We are very grateful to Dr. Levinthal for his valuable suggestions and help in this work. We are also indebted to the "W. H. Rose Foundation (Scotland) for the Prevention of Blindness," who have defrayed the expenses of this investigation; to Sir Henry Dale, who kindly gave us some of his supply of tyrothricin; to Dr. Trevan, of the Wellcome Research Foundation, who kindly supplied us with penicillin made by Dr. Pope and Miss Stevens; and to Mr. Edwards, of British Schering, Ltd., who kindly supplied us with sodium sulphacetamide (Albucid soluble).

REFERENCES.


A PURE STRAIN OF ROUS SARCOMA CELLS.

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Received for publication February 26, 1943.

The usual method of cultivation of Rous chicken sarcoma is that of Fischer (1925), who keeps the sarcoma cultures permanently alive and virulent by the regular addition of normal tissue to the tumour-cell colonies. Fischer assumes that the added normal tissue furnishes the necessary mechanical support to the outwandering sarcoma cells, which by liquefying the plasma clot deprive themselves of such support. According to him the added tissue is digested and assimilated by the neoplastic cells.

Besides mechanical and nutritive functions the added normal tissue has another significance. The causative agent, present in the sarcoma cell cultures, combines with the normal cells and provokes their malignant transformation (Ludford, 1937; Halberstaedter and Doljanski, 1939; Halberstaedter, Doljanski and Tenenbaum, 1941). Thus in these cultures apart from the multiplication of the sarcoma cells a permanent process of cancerization of the added normal tissue takes place.

It is evident that sarcoma cultures maintained according to Fischer's method cannot be considered as pure colonies of sarcoma cells; they contain malignant cells, normal cells and elements involved in a process of gradual cancerization. Most investigations dealing with sarcoma cells in vitro were carried out of necessity on such very heterogeneous cell material, ill adapted to either morphological or physiological study. It seemed therefore desirable to attempt the culture of Rous sarcoma cells in vitro without addition of normal tissue. As we show below, Rous sarcoma cells can be kept easily under these conditions as a pure culture over a long period of time (4–6 months).

MATERIAL AND TECHNIQUE.

Our sarcoma strains originated from sarcoma cultures cultivated through 520 passages according to Fischer's method. The cultures were carried out by the standard
Fig. 1.—Pure Rous sarcoma culture 18th passage, living. × 71.
Fig. 2.—" " " 20th passage, living. × 59.
Fig. 3.—" " " 31st passage. Giemsa stain. × 50.

Tenenbaum.
hanging drop method in equal parts of fowl plasma and diluted embryonic extract. Passages were made every second or third day.

RESULTS.

The behaviour of colonies of sarcoma cells when cultivated for several passages without addition of normal tissue is as follows: Soon after transferring, numerous spindle-shaped and round cells begin to migrate from the original fragment. They form at first a compact growth area composed chiefly of spindle cells and scattered round cells. This outgrowth pattern is usually not maintained for long. The strong proteolytic activity of sarcoma cells interferes with the maintenance of a continuous growth area. Very soon after transferring the fibrin clot undergoes liquefaction, the growth area is split up, and the cells are separated one from another. As a consequence of this development we find, as early as 2 to 24 hours after transferring, the original fragment surrounded by a large liquefied area, occupied by numerous single rounded cells. The area of liquefaction is always outlined by a ring composed chiefly of connected spindle cells. At some places in this ring increased proliferation of the cells may result in the formation of nodule-shaped swellings, which often attain a considerable size. During the process of cultivation the central piece becomes completely rounded and acquires a glassy appearance. As a rule it presents itself as a semi-transparent lens-like body. The liquefied plasma around the original fragment takes on a viscous consistency, and a vigorous multiplication of free rounded cells takes place in this slimy medium.

The colony of sarcoma cells cultivated without addition of normal tissue possesses a peculiar and characteristic appearance which is maintained over a prolonged time (Figs. 1 and 2). The multiplication of cells is vigorous, and the cell colonies could be divided at every second or third passage. Thus it is possible to obtain in a short time from a few initial Rous sarcoma cultures large numbers of cell colonies.

The characteristic appearance of sarcoma-cell cultures depends to a high degree on the presence of an adequate amount of embryonic extract in the culture medium. When cultivated without or with a small quantity of embryonic extract the cultures show an entirely different aspect. As liquefaction does not occur under these conditions, the spindle cells that proliferate from the central piece are able to form an extensive continuous growth area. This is to be seen after 24–48 hours’ development as a rich layer consisting of a network of connected spindle and star-like cells, the majority of which are flattened (Fig. 3). After transferring to a medium containing sufficient amount of embryonic extract, such a culture reverts to the typical pattern.

Cultivation of sarcoma cells without addition of normal tissue is possible over a long period of time. We have kept several cell strains in culture over a period of 4–6 months each (40–50 passages). It has not proved possible, however, to maintain such cell strains for longer than this time.

All our efforts to obtain a permanent pure strain of Rous cells were without success, and we are inclined to conclude that this objective is not realizable. The obstacles in the way of this undertaking were not of a technical nature but intrinsic. During the first 30 passages the sarcoma-cell colonies grow well and active multiplication of cells occurs, but in later passages changes set in which interfere with a normal development of the cultures. After the period of undisturbed growth the vigorous development ceases and the cultures become gradually poorer in cells. This process ultimately leads to the death of the cell colonies. We propose to describe elsewhere the peculiar pathological changes which constantly develop in sarcoma cells after a certain number of passages. These changes interfere with permanent culture and render it impossible.
The sarcoma cells in pure culture maintain their malignant properties during the entire period of cultivation. Inoculation of such colonies into chickens always produced typical tumours. Altogether we have inoculated a total of 16 cultures, ranging in age from 9–30 passages, and in all cases obtained a positive result. The tumours were accompanied by metastases.

In conclusion we may mention that Carrel (1912), in his earlier tissue-culture investigations, made attempts to cultivate sarcoma-cell cultures. He was able to maintain the sarcoma-cell strains for 46 days. At the end of this time the cultures died "without any apparent cause."

**SUMMARY.**

The cultivation of Rous sarcoma colonies as a pure cell strain is reported. The pure sarcoma-cell cultures can be maintained in vitro for a period up to six months. During a prolonged time the cells proliferate vigorously, but ultimately they undergo pronounced alterations, progressively degenerate and die. The aspect and behaviour of pure Rous sarcoma-cell strains is described.

**REFERENCES.**


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**THE MECHANISM OF RED BLOOD CELL DESTRUCTION.**


*From a Military Laboratory.*

Received for publication March 18, 1943.

It is generally believed that the reticulo-endothelial system is responsible for destruction of red blood cells in the normal body, but the exact factors controlling the rate and extent of this destruction are not accurately known. It occurred to one of us (B. G. M.) that lysis of erythrocytes might be a biological process associated with the destruction of the red cell membrane or substance by an enzyme, in a manner similar to the break-up of certain bacterial bodies in the presence of lysozyme, where one particular fraction is broken down by the specific enzyme (Epstein and Chain, 1940).

A series of experiments was therefore designed to investigate red cell destruction from this angle. The technique and results of these experiments are described in this communication.

Experimental results so far demonstrate the presence of a specific lytic agent in animal tissues. Although complete proof of its nature is not yet available, it is considered that this lytic agent is probably an enzyme.

The existence of substances capable of inhibiting the action of the lytic agent has also been demonstrated both in the tissues and in the blood serum. It is suggested that the balance between the lytic agent and the inhibitory factor controls the degree