MICROBIOLOGY AND IMMUNITY

CULTIVATION OF TOXOPLASMA GUNDI IN TISSUE CULTURES

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The method of tissue culture has been applied only comparatively recently to the study of the pathogenic protozoa and of the diseases which they cause. However as we know this method is widely used at the present time for all manner of work on different viruses, pathogenic to man and animals.

It is only in recent times that the method of tissue culture has begun to find application in the study of toxoplasmosis: the suitability of tissue cultures for the diagnosis of toxoplasmosis [5]; the quest for effective drugs for the treatment of toxoplasmosis; investigation of the action of toxoplasms on the cell [4]; the length of survival of toxoplasms in tissue culture [6] and so on. Despite the fact that still comparatively few investigations have been carried out in this field it is to be expected that this method will in future be used for a variety of purposes: maintenance of strains of toxoplasms; the study of the cytology, biology and metabolism of toxoplasms and so on; production of antigen for the complement fixation reaction and a skin test used in the diagnosis of toxoplasmosis; the diagnosis of toxoplasmosis by infection of tissue cultures with material from sick persons and animals; the study of some of the problems of pathogenesis and immunity, etc. in toxoplasmosis; research into effective chemotherapeutic drugs for use in toxoplasmosis.

EXPERIMENTAL METHOD

For this research we used cultures of various tissues from chick embryos.* Pieces of skin, muscle and heart were taken from 7-11 day old chick embryos. The tissues were grown on cover glasses in a nutrient medium of the following composition: Hanks' solution — 50%, chick embryonic extract — 10%, chick serum — 40%.

To 10 ml of this medium was added 1 drop of penicillin (2 ml of physiological saline to 200,000 units). A small droplet of chick plasma was placed on the cover glass and smeared over the surface. Pieces of chick embryonic tissue were carefully ground up in physiological saline and a few of the tiny fragments of tissue were then affixed to the cover glass prepared in the manner described above.

The coverglass and adherent tissue fragments was placed into a penicillin flask and into each flask was poured 1-1.5 ml of the nutrient medium composed as described above. The flasks were carefully closed with rubber stoppers and placed in an incubator (at a temperature of 37°C) at such an angle that the nutrient medium covered the glass with the tissue fragments. After 3-4 days this nutrient medium was poured away and replaced by fresh medium of the same composition. After 1-2 days a large zone of growth of fibroblasts was formed around the fragment. Cultures produced in this way were inoculated with toxoplasms from the peritoneal exudate of mice.

* The work was performed under the direction of Dr. of Biological Sciences D. N. Zasukhin.

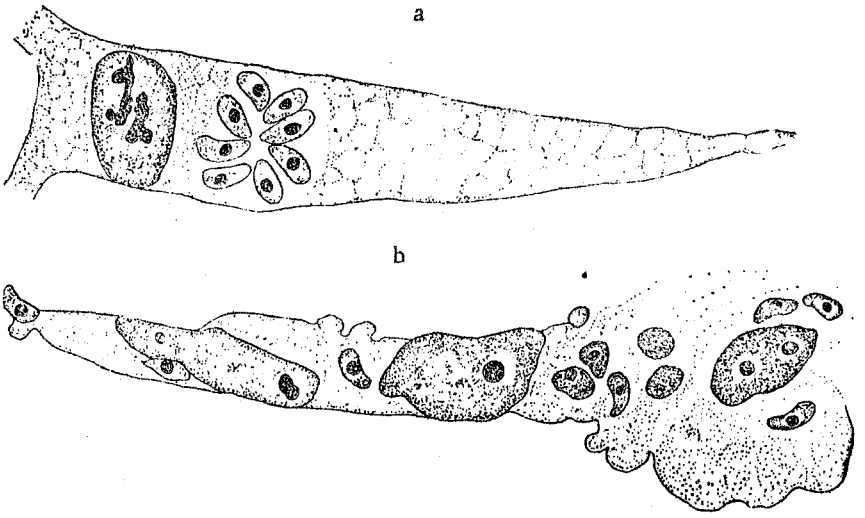

Fig. 1. A fibroblast in tissue culture 24 hours after infection. Toxoplasms arranged in rosette form in the cell; the protoplasm of the fibroblast is slightly vacuolated.
a) Destruction of a cell infected by a toxoplasm. Absorption of the protoplasm and karyorrhexis of the nucleus (b).

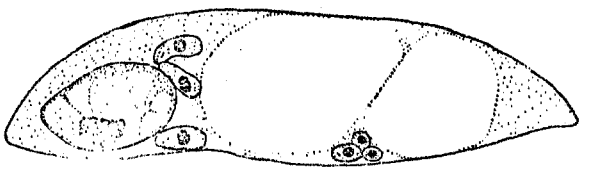

Fig. 2. A fibroblast infected with toxoplasm from a chick embryo. In the cell large vacuoles appear which gradually fill the whole cell and displace and distort the nucleus.

Before inoculation, the old nutrient medium was aspirated from the flasks. Next, 0.1 ml of exudate was added to the growing tissue and left for 1-1½ hours at room temperature. 1-1.5 ml of fresh nutrient medium was then added to the flask. In some flasks the medium was changed regularly every 3-4 days and in others it was not changed at all.

The material was fixed in alcohol and ether (50:50) 2, 3, 6, 23 and 29 hours and 1, 2, 3, 4, 5, 6 and 7 days after inoculation. The cultures were stained by the Romanowsky method (for 30 minutes at a temperature of 37°C). The film was taken through acetone mixtures and mounted in neutral Canada balsam. Control films from noninfected tissue cultures were made concurrently by the same method.

Besides experimental inoculation of healthy tissue we also carried out experiments in which tissue from chick embryos infected with toxoplasmosis was cultivated. For this purpose, 8-day chick embryos were opened up and fragments of muscle or heart explanted by the method described above. For cultivation a nutrient medium of the same composition was used. The material was fixed every 24 hours after inoculation.

EXPERIMENTAL RESULTS

Thirty-three experiments were performed, i. e. about 300 films were examined. Fixation was carried out, as has been pointed out, starting from 3 hours after inoculation of the tissue culture with toxoplasms.

After 2-3 hours we found toxoplasms of typical form in the cells. The infected cells were still very small, but individual cells sometimes were intensively infected. The parasites in the cells were solitary but in a few cases they reached 7 in number. The toxoplasms were arranged haphazardly in the protoplasm. No vacuoles were yet to be seen around them. No dividing parasites were observed at this period. In the infected cells we often saw mitoses of the nucleus and attenuation of the protoplasm of the cell leading, as we shall see later, to its absorption.

After 6 hours there were more toxoplasms in the growing fibroblasts. The average number of parasites per cell was 8. From time to time cells were encountered in which toxoplasms were to be found not only in the center of the cell but also in its numerous processes; in such cases they were 20-25 in number. The toxoplasms were arranged haphazardly in the cell protoplasm. Often accumulations of them deformed the nucleus; it was often markedly indented. The nucleus was often displaced towards the periphery of the cell.

On examination of a large number of films some areas presented a distinctive picture: toxoplasms (2-5 in number) lay directly against the nucleus. Whether this is due to penetration of the parasites into the nucleus or the result of superimposition of the parasites on the nucleus cannot yet be decided.

After 24 hours the toxoplasms occupied almost $^1/_3$ of the cells. In many of the parasites a vesiculiform nucleus could be clearly distinguished. They were mainly arranged haphazardly in the cell but in some cells they formed rosettes (Fig. 1) or were arranged in a single row. The rosettes were usually made up of an even number of toxoplasms – 4, 6, 8 or 10. Parasites arranged singly or in pairs were often surrounded by vacuoles. Toxoplasms in a state of division were seen. At this time some cells were already undergoing destruction: all the protoplasm appeared "frothy" and its separate areas were becoming attenuated – the protoplasm was apparently being absorbed (Fig. 2).

After 48 hours the maximum number of toxoplasms was observed both inside and outside the cells. Before the maximum cytopathogenic effect had been achieved, the explantate contained a large number of free parasites. Those arranged in the form of rosettes were particularly numerous. The parasites were usually surrounded by vacuoles and the rosettes were always so surrounded. The bulk of the toxoplasms had undergone division: they were arranged in pairs unless division was not yet complete. Multinucleated fibroblasts (2-7 nuclei) were seen. Intensive cell destruction was observed and the further growth of the cells was retarded.

After 72 hours the majority of cells were now destroyed. The protoplasm was being absorbed and eventually only the bare nuclei were left. Often the nucleus itself was broken up into several segments. Those cells which had not yet disintegrated were highly vacuolated. Often a single, large vacuole occupied the center of the cell, and both protoplasm and nucleus (or nuclei) were displaced to the periphery.

There were many free toxoplasms in the center of the fragment, but in the zone of growth – now the zone of destruction – hardly any were present. Parasites which had escaped from the disintegrating cells found the conditions in the medium outside the cells unfavorable for life.

After 4-7 days the cells were completely destroyed and the toxoplasms had almost completely disappeared.

The observations described above were made on cultures in which the nutrient medium was not changed before and including the 4th day. We made a parallel study of control films in which none of the above changes were observed.

An insufficient number of experiments to cultivate tissue from infected chick embryos (see above) were carried out. At this stage we can only state that the cultivation of toxoplasms in this tissue is possible.

After 48 hours the greatest number of parasites was concentrated in the center of the fragment. However toxoplasms also appear in newly growing fibroblasts. It is true that there are very few infected cells. Inside the cells the toxoplasms are arranged either in a single row or, more often, haphazardly (Fig. 2).

On the 3rd day the toxoplasms develop most violently; their arrangement inside the cell is the same as on the 2nd day. The rosettes of parasites, which we saw in the experimental infection of normal chick tissue, are not observed here. Toxoplasms are also seen outside the cells. However on the 5th day after setting up the culture the number of toxoplasms is already decreasing and the cells are highly vacuolated. Free toxoplasms are encountered.

The methods described may be used in the future in the study of toxoplasms and of various problems in connection with the diseases caused by these parasites.

SUMMARY

It was demonstrated that toxoplasms may be successfully grown in the tissue cultures, causing a characteristic cytopathogenic effect. The parasites penetrate into the cells and by multiplying gradually fill them

completely. The nuclei are subjected to pyknosis and kariorrhexis; the protoplasm becomes vacuolized and is gradually resolved; the cell degenerates. Toxoplasms escape from the cell and penetrate intact, uninfected cells. The parasites perish when the tissue is completely destroyed.

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* In Russian.