THE CULTIVATION OF MALIGNANT TUMOR CELLS
INDEFINITELY OUTSIDE THE BODY

ALBERT FISCHER

From the Institute of General Pathology of the University of Copenhagen, Denmark

Many investigators have studied the malignant tumor cells in vitro. Lambert and Hanes (1) have cultivated the rat sarcoma; Carrel and Burrows (2) cultivated the Rous chicken sarcoma, the rat sarcoma, human sarcomata, carcinomata from the dog, the Flexner-Jobling rat carcinoma and several human carcinomata. Losee and Ebeling (3) cultivated human sarcomata. Carrel and Burrows, Losee and Ebeling stated that sarcomatous tissue grew as well during a few days as normal fibroblasts, but afterwards the rate of growth became less rapid and the tissue could not be kept alive for more than about two months. The life of the cultures is short, which may be due to technical factors. Liquefaction of the plasma clot occurred generally and no further growth and increase of the mass of the tissue takes place. It is difficult to prevent the liquefaction of the coagulum. Recently Carrel (4) found that the addition of small amounts of serum and trace of sodium linoleate to a fibrinogen-tyrode medium partly prevented the liquefaction of this medium when normal fibroblasts were cultivated in it. This was tried also in our cultures of sarcomatous tissue cells, but showed not to prevent the extensive liquefaction of the medium. The best results of this kind were obtained by reducing the amount of embryonic tissue juice in the culture medium.

The method of cultivation of sarcoma cells in vitro which is reported in this paper, is built on the idea that if the malignant cells were able to metastasize in vivo, there is no reason why they should not in vitro.

TECHNIQUE

The Rous chicken sarcoma served as material for the experiments reported here. It was in all respects the same as described by Rous (5).
The tissue for cultivation was excised under ether anesthesia from the tumor-bearing animal, about 6 weeks after inoculation. Sometimes the tissue was taken from metastases in the lungs shortly after the spontaneous death of the animal.

The tissue was cut into small pieces and placed in the culture medium, usually consisting of two volumes of chicken plasma and a trace of fresh prepared embryonic tissue juice. The growth in this medium was the same as described earlier by Carrel and Burrows (2). The cells are actively ameboid and wander far out in the plasmatic medium. After a few hours cultivation, a very extensive liquefaction occurs and the migrated ameboid cells can now be seen as spherical bodies, floating around in the lake of liquefied plasma clot.

To such cultures of sarcomatous tissue small bits of muscle are now added. These are excised either freshly from normal adult chickens or from muscle tissue which has been stored in Ringer's solution for a long time in the refrigerator. The small muscle fragments, about the size of the explanted tissue itself, are placed almost in contact with the fragment of sarcoma in the culture medium.

The stock muscle tissue is taken from the muscles of the neck, when the chickens are bled to obtain the plasma. The muscle fragment is quickly placed in a small jar with Ringer's solution and stored in the refrigerator. When it is to be used, it is taken out and cut into small pieces. These are then embedded in the usual culture medium and incubated for about 2–4 days and tested out this way for infection and to see if any outgrowth takes place. No cells have been seen growing out from such a piece of pure muscle tissue. The tissue used is always selected of as pure muscle as possible, without tendines or connective tissue. By storing the muscle fragments for about 14 days in the refrigerator, all the cells die and hence are not able to grow in cultures.

After having tested the fragments of normal muscle, they are cut out of the plasma clot, washed in Ringer's solution for about half a minute and a single piece is placed together with the sarcomatous tissue side by side in the culture medium. When
the culture, containing the little fragment of normal muscle tissue and the sarcoma has to be renewed and transferred, the two fragments are picked up by the point of the needle or knife from the liquefied medium, washed in Ringer's solution and replaced in a fresh culture medium. After a few passages, the two fragments generally adhere to each other and can be transferred as one piece.

After about 4–6 passages the muscle tissue is entirely destroyed and a new piece must be added, but before the muscle destruction is complete, the mass of tissue can be divided and subcultures made.

RESULTS

When a small fragment of the Rous chicken sarcoma is cultivated in the usual way in chicken plasma and embryonic tissue,
juice, an extensive liquefaction takes place of the surrounding medium within a few hours and the fragment is found after 24 hours to be floating in the medium surrounded by numerous spherical cells.

After a few passages, the fragment gets smaller and smaller and finally dies. The liquefaction of the culture medium is preventing the actual increase of the mass and the tissue cannot be divided and the cultures multiplied, as can be done for the fibroblasts and epithelium.

In adding a piece of solid muscle tissue to the sarcomatous tissue in the culture, the sarcoma cells are observed to migrate abundantly to the muscle before any liquefaction of the medium has taken place. After the liquefaction has begun, many cells have already migrated over in the muscle where they multiply very extensively, independent of the liquefaction going on.

After a few passages, sometimes already after the first passage, the normal muscle fragment and the sarcomatous tissue adhere to each other, so that they can be transferred as one piece of
tissue. After 2–4 passages, the muscle tissue is so invaded by sarcoma cells that it can be separated from the original tissue and transferred to a separate culture, it is able to grow independently and behave as the original fragment of sarcomatous tissue, i.e., it liquefies the culture medium in the same peculiar way as did the original explanted tumor tissue by producing a very mucous secretion and ameboid cells migrate out from the muscle, representing the same types of cells as found in the original piece.

![Image](image.png)

**Fig. 3. A Section through a Culture of Rous Chicken Sarcoma**
A small remnant of muscle tissue can be seen in the middle of the culture surrounded by sarcomatous tissue cells.

By and by the muscle gets thick, opaque and debris of decayed muscle bundles can be seen floating around together with the spherical cells in the liquefied plasma. Before the muscle fragment is entirely destroyed, a new piece may be added or the old fragment divided up and subcultured with addition of fresh muscle.

This method of cultivation of malignant tumor cells is so simple; not much training in culture technique is necessary to carry on such cells indefinitely.
The histological examination of the cultures shows that the muscle tissue added to the sarcomatous tissue is completely invaded by the sarcoma cells in the way typical for this tumor in vivo.

Figs. 1, 2, 3, 4, 5 and 6 represent sections through such cultures of the Rous chicken sarcoma after a month's cultivation. Shortly before the fixation and sectioning, a new piece of muscle tissue was added and now it can already be seen invaded by sarcoma cells. In Figs. 1, 2, and 3, a part of the muscle formerly added can be seen perfectly infiltrated and over-filled with the typical polymorphous cells, characteristic for this tumor. The arrangement of the cells, bundles of fibroblastic cells crossing in several directions, gives a picture which can be diagnosed as sarcoma.

The cells seem to grow abundantly and multiply rapidly in the
muscle tissue and finally replace the muscle bundles. The sarcoma cells seem to be able to transform the protoplasm of the muscle tissue cells into protoplasm of their own. Only a trace of embryonic tissue juice is added to the culture medium in order to get a quick coagulation and a firm clot. The amount of embryonic tissue juice added to the cultures is probably not responsible for the multiplication of the sarcoma cells. Experiments are now under way to see if the sarcoma cells are able to

Fig. 5. A section through a culture of the Rous chicken sarcoma after its 20th passage

On the right side can be seen a broad invasion of the sarcomatous tissue. In between the muscle bundles can be seen the sarcoma cells. The picture is somewhat similar to an illustration by Rous in his first original paper, which shows the invasion of sarcomatous tissue between the muscles of the host.

live in cultures without any embryonic tissue juice, but only on muscle tissue.

It is not yet known if the fresh excised living muscle is better than old. So far it has been possible to cultivate the Rous chicken sarcoma in vitro for more than two months with the addition of muscle tissue, stored in the refrigerator, the mass
of the tissue being increased very much during that time. It can therefore be concluded that the sarcoma cells of the type of the Rous chicken sarcoma can be cultivated indefinitely that way.

Fig. 6. A Section through the same culture as opposite. It shows also the extensive invasion of sarcomatous tissue in the muscle fragment.

Experiments on reinoculation of the cultures of tumor cells into normal chickens are going on, but nothing definite can be said about the malignancy of the cultivated cells. There are indications, however, that the tumor cells are able to develop tumors in chickens after inoculation. The histological picture of the cultures indicates that the invasive and destructive power of the sarcoma cells is preserved.

SUMMARY AND CONCLUSIONS

A method has been developed by which it is possible to cultivate sarcoma cells of the type of Rous chicken sarcoma for a long time outside the body. Hitherto this has been impossible.
The technique is simpler than any other for keeping strains of tissue cells in vitro.

By placing a piece of muscle tissue from an adult normal chicken side by side with the fragment of sarcomatous tissue in the culture, the muscle tissue is invaded by the destroying and infiltrating sarcoma cells, which grow and multiply within the muscle fragment. After a few passages the muscle tissue is practically destroyed and a new piece must be added. It is possible to get several pieces of normal muscles contaminated by sarcoma cells at one time, after which they can be separated and cultivated in individual cultures and again contaminate other muscle fragments.

Numerous problems of great importance are rendered capable of investigation by means of this method.

If it is true that the sarcoma cells are able to transform the protoplasm of other cells into protoplasm of their own, it brings light into many questions about the nature of the cells.

There is no reason why it is not possible to cultivate other tumor cells in the same way as reported here. As long as the liquefaction does not play any rôle in the cultivation of the sarcoma cells or other malignant cells, it is very probable that human tumor cells can be cultivated in the same way.

REFERENCES


