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ISOLATION AND CHARACTERISTICS OF A CONTINUOUS CELL CULTURE FROM HUMAN SPLEEN

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A continuous line of human spleen cells was obtained. Important factors in the isolation of this strain were seeding a large number of cells and prolonged incubation of the culture; these two factors created conditions for gradual modification of cellular metabolism, without which the cells could not have adapted themselves to prolonged culture. A continuous culture could not be obtained from one colony and a "shaking" method had therefore to be used. Cultures were obtained only after the addition of 1% phytohemagglutinin to the nutrient medium.

KEY WORDS: *Spleen — continuous culture; "shaking" method; phytohemagglutinin.*

How to increase the resistance of the body is one of the most important problems in the general and infectious pathology of man and animals. Resistance of the body to pathological agents of various sorts is largely determined by the state of the reticuloendothelial system. The method of growing cells *in vitro* offers wide opportunities for the study of the mechanisms of antiviral immunity, cell resistance, and so on.

The object of this investigation was to obtain a continuous cell line from adult human spleen.

EXPERIMENTAL METHOD

The spleen was removed at operation from a patient with lymphogranulomatosis. The tissue was trypsinized by the usual method 1-2 h after the operation. The cell concentration was adjusted to 2,000,000/ml and the suspension transferred to 1-liter flasks. Altogether 14 such flasks were seeded. The primary culture medium (No. 1) consisted of 20% calf serum, 30% lactalbumin hydrolysate, and 50% Eagle's medium for diploid cells. The sera were inactivated. The medium for further growth of the culture (No. 2) consisted of 30% conditioned medium (medium No. 1 in which the cells had been grown for 3 days), 10% calf serum, 10% nor-

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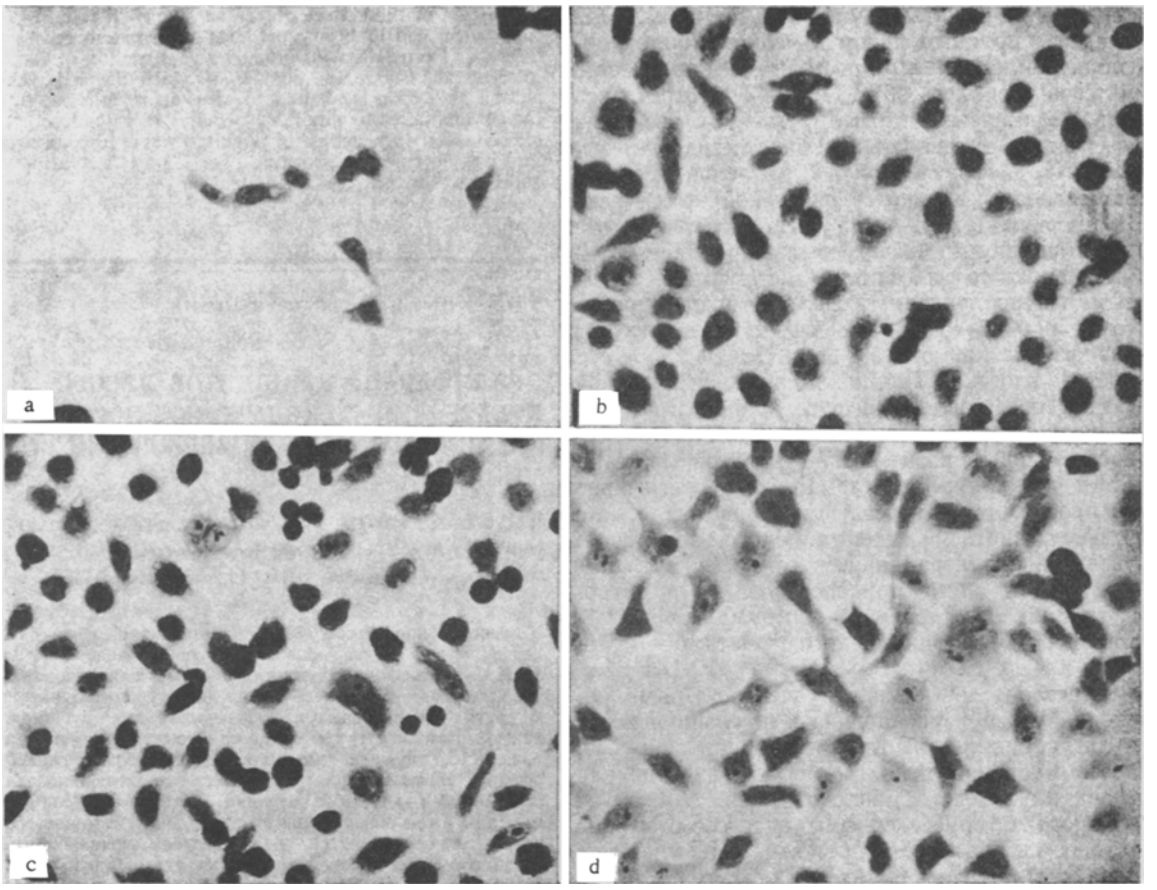

Fig. 1. General appearance of culture at different passages: a) 3rd, b) 9th, c) 19th, d) 49th passage. Fixation in Bouin's fluid, stained with hematoxylin and eosin, 350×.

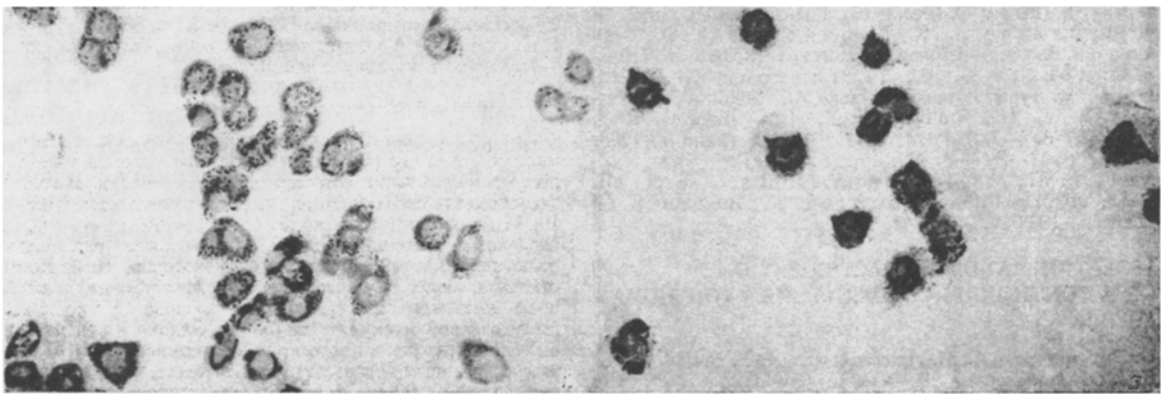

Fig. 2 Fig. 3

Fig. 2. NADH-diaphorase activity in cells. Burstone's method, 350×.

Fig. 3. Detection of neutral lipids in cell cytoplasm. Sudan Black B, 350×.

mal bovine serum, 20% lactalbumin hydrolysate, and 30% Eagle's medium for diploid cells. Phytohemagglutinin (PHA), which can stimulate division of lymphocytes and monocytes both free from the peripheral blood and from the spleen in vitro [4, 6, 7, 9], was used as the activating factor. PHA was added in a dose of 1% (v/v) of the standard solution.

The morphological and histochemical study of the culture was carried out by the usual method [1, 3, 8]. The karyological investigation was undertaken by the method of Moorhead et al. [8] with certain modifications determined by the nature of the test material.

TABLE 1. Sensitivity of Continuous Culture of Human Spleen Cells (C_2) to Coxsackie B_5 and Vesicular Stomatitis (VSV) Viruses

Viruses	Cells used for passage of viruses	Passage of viruses							
		1st	2nd	3rd	4th	5th	6th	7th	8th
		Passage of C_2 cells							
		42nd	43rd	44th	45th	46th	47th	48th	49th
VSV	L	10^5	10^5	10^5	10^6	10^6	10^6	10^7	10^7
	C_2	0	0	0	10	10^2	10^2	10^3	10^3
Coxsackie B5	J96	10^4	10^4	10^5	10^6	10^6	10^6	10^6	10^6
	C_2	0	0	10^2	10^2	10^2	10^3	10^3	10^3

EXPERIMENTAL RESULTS

After cultivation for 3 days nearly the whole surface of the glass was covered with cells in only eight of the 14 flasks. The nutrient medium was removed from these eight flasks centrifuged 3 times at 3000 rpm for 1 h and used during subsequent cultivation as the "conditioned" medium. Cells remaining on the glass were washed off with Hanks's solution and added to medium No. 2. The culture was incubated for 1 week until a monolayer had formed. During subculture PHA was added to five flasks and the rest were grown in medium No. 2 only. The culture was monitored under the microscope in the ordinary way starting from the third day of growth and until the second week. The cells were found to adhere in only three of the five flasks whose nutrient medium contained PHA. Growth of the adherent cells was severely retarded. The culture was incubated without subculture for about 2 months, in the course of which half of the total volume of medium was removed from the flasks and replaced by fresh medium with 1% PHA every 6-8 days. Fibroblast-like cells were mainly observed in the monolayer. Toward the end of the second month of cultivation much of the monolayer had degenerated, leaving behind cells joined together by processes forming a type of network with numerous round cells in its loops. Further observations showed that in two of the three falsks with PHA the round cells were actively dividing to form colonies of irregularly shaped cells. These colonies had a loose structure and individual cells broke away from them during a change of medium. It was impossible to obtain a continuous culture from one such colony. By the use of the shaking method and combining all the colonies a continuous subculture could be obtained. Several dozen colonies of different sizes could be seen 7-10 days later in the flask. The procedure of combining the colonies was repeated four times. The fibroblast-like cells were gradually replaced by polygonal cells and the colonies united to form a monolayer. The cells thus obtained had high growth potential; for that reason, they were removed from the glass with versene solution containing trypsin in the ratio of 3:1 every 3-4 days. Starting from the 28th-32nd passage the cells acquired a definite polygonal shape, good adhesive properties, and a stable reproduction cycle and they were designated as C_2. The C_2 cells are at present at the 156th passage, having been subcultured every 5-6 days without a change of medium, and the cell harvest at that time has been three to four times greater than the seeded dose (Fig. 1). Morphological investigations have shown that these are polygonal cells with a large nucleus and a finely granular cytoplasm. On the first day of growth the mean number of mitoses was $26^{\circ}/_{oo}$, but on the second to fourth day only solitary mitoses were found. Moderate succinate dehydrogenase, NADH-diaphorase (Fig. 2), and acid phosphatase activity and weak alkaline phosphatase activity was discovered in the cells. The cytoplasm of the cells was rich in neutral lipids detected as large drops throughout the cytoplasm (Fig. 3). The culture is heteroploid and its modal number of chromosomes is 58-59. In their morphology the chromosomes are analogous to those of continuous human cell lines. This culture is interesting to us as a substrate for the reproduction of viruses. As Table 1 shows, reproduction of Coxsackie B5 and vesicular stomatitis viruses, studied in the course of eight passages, was accompanied not only by the development of a cytopathic effect, but also by an increase in the titer of the viruses from one passage to the next. Cultures of J96 cells were used as the control for passage and titration of Coxsackie B5 virus and cultures of L cells in the case of vesicular stomatitis.

The preliminary results of electron microscopy showed the presence of particles of oncornavirus type in the cell culture obtained as described above; it can accordingly be used for study as a tissue of tumor origin taken from a patient with a known diagnosis. The culture of C_2 cells can also be used as lymphoid tissue in experiments to study cellular resistance.

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PROLIFERATION OF MYOCYTES OF THE LEFT ATRIUM AND VENTRICLE AFTER VARIOUS TYPES OF MYOCARDIAL INJURY IN ADULT RATS

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An infarct of the myocardium of the left ventricle was produced in adult rats weighing 120-160 g by ligating the left coronary artery at different levels: in the atrial region, at the level of the first third of the left ventricle, and in its middle. In other series of experiments the left atrium was damaged by applying a ligature to its anterior wall or to the auricle. Animals undergoing a mock operation during which the pericardium was removed acted as the control. The left half of the heart was investigated on the 5th day after the operation. Mitotically dividing myocytes were found in the atrium or auricle of the animals in those series of experiments in which these parts of the heart had been directly injured and had a thickened epicardium (in 35 of 49 cases); the mitotic index varied from 0.9 to 10%. After litigation of the coronary artery in the middle of the ventricle mitoses were not found in the myocytes of the atrium and auricle. In all series of experiments mitoses were rare in the myocytes of the ventricle (in seven of 49 cases) and were located at a distance from the infarct, in subepicardial zone; the mitotic index there varied from 1 to 2%.

KEY WORDS: *Myocardial infarct; division of cardiomyocytes; mitotic index.*

The problem of the degree of proliferation of the muscle cells of the myocardium during its repair after injury in adult mammals has not yet been solved. Experimental data both confirming [3, 4, 10, 13] and refuting [9, 11, 12] the possibility of repeated divisions of the cardiomyocytes after various types of myocardial injuries have been published. In some reports well-marked proliferative activity of the atrial myocytes has been observed by comparison with the myocytes of the ventricular musculature [5-7]. However, these observations have recently been questioned [2]. It has accordingly been postulated that differences in the response of myocytes in different parts of the myocardium to trauma may depend on the size and location of the defect arising in the organ.

The object of this investigation was to study the level of proliferation of myocytes of the left half of the heart (left ventricle, atrium, and auricle) after infliction of injuries differing in intensity and location.

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