2 units in the whole culture (i.e. in supernatant and homogenate together). The titer of EMC-virus increased also in axenic cultures of *T. pyriformis* from the initial 0.065 hemagglutination units per protozoon to 2.4 units in the homogenate of the ciliates, and up to 19 units in the whole culture.

As the titer of hemagglutinins of EMC-virus in control media remained at the initial level, the authors drew the conclusion that the increase of the hemagglutinins' titer of viruses both in yeast cultures and *T. pyriformis* was the result of the replication of the virus in these protista.

The second series of experiments was carried out with very highly purified infectious RNA of poliovirus, which had been grown either on cells of monkey's kidneys or on cells HeLa and Fl. 24 hr after adding RNA of poliovirus to the cultures of *S. cerevisiae* and *T. pyriformis* the protista were destroyed as a result of repeated washing, and the obtained homogenates inoculated in cell cultures.

In all four analogical experiments with yeast and five experiments with ciliates, the authors succeeded in reisolating complete poliovirus from homogenates of these protista. The poliovirus had complete cytopathogenic effect on cell cultures and could be neutralized by type-specific antiserum.

Thus, it took 25 years for the hypothesis of Kling et al. (1942a, b) on the replication of poliovirus either in protozoa or yeast cells to be experimentally proved. At the same time it was proved that, beside poliovirus and EMC-virus, also other types of viruses may replicate in these protista. From the given article by Kovács and Bucz (1967) as well as other subsequent reports by Kovács (1967—1968, 1969) we learn that in Zoology Department of Toronto University the replication of polyoma virus in the organisms of *T. pyriformis* and *S. cerevisiae* was also established, whereas the virus reisolated from the protozoa after inducing them both with complete virus and highly purified DNA. The methods used were the same as in case of poliovirus and EMC-virus.

Besides, the authors noticed that the adding of the carcinogen urethan to the system when inducing *T. pyriformis* with complete virus, and dimethylsulfoxide when inducing it with infectious DNA, facilitated the replication of this virus. Discussing possible mechanisms of the penetration of the virus into protista's organism, Kovács (1969) suggested that in case of inducing protozoa and yeast with complete virus, only nucleic acid penetrates into the cell after being released from external protein membrane.

Besides the three above-mentioned types of viruses, i. e. poliovirus, EMC-virus and plyoma virus, Kovács and Kolompar (1969) described also the replication of a vaccine strain of measles virus in the organism of *T. pyriformis*, which had been established by hemadsorption reaction

using Lennet's method. In these experiments the authors used axenic cultures of *T. pyriformis* incubated for 24, 48 and 72 hr in Scherbaum-Zeuthen medium together with measles virus, and a 4% suspension of washed erythrocytes of guinea pigs and monkeys. As absorption of erythrocytes took place only on the ciliates that had been incubated with viruses no less than 24 hr, the authors came to the conclusion that the virus, penetrating into *T. pyriformis*, began to replicate intensively. Such conclusion is supported by the fact that when the authors used for the induction of *T. pyriformis* the measles virus that had been previously treated with reconvalescence serum, no absorption of erythrocytes was observed. But in case the virus had been treated with rabbit or horse sera before adding to ciliates, absorption was quick to follow.

During these experiments the authors paid special attention to the vitality of the ciliates induced with measles virus; alongside with the decreased mobility and retarded replication cycle they also observed certain changes in the size and form of the protozoa in the cultures accompanied by reduction in number (to 29%). Relying on this the authors supposed that the penetration of measles virus into the organism of *T. pyriformis* was followed not only by the replication of the former, but also by the stimulation of the biosynthesis of hemagglutinative and haemolytic factors as well as by increased activity of lipolytic enzymes.

According to Kovács et al. (1967) and Kovács (1969), deviations in the life cycle and multiplication of *T. pyriformis*, *S. cerevisiae* and *Candida albicans* occur also in case of the penetration of EMC-virus into their cells. These deviations increase correspondingly to the virus titer in protista. Thus, for instance, the number of *T. pyriformis* in the cultures induced with EMC-virus was in 24 hr on an average 20% and in 48 hr even 45% smaller than in the control populations of ciliates. During that time both the titer of the infectiousness of the virus and that of hemagglutinative units per individual grew approximately twofold.

The next to succeed in proving that viruses can penetrate into protozoan organism and replicate there was a group of scientists of the Protozoology Departmant of Experimental Biology Institute of the Estonian Academy of Sciences in Tallinn, who have been investigating the problem since 1970.

They used mainly the method of direct immunofluorescence and cell cultures to detect virus in the organism of protozoa.

At first 26 species of protozoa and five types of viruses were studied (Teras et al., 1974, 1977). Six species of the protozoa were parasitic (*Trichomonas vaginalis*, *T. tenax*, *Pentatrichomonas hominis*, *Trypanosoma cruzi*, *Giardia duodenalis*, *Toxoplasma gondii*), and 20 species were free-living, five of which belonged to *Sarcodina* class (*Naegleria gruberi*, *N. aerobia*, *N. fowleri*, *Amoeba proteus*, *A. dubia*), 15 species to *Ciliata* class (*Coleps hirtus*, *Dileptus anser*, *Didinium nasutum*, *Tillina magna*, *Tetrahymena pyriformis*, *Paramecium caudatum*, *P. multimicronucleatum*, *P. jenningsi*, *P. aurelia*, *Climacostomum virens*, *Stentor coeruleus*, *Spirostomum ambiguum*, *Blepharisma japonicum*, *Spirostomum minus*, *Condylostoma* sp.). All these species of protozoa were cultivated in widely known media. The populations of *Toxoplasma gondii* were obtained from the peritoneal exudate of infected white mice. Four types of viruses out of the five used were RNA-viruses, three of which were picornaviruses (Coxsackie B-3 and B-5, Echovirus-11) and one—ortomyxovirus (influenza A/H_3N_2), of DNA-viruses adeno-3 was chosen. For the replication of the ortomyxovirus 9—11 days old hen embryos were used, for other viruses—cell cultures HEp-2 and Rh.

While examining possible associations, the authors added the suspension of one of the viruses to the medium with the protozoa population.

After that the experimentally created system protozoon+virus was incubated at the necessary temperature for 15 days, depending on species. The results of the experiments were usually analyzed immediately after establishing the system. For obtaining preliminary data, preparates for direct immunofluorescent study were made from the protozoa induced with viruses. Homologous virus-specific rabbit antisera marked with fluorescein-isotiocyanate were used for that purpose. In order to prevent possible non-specific fluorescence of the protozoa, the antisera were treated with an acetonized powder, made from protozoa biomass. Having first established that none of the protozoan species under examination gave fluorescence after treatment with such virus-specific antisera, the authors repeatedly studied all the 130 combinations (5 virus types and 26 protozoan species) of the experimentally created systems protozoon+virus using the method of direct immunofluorescence.

It became evident that in 25 systems out of the 130 the protozoa manifested fluorescence after having been incubated with viruses and then treated with virus-specific antisera. This was detected only in nine out of the 26 investigated species of protozoa (Table 1). Among these were eight free-living species (belonging to the *Ciliata* class) and only one parasitic (*Giardia duodenalis*). Fluorescence was observed more often in the protozoa induced with either DNA-virus adeno-3 (in eight species), or picornaviruses Coxsackie B-3 and B-5 (in seven species). After contact with influenza virus, the virus-specific fluorescence was acquired only by *Climacostomum virens*, and after contact with Echo-11 virus — by *Dileptus anser* and *Tillina magna*.

Experiments with Coxsackie B-3 and B-5 viruses produced analogical results, which can be explained by a close similarity of these two types of RNA-viruses. But the affinity of the protozoan species had no effect on the results of these experiments, for related species of the *Paramecium* family reacted to the contact with viruses in different ways. While no type of virus caused any fluorescence in *P. multimicronucleatum* after they had been incubated together, *P. jenningsi* and *P. aurelia* did give virus-specific fluorescence after contracting adeno-3 virus and *P. caudatum*—besides this virus also after contact with Coxsackies B-3 and B-5. These data indicate to a strict species-specificity of interaction between protozoa and viruses.

Table 1

Systems protozoon+virus in which the penetration of viruses into protozoa was detected (Tepac, 1981; Teras et al., 1977)

Species of protozoa	Type of virus used for inducing protozoa				
	Cox. B-3	Cox. B-5	Echo-11	M. infl. A_2	Adeno-3
Lamblia duodenalis	+	+	–	–	+
Tetrahymena pyriformis	+	+	–	–	+
Paramecium caudatum	+	+	–	–	+
P. jenningsi	–	–	–	–	+
P. aurelia	–	–	–	–	+
Dileptus anser	+	+	+	–	+
Tillina magna	+	+	+	–	+
Climacostomum virens	+	+	–	+	–
Condylostoma (sp.)	+	+	–	–	+

"+" penetration of the virus into protozoa was proved;
"–" penetration of the virus into protozoa was not detected.

In order to elucidate whether the use of active or inactivated type of viruses and live or killed cultures of protozoa for creating a system protozoon+virus influenced the appearance of the virus-specific fluorescence, the authors carried out one series of experiments with live cultures of *P. caudatum* and Coxsackie B-5 virus, inactivated by ultraviolet rays, and another — with ciliates killed by heating, freezing or formaline and an active virus.

Fluorescence was not observed in either series of experiments, and the authors carried out some extra studies on the effect of the widely known inhibitors of the replication of viruses, adding leucocytic interferon, aurantine or guanidine to the cultures of *P. caudatum* before the inoculation of viruses. It turned out that only the ciliates of the cultures to which leucocytic interferon had been added gave fluorescence.

Therefore, all the data serve as evidence of the fact that virus-specific fluorescence, which appeared in nine species of the protozoa in all the 25 above-mentioned combinations of protozoon+virus systems, could be caused only by the viruses penetrating into unicellular organisms.

As it seemed too rash to say anything certain about the replication of viruses in these protozoa on the basis of the results of immunofluorescence only, the authors watched simultaneously also the dynamics of the titer of Coxsackie B-5 virus in system cultures with *P. caudatum* and *T. pyriformis*. In order to ascertain viral infection in these protozoa, both lysates of the ciliates and supernatants of the system cultures were used for contaminating the cell cultures HEp-2. The experiments with *P. caudatum* lasted for 11 and with *T. pyriformis* for 15 days; the titer of the virus was determined every 24 hr.

The results revealed that the titer of the cytopathogenic effect (TCD_{50}) of the lysates of the ciliates as well as the supernatants of the cultures of both systems protozoon+virus exceeded at the end of the observation period by far that of the control medium, which contained merely virus. The titer remained almost at the initial level during the first experiment with *P. caudatum* and *T. pyriformis*, increasing, though, even by 3 log in the lysates of the ciliates during the second experiment with *P. caudatum*.

These experiments proved that the penetration of Coxsackie B-5 virus into the organisms of *P. caudatum* and *T. pyriformis* is followed by the replication of the virus in these ciliates.

The authors regarded the results as preliminary, which would enable to choose suitable models and work out necessary methods for further study of the interaction between protozoa and viruses.

Alongside with *T. pyriformis*, the same group of researchers used also *Giardia lamblia* as a model for investigating the relationships between protozoa and viruses. The reason was the fluorescence-specificity of Coxsackie B-5 virus in these protozoa after their contact with that virus (see Table 1). Having first elucidated the effect of *G. lamblia* on Coxsackie B-5 virus on cell cultures, the following experiments showed that the virus penetrated into this protozoon immediately after the system protozoon+virus was established (Lakhonina et al., 1981; Лахонина и др., 1983). During these experiments the authors observed not only the persistence of the virus, but also its replication in the new host. It was firstly proved on the cell cultures by higher titers of cytopathogenicity of the lysates of the protozoa induced with viruses, and secondly, by the rising degree of pathogenicity of the same protozoon on intracerebrally infected animals. Bearing in mind that after passaging *G. lamblia* in a virus-free medium their lysates retained cytopathogenic action on cell cultures and pathogenic effect on new-born mice, and taking into consideration the fact of the reisolation of the virus from the protozoa subcultivated in a

Data on the experimentally investigated systems protozoa+virus published in literature

Virus type	Species of protozoa and type of interaction
1	2
Poliovirus	**Replication in:** *Tetrahymena pyriformis* (Kovács and Bucz, 1967); **Inactivation by:** *Entamoeba moshkovskii* (Blawat and Kowalska, 1963); **Indifferent to:** *Oikomonas* (sp.), *Monas* (sp.), *Bodo* (sp.), *Pleuromonas* (sp.), *Uronema* (sp.) (Evans and Osterud, 1946); *Amoeba proteus* (Toomey et al., 1948); *Acanthamoeba castellani* (Blawat and Kowalska, 1963; Baron et al., 1980); *A. hartmanelli* (Möse et al., 1970); *Trypanosoma gambiense, Leishmania donovani, Chilomastix mesnili, Entamoeba coli* (Brutsaert et al., 1946); *E. histolytica* (Young et al., 1949).
Coxsackie B-3 and B-5	**Replication in:** *Dileptus anser, Paramecium caudatum, Tillina magna, Tetrahymena pyriformis, Condylostoma* (sp.), *Climacostomum virens, Giardia lamblia* (Teras et al., 1977); **Inactivation by:** no data; **Indifferent to:** *Naegleria gruberi, N. fowleri, N. aerobia, Amoeba proteus, A. dubia, Coleps hirtus, Didinium nasutum, Paramecium aurelia, P. multimicronucleatum, P. jenningsi, Spirostomum ambiguum, S. minus, Blepharisma japonicum, Stentor coerulues, Trypanosoma cruzi, Pentatrichomonas hominis, Trichomonas tenax, T. vaginalis, Toxoplasma gondii* (Teras et al., 1977).
Echovirus 11	**Replication in:** *Dileptus anser, Tillina magna* (Teras et al., 1977); **Inactivation by:** no data; **Indifferent to:** *Naegleria gruberi, N. fowleri, N. aerobia, Amoeba proteus, A. dubia, Didinium nasatum, Coleps hirtus, Paramecium caudatum, P. aurelia, P. multimicronucleatum, P. jenningsi, Tetrahymena pyriformis, Spirostomum ambiguum, S. minus, Blepharisma japonicum, Condylostoma* (sp.), *Stentor coeruleus, Trypanosoma cruzi, Pentatrichomonas hominis, Trichomonas tenax, T. vaginalis, Toxoplasma gondii* (Teras et al., 1977).
Encephalomyocarditis virus (Mengo virus)	**Replication in:** *Tetrahymena pyriformis* (Kovács and Bucz, 1967); **Inactivation by:** no data; **Indifferent to:** *Trichomonas vaginalis* (Eva et al., 1979).
Influenza virus	**Replication in:** *Climacostomum virens* (Teras et al., 1977); **Inactivation by:** *Tetrahymena pyriformis* (Groupé and Pugh, 1952; Möse et al., 1970; Perez-Prieto and Garcia-Gancedo, 1975; Teras et al., 1977; Tepac, 1981), *Naegleria* (sp.) (Knorr et al., 1956), *Vahlkampfia limax* (Bauer, 1961); **Indifferent to:** *Euglena gracilis, Astasia klebsi, Glaucoma scintillans* (Groupé et al., 1955), *Acanthamoeba castellani* (Möse et al., 1970), *Naegleria gruberi, N. fowleri, N. aerobia, Amoeba proteus, A. dubia, Didinium nasatum, Coleps hirtus, Dileptus anser, Paramecium caudatum, P. aurelia, P. multimicronucleatum, P. jenningsi, Tillina magna, Spirostomum ambiguum, S. minus, Blepharisma japonicum, Condylostoma* (sp.), *Stentor coeruleus, Trypanosoma cruzi, Pentatrichomonas hominis, Trichomonas tenax, T. vaginalis, Toxoplasma gondii* (Teras et al., 1977).

Table 2 (continued)

1	2
Newcastle virus	**Replication in:** *Eimeria tenella, E. necatrix* (Sibalić et al., 1978); **Inactivation by:** *Tetrahymena pyriformis* (Groupé et al., 1955); **Indifferent to:** no data.
Measles virus	**Replication in:** *Tetrahymena pyriformis* (Kovács and Kolompar, 1969); **Inactivation by:** no data; **Indifferent to:** no data.
Human adenovirus type 3	**Replication in:** *Dileptus anser, Paramecium caudatum, P. aurelia, P. jenningsi, Tillina magna, Tetrahymena pyriformis, Condylostoma* (sp.), *Giardia duodenalis* (Teras et al., 1977); **Inactivated by:** no data; **Indifferent to:** *Naegleria gruberi, N. fowleri, N. aerobia, Amoeba proteus, A. dubia, Didinium nasutum, Coleps hirtus, Paramecium multimicronucleatum, Spirostomum ambiguum, S. minus, Blepharisma japonicum, Stentor coeruleus. Climacostomum virens, Trypanosoma cruzi, Pentatrichomonas hominis, Trichomonas tenax, T. vaginalis, Toxoplasma gondii* (Teras et al., 1977).
Polyoma virus	**Replication in:** *Tetrahymena pyriformis* (Kovács 1967—1968); **Inactivation by:** no data; **Indifferent to:** no data.
Vaccinia virus	**Replication in:** no data; **Inactivation by:** *Tetrahymena pyriformis* (Möse et al., 1970; Perez-Prieto and Garcia-Gancedo, 1975; Jareño et al., 1985); *Onychodromus acuminatus* (Perez-Prieto and Garcia Gancedo, 1981; Jareño, 1987); **Indifferent to:** *Acanthamoeba hartmanelli* (Möse et al., 1970).
Cowpox virus	**Replication in:** no data; **Inactivation by:** *Naegleria* (sp.) (Knorr et al., 1956), *Vahlkampfia limax* (Bauer, 1961); **Indifferent to:** no data.
Pseudorabies virus	**Replication in:** no data; **Inactivation by:** *Trichomonas vaginalis* (Chýle et al., 1971); **Indifferent to:** no data.
Blue tongue virus (Arbovirus Theileri)	**Replication in:** no data; **Inactivation by:** no data; **Indifferent to:** *Oikomonas* (sp.), *Monas* (sp.), *Bodo* (sp.), *Pleuromonas* (sp.), *Tetrahymena pyriformis, Uronema* (sp.) (Evans and Osterud, 1946).
Sindbis virus, vesicular stomatitis virus, herpes 1 and 2 viruses	**Replication in:** no data; **Inactivation by:** *Tetrahymena pyriformis* (Perez et al., 1985); **Indifferent to:** no data.
Coliphage	**Replication in:** no data; **Inactivation by:** *Vahlkampfia limax* (Bauer, 1961); **Indifferent to:** *Naegleria* (sp.) (Knorr et al., 1956).

virus-free medium, the authors assume that the penetration of Coxsackie B-5 virus into *G. lamblia*, as well as the penetration of the same virus into *T. pyriformis*, has a constant character.

Except for the above-given complex experimental data on the penetration, replication and persistence of the mammalian viruses in the cell of protozoa we could find but a few reports on this problem in literature. So, Šibalić et al. (1978) carried out a comprehensive research for elucidating the relationships between protozoa and viruses, including parasitological, virological and serological methods of investigation, as well as experiments *in vivo*. The authors used the *Coccidia Eimeria tenella* and *E. necatrix*, and a vaccine strain of Newcastle virus, belonging to paramyxovirus family, as a model. In their short report the authors state that Newcastle virus is able to penetrate into *Coccidia* at any stage of endogenic development in hens, but the virus is detectable only in oocysts. It remains active in them for at least eight months at 4 °C. When inducing the oocysts of *E. tenella* and *E. necatrix* to hens per os or parenterally, both strains of *Coccidia* and Newcastle virus could be reisolated from them. Unfortunately we did not find more detailed data on this research in literature. Judging by the materials presented by the authors on the VIth International Congress of Protozoology in Warsaw, they appear to be the first ones to have succeeded in establishing the induction of protozoa with viruses *in vivo*.

Here we should also mention the work of Eva et al. (1979), who detected the penetration of complete virus into the organism of a flagellate in the experimentally created system *Trichomonas vaginalis*+Mengo virus (EMC-virus). But attempts to infect trichomonads with the infectious RNA of the virus failed. The penetration of the complete virus was not followed by its replication and therefore the authors assumed that Mengo virus does not develop in the organism of *T. vaginalis* because viral RNA is not released or else it is not translated by the system of trichomonad protein synthesis.

Although the data on the penetration, replication and persistence of viruses in protozoa are still scarce, they provide enough convincing evidence for treating protozoa as possible hosts of mammalian viruses, and, consequently, also as potential sources and carriers of virus infections of both man and animals, including those of natural foci. In studying the ecology of viruses one should take into account alongside other factors also possible associations of viruses with protozoa. Attention should likewise be paid to possible changes of genetic information of protozoa after their interaction with viruses as a result of which free-living unicellular organisms themselves may turn into pathogenic forms. This may occur due to integration of RNA- or DNA-virus with protozoan genome as well as in consequence of chromosome reorganization in the genetic material of protozoa caused by mutagenous effect of viruses.

Conclusion

Beside the forms of interaction between protozoa and viruses discussed above and in our three previous reports (Teras, Kesa 1988 a, b; 1989) the existence of an indifferent form of relationships between unicellular organisms and viruses also deserves mentioning. As can be seen in Table 2, compiled on the basis of literature, the majority of information was received about those systems in which no interaction was observed between protozoa and viruses. It should be born in mind that up to the present only 17 types of viruses have been investigated, nine of which can replicate or persist in the organism of protozoa and nine types are

inactivated by protozoa. Therefore it becomes obvious that both these forms of interaction between protozoa and viruses work only in certain systems, but not in any combination of protozoon+virus. Thus, only one out of the 17 investigated types of viruses, i. e. blue tongue (Theileri) virus, gave indifferent systems of protozoon+virus. But even these data cannot be considered final.

Möse et al. (1970) also agree that the detected indifference of protozoa and viruses has to be treated carefully. They observed relationships of *Tetrahymena pyriformis* and poliovirus for eight days, having detected no replication or inactivation of the virus in the organism of this infusor, they regarded that system as an indifferent one. But Kovács and Bucz (1967) discovered the replication of viruses in protozoa in the very same system.

Thus, we cannot speak of the indifference of viruses to protozoa and, vice versa, as of a certain biological phenomenon without taking into consideration concrete circumstances. There is already some foundation for the hypothesis that if not all the types of viruses, then at least the majority of them have a corresponding species of a protozoon in their environment, in the organism of which the given virus can either replicate or persist, as well as a species of a unicellular organism which inactivates namely this type of virus.

Judging by literary data, up to now three types of viruses out of the 17 investigated have been established to have such protozoa with the opposite effect (Table 2).

Thus, according to experiments, poliovirus is able to replicate in the organism of *T. pyriformis* (Kovács and Bucz, 1967), whereas in the medium containing *Entamoeba moshkovskii* it is inactivated (Blawat and Kowalska, 1963). Influenza virus, which most probably replicates in the organism of *Climacostomum virens* (Teras et al., 1977), is, in turn, inactivated in *T. pyriformis* (Groupé and Pugh, 1952; Knorr et al., 1956; Möse et al., 1970; Teras et al., 1974; Perez-Prieto and Garcia-Gancedo, 1975) as well as in *Vahlkampfia limax* and *Naegleria* (sp.) (Knorr et al., 1956).

Newcastle virus is inactivated in the organism of *T. pyriformis* (Groupé et al., 1955), but replicates in the organism of *Eimeria tenella* and *E. necatrix* (Šibalić et al., 1978).

Because of lack of valid data and even hypotheses on the mechanisms of the replication and persistence of viruses in a protozoan organism and likewise of the inactivation of viruses by unicellular organisms, it cannot yet be explained why one and the same protozoan species appears to be a host for one type of virus and intensively inactivates another. But still, despite all this as well as the fact that the problem of the interaction of protozoa with viruses has been comparatively little studied and is mainly at the level of phenomenology, even on the basis of the results obtained, there is no doubt about the enormous practical and theoretical value of the investigation of this problem.

Beside elucidating possible roles of protozoa in the ecology of viruses, the spread of virus infections and clearing water reservoirs and sewage water from viruses, investigation of the interaction between protozoa and viruses can certainly provide valuable information on the mutagenesis, genetics, pathogenicity and changeability of biological properties of both protozoa and viruses as well as clarify some essential problems of the pathogenesis and immunology of protozoan diseases of man, animals, birds, and fish. No doubt, in the near future the model protozoon+virus will become an essential model also in molecular biology, including biotechnology.

REFERENCES

Baron, D., Danglot, C., Vilagines, R. Rôle d'une amibe libre de l'eau, *Acanthamoeba castellani*, dans le transport de virus animaux nus ou encelopés. — Comp. rend. Acad. Sci., 1980, D 291, 7, 629—632.

Bauer, L. Ein Beitrag zur Klärung der Beziehungen zwischen Amöben und Virus im Wasser. — Arch. Hyg., Bact., 1961, **145**, N 1, 12—20.

Blawat, F., Kowalska, Z. Investigations in vitro on the influence of some amoeba on survival of poliomyelitis virus. — Bull. Inst. Marine Med. in Gdansk, 1963, **14**, 15—24.

Brutsaert, P., Jungeblut, C. W., Knox, A. Attempts to propagate murine poliomyelitis virus on various intestinal bacteria and protozoa. — Proc. Sox. Exp. Biol. Med., 1946, **61**, 163—168.

Chýle, M., Chýle, P., Stepan, J., Patečka, T. Some enzymes and isoenzymes of *Trichomonas vaginalis* and changes in their activities after inoculation with a live mammalian virus. — Časopis Lékařu Českých, 1971, **110**, 234—236.

Eva, A., Viano, I., Martinotti, M. G., Cappucinelli, P. Trichomonas vaginalis resistance to *Mengo* virus infection. — J. Protozool., 1979, **26**, 2, 249—252.

Evans, C. A., Osterud, K. L. The failure of poliomyelitis virus to grow in certain protozoa of sewage. — Science, 1946, N. 2690, **104**, 51—53.

Groupé, V., Herrmann, E. C., Rauscher, F. J. Ingestion and destruction of influenza virus by free-living ciliate *Tetrahymena pyriformis* (21625). — Proc. Soc. Exp. Biol. Med., 1955, **88**, 479—482.

Groupé, V., Pugh, L. Inactivation of influenza virus and of viral hemagglutinin by the ciliate *Tetrahymena geleii*. — Science, 1952, 115, 307—308.

Jareño, M. A. Some ultrastructural changes produced by vaccinia virus in *Onychodromus accuminatus* (Ciliata, Hypotricha). — Acta Protozool., 1987, **26**, N 1, 9—14.

Jareño, M. A., Perez, S., Gancedo, A. The ciliates as useful tools in cytological studies of cell-virus interactions. — In: Abstr. III Intern. Congr. Protozool. Nairobi, 1985, 30.

Kling, C. Sur la présence du virus poliomyelitique dans les eaux d'égout. — Bull. Acad. Medicine, 1940, **123**, 335.

Kling, C., Olin, G., Fahraeus, J., Norlin, G. Sewage as a carrier and disseminator of poliomyelitis virus. I. Searching for poliomyelitis virus in Stockholm sewage. — Acta med. scand., 1942a, **112**, 217—249.

Kling, C., Olin, G., Fahraeus, J., Norlin, G. Sewage as a carrier and disseminator of poliomyelitis virus. II. Studies on the conditions of life of poliomyelitis virus outside the human organism. — Acta med. scand., 1942b, **112**, 250—263.

Knorr, M., Klöhr, K., Kracher, H. Das Schicksal des Virus im Flußwasser und im Boden. II. Mitteilung: Virus und Amöben. — Arch. Hyg., 1956, **140**, N 1/2, 181—207.

Kovács, E. Visualization and propagation of mammalian viruses on their nucleic acids in protista. — J. Cell. Biol., 1966, **31**, 2, 62A, 123.

Kovács, E. Propagation of mammalian, especially tumour viruses in protista and mycoplasma. — In: Annual Report National Cancer Institute of Canada, 1967—1968, 115.

Kovács, E. Enhancing effect of dimethyl sulfoxide on the transfer of polyoma virus to protista with isolated viral genome. — J. Cell. Biol., 1969, **43**, N 2, 73.

Kovács, E., Bucz, B. Propagation of mammalian viruses in protista. II. Isolation of complete virus from yeast and *Tetrahymena* experimentally infected with picorna viral particles or their infectious RNA. — Life Sciences, 1967, **6**, 347—358.

Kovács, E., Bucz, B., Kolompar, G. Propagation of mammalian viruses in protista. I. Visualization of fluorochrome labelled EMC virus in yeast and *Tetrahymena*. — Life Sciences, 1966, **5**, 2117—2176.

Kovács, E., Kolompar, G. Hemadsorption reaction in *Tetrahymena pyriformis* incubated with measles virus. — Life Sciences, 1969, **8**, II, 1089—1097.

Kovács, E., Kolompar, G., Bucz, B. Propagation of mammalian viruses in protista. III. Change in population densities, viability and multiplication of yeast and *Tetrahymena* experimentally infected with encephalomyocarditis virus. — Life Sci., 1967, **6**, 2359—2371.

Lakhonina, G., Teras, J., Kesa, L. On the interaction of Giardia duodenalis and picornaviruses in vitro. — In: Abstr. VI Intern. Congr. Protozool. Warszawa, 1981, 210.

Möse, J. R., Dostal, V., Wege, H. Inaktivierung von Viren durch Abwasserprotozoen. — Arch. Hyg. und Bacteriol., 1970, **154**, N 4, 319—330.

Paul, J., Trash, J., Culotta, S. Poliomyelitis virus in urban sewage. — Science, 1939, **90**, N 3350, 258.

Perez-Prieto, S., Garcia-Gancedo, A. Interaction of *Tetrahymena pyriformis* and mammalian viruses. — In: Abstr. III Intern. Congr. for Virology. Madrid, 1975, 249.

Perez-Prieto, S., Garcia-Gancedo, A. Uptake of vaccinia virus by *Tetrahymena pyriformis*. — Microbiol, exp., 1981, **34**, N 1, 29—43.

Perez, S., Garcia-Gancedo, A., Jareño, M., Vilas, P. On the experimental cocultures of *Tetrahymena pyriformis* with several RNA and DNA viruses. — In: Abstr. VII Intern. Congr. Protozool. Nairobi, 1985, 133.
Sibalič, S., Mihajlovič, B., Tomanovič, B., Kuzmanovič, N. Coccidias as potential carriers of the viruses. — In: Abstr. IV Intern. Congr. Parasitol. Warszawa, 1978, Sect. C, 44.
Teras, J., Jõgiste, A., Kallas, E. Theoretical aspects of the pathogenicity mechanisms of free-living protozoa. — In: Abstr. III Intern. Congr. Parasitol. Munich, 1974, 1, 185.
Teras, J., Kesa, L., Kallas, E., Jõgiste, A. On the relationship between some free-living and parasitic protozoa and RNA and DNA viruses. — In: Abstr. V Intern. Congr. Protozool. New York, 1977, 446.
Teras, J., Kesa, L. The relationships between protozoa and viruses. 1. Protozoan viruses. — Proc. Acad. Sci. ESSR, Biol., 1988a, **37**, N 1, 17—26.
Teras, J., Kesa, L. The relationships between protozoa and viruses. 2. Virus-like particles and cytopathogenic agent in protozoa. — Proc. Acad. Sci. ESSR. Biol., 1988b, **37**, N 3, 185—194.
Teras, J., Kesa, L. The relationships between protozoa and viruses. 3. Protozoa as inactivators of viruses. — Proc. Estonian Acad. Sci. Biol., 1989, **38**, N 4, 289—295.
Toomey, J. A., Takacs, W. S., Schaeffer, M. S. Attempts to infect *Amoeba proteus* with poliomyelitis virus. — Am. J. Dis. Children, 1948, **75**, 11—14.
Young, V. M., Felsenfeld, O., Byrd, C. L. Behavior of virus of poliomyelitis in a culture of *Entamoeba histolytica*. — Clin. Pathol., 1949, **19**, N 12, 1135—1138.
Лахонина Г. М., Терас Ю. Х., Кеса Л. Ю. О взаимодействии *Lamblia duodenalis* с вирусом *Coxsackie* B-5 в экспериментальной системе. — В кн.: Резюме IV Национальной конфер. паразитол. Варна, 1983, 62—63.
Терас Ю. Х. Формы взаимоотношений простейших с вирусами. — В кн.: Взаимоотношения простейших с вирусами. Л., 1981, 21—55.

Estonian Academy of Sciences,
Institute of Experimental Biology

Received
April 11, 1990

Jüri TERAS, Leida KESA

ALGLOOMADE JA VIIRUSTE VAHEKORD

4. Algloomad kui imetajate viiruste peremehed

Märkimisväärsete edusammude tõttu algloomade viiruste, algloomades leiduvate viirusesarnaste partiklite ja tsütopatogeense materjali, samuti algloomade kui viiruste inaktiveerijate uurimisel (autorid on käsitlenud neid teemasid oma artikli kolmes publitseeritud osas: Teras, Kesa, 1988 a, b; 1989) on viimastel aastatel hakatud taas tähelepanu pöörama ka algloomadele kui imetajate viiruste võimalikele reservuaaridele.

Alguse sai selle probleemi uurimine juba umbes 50 aastat tagasi, ajendiks rootsi viroloogi C. Klingi ja tema kaastöötajate artiklid (Kling jt. 1942 a, b), milles autorid, analüüsides polioviiruse pideva leidumise põhjusi poliomüeliidi epideemia vahelistel perioodidel Stockholmi kinnise kanalisatsioonisüsteemi heitvetes, püstitasid hüpoteesi polioviiruse võimalikust ja tõenäolisest replitseerumisest kanalisatsioonitorustikes elunevates algloomades.

C. Klingil ja tema kaastöötajatel endil seda hüpoteesi tõestada ei õnnestunud, kuid nende töö leidis poliomüeliidi erakordse aktuaalsuse tõttu sel perioodil siiski vajalikku tähelepanu. Samaaegselt polioviirust sisaldavate algloomade otsimisega reovetes püüti selgitada ka eksperimentaalselt, kas ja millistes algloomades see viirus replitseeruda ja persisteeruda üldse võib (Evans, Osterud, 1946; Brutsaert jt., 1946; Toomey jt., 1948; Young jt., 1949).

Tõestuse leidis hüpotees imetajate viiruste penetreerumisest algloomadesse alles 25 aastat pärast C. Klingi ja tema kaastöötajate artiklite publitseerimist. Nii õnnestus Kanada teadlastel eesotsas E. Kovácsiga kindlaks teha, et mitte ainult kompleetne viirus, vaid isegi selle infektiivne RNA ja DNA on suutelised sisenema algloomadesse ja seal tõenäoselt ka replitseeruma (Kovács, Kolompar, 1969). Selliste viiruste hulgas olid nii polioviirus ja sellega väga sarnane entsefalomüokardiidi viirus kui ka onkogeense viirusena tuntud polioomiviirus.

Tähelepanuväärseid tulemusi on imetajate viiruste ja algloomade vahekorra uurimisel eksperimentaalsetel mudelitel saadud ka Eesti Teaduste Akadeemia Eksperimentaalbioloogia Instituudi protozooloogia osakonnas. Sellesuunaliste tööde algetapil püüti leida sobi-

vaid mudeleid nii RNA- kui ka DNA-viiruste uurimisel nii vabalt elavate kui ka parasiteerivate algloomade hulgas. Uuringud tehti algloomade 26 liigi ja viiruste 5 tüübiga, kusjuures algloomade 20 liiki olid vabalt elavad, ülejäänud 6 parasitaarsed.

Kasutades vahekorra algloom—viirus uurimiseks rakukultuure ja immunofluorestsentsmeetodit tehti eksperimentaalselt *in vitro* loodud 130 süsteemis penetreerumine algloomadesse kindlaks 25 süsteemis (Tepac jt., 1976).

Edasistest uuringutest, milleks kasutati peamiselt Coxsackie B-5-viirusega eksperimentaalselt indutseeritud *T. pyriformis*'e ja *Giardia lamblia* mudeleid, selgus, et see viirus ei ole suuteline mitte ainult tungima algloomadesse, vaid seal ka replitseeruma ja persisteeruma, säilides selliselt algloomas infektsioonivõimelisena väga kaua.

Algloomade vahekorda imetajate viirustega on uuritud ka mitmesugustel teistel mudelitel, kusjuures on selgunud, et see vahekord oleneb väga suurel määral algllooma liigist ja viiruse tüübist. Seda illustreerib kujukalt artikli lõpul esitatud tabel, mis on koostatud kirjanduses leiduvate andmete põhjal imetajate viirustega eksperimentaalselt nakatatud algloomade uurimistulemuste kohta.

Юрий ТЕРАС, Лейда КЕСА

ВЗАИМООТНОШЕНИЯ МЕЖДУ ПРОСТЕЙШИМИ И ВИРУСАМИ

4. Простейшие как хозяева вирусов млекопитающих

В значительной мере благодаря заметным успехам исследований вирусов простейших, вирусоподобных частиц и цитопатогенного материала, обнаруженных в простейших, а также простейших как инактиваторов вирусов, рассмотренных нами уже ранее (Teras, Kesa, 1988a, b; 1989), в последние годы стали уделять внимание простейшим, как возможным резервуарам вирусов млекопитающих.

Исследование этой проблемы было начато около 50 лет назад. Поводом для этого послужили статьи шведского вирусолога К. Клинга и его сотрудников (Kling et al., 1942a, b), в которых авторы, анализируя причины постоянного обнаружения полиовируса в межэпидемические периоды в сточных водах закрытой канализационной системы Стокгольма, выдвинули гипотезу о возможной и вероятной репликации полиовируса в обитающих в канализационных трубах простейших.

К. Клингу и его сотрудникам самим не удалось доказать эту гипотезу, однако их работе, благодаря особой актуальности проблемы полиомиелита в то время, было уделено необходимое внимание. Одновременно с поисками в сточных водах простейших, содержащих полиовирус, были предприняты попытки выяснить также и экспериментально, может ли вообще и в каких простейших этот вирус реплицироваться и персистировать (Evans, Osterud, 1946; Brutsaert et al., 1946; Toomey et al., 1948; Young et al., 1949).

Гипотеза о пенетрации вирусов млекопитающих в простейшие была доказана лишь спустя 25 лет после опубликования статей К. Клинга и его сотрудников. Так, канадским ученым во главе с Е. Ковачем удалось установить, что не только комплетный вирус, но даже инфективные РНК и ДНК последнего способны проникать в простейшие и, вероятно, реплицироваться в них (Kovàcs, Kolompar, 1969). К числу таких вирусов относится как полиовирус и очень схожий с ним вирус энцефаломиокардита, так и онкогенный вирус полиомы.

Заметные успехи в исследовании взаимоотношений вирусов млекопитающих и простейших получены также в отделе протозоологии Института экспериментальной биологии Академии наук Эстонии на модельных объектах. На начальном этапе этих исследований изыскивались подходящие модели РНК- и ДНК-вирусов для опытов как со свободноживущими, так и паразитическими простейшими. Исследования были проведены с 26 видами простейших и 5 типами вирусов, среди которых 20 видов простейших были свободноживущими, остальные шесть — паразитическими.

Для исследования взаимоотношений простейшее—вирус были использованы культуры клеток и метод иммунофлуоресценции, с помощью которых из 130 экспериментально созданных *in vitro* систем пенетрация вирусов в простейшие была установлена в 25 (Терас и др., 1977).

Из дальнейших опытов, в которых в основном в качестве моделей использовали *T. pyriformis* и *Giardia lamblia*, экспериментально индуцированные вирусом Coxsackie B-5, выяснилось, что этот вирус способен не только пенетрировать в простейшие, но и реплицироваться и персистировать в них, сохраняясь, таким образом, в простейших инфекционноспособным очень долго.

Взаимоотношения между простейшими и вирусами млекопитающих исследовались также и на некоторых других моделях, причем выяснилось, что эти взаимоотношения в очень большой мере зависят от вида простейшего и типа вируса. Наглядно это иллюстрирует таблица, составленная на основе имеющихся в литературе данных о результатах исследования простейших, экспериментально ифицированных вирусами млекопитающих.