MONOLAYER CELL CULTURES FROM ANIMAL THYMUS AND SPLEEN TISSUES

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Monolayer cultures were obtained from thymus and spleen cells from embryos and young animals (guinea pigs and calves). The cell composition and dynamics of the changes in the primary cultures and subcultures were studied. Monolayers of a primary thymus culture consist of lymphoblast-like and fibroblast-like cells, whose presumptive ancestors are thymocytes and reticulum cells, respectively. The monolayer culture of the spleen consisted mainly of fibroblast-like cells with a few giant multinuclear cells. The cultures obtained preserve their morphological, cultural, and biological properties for 8-10 days without a change of medium. It was shown that the thymus and spleen cells can be subcultured for a long time (10-16 passages). Cultures of lymphoid tissue cells are sensitive to RNA- and DNA-containing viruses. A monolayer culture of thymus and spleen tissue cells can be used on a wide scale in many different branches of biology, medicine, and veterinary medicine.

The cultivation of lymphoid tissue cells is of considerable interest. There are reports of the cultivation of spleen, lymph gland, thymus, and bone marrow tissues in organ cultures, using Millipore filters [1-9]. However, no investigations on the preparation of monolayer cell cultures from lymphoid tissue suitable for routine scientific and practical purposes are known to the authors.

The object of the investigation described below was to prepare a monolayer culture of cells from the thymus and spleen of laboratory and farm animals.

EXPERIMENTAL

The thymus of guinea pigs and Syrian hamsters (aged 1-2 months), calves (4-6 months), and of calf and pig embryos (aged 2-5 months) was used to obtain cell cultures.

The thymus and spleen were removed from the animals under aseptic conditions, washed three times in Hanks' solution with antibiotics, the capsule was removed, and the organ cut up with scissors into small fragments and disintegrated by means of 0.25% trypsin solution on a magnetic mixer. The minced thymus and spleen tissues were trypsinized at 37°C until all the tissue was used up. The suspension of trypsinized cells was centrifuged for 10 min at 800 rpm. The cell residue was resuspended in medium No. 199 with 10% bovine serum. The cell suspension was seeded into tubes and small and large flasks and incubated at 37°C. The suspension of thymus cells consisted chiefly of reticulum cells and lymphocytes (thymocytes). On treatment with 0.5% aqueous solution of trypan blue the reticulum cells stained a dark color. They were 2-3 times larger than the lymphocytes, which were unstained.

When the cell concentration in the original suspension was $1-1.5 \cdot 10^6$/ml monolayer cultures were formed in 4-6 days without a change of medium. Altogether 24 experiments were carried out with thymus

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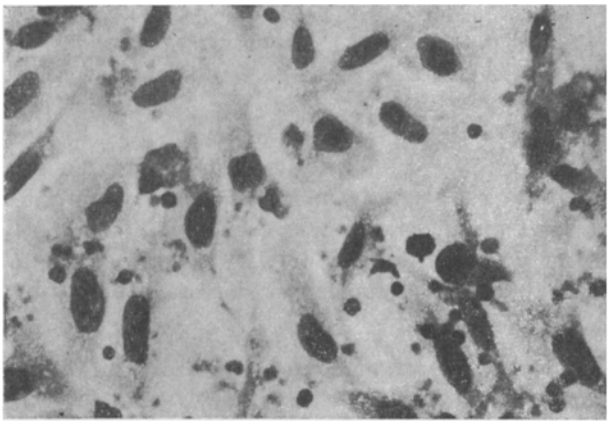

Fig. 1

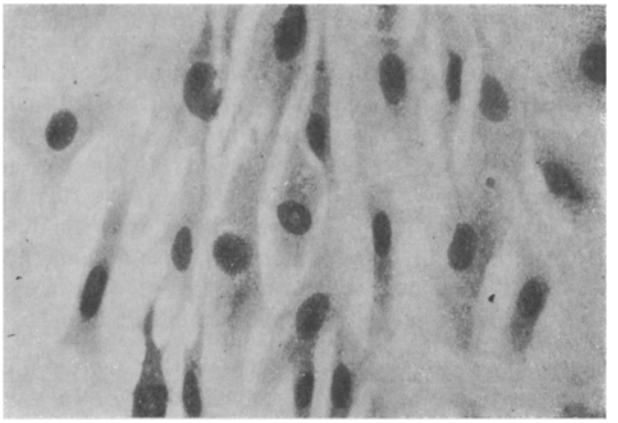

Fig. 2

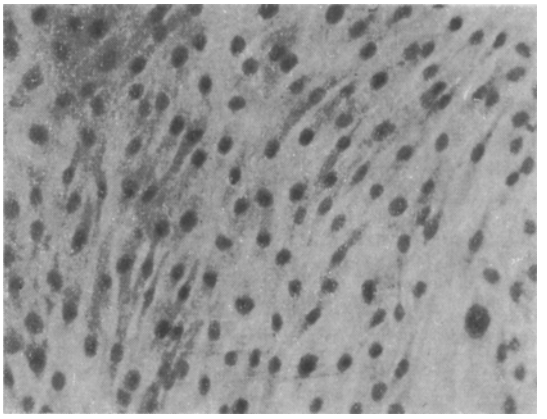

Fig. 3

Fig. 1. Primary culture of guinea-pig thymus cells after cultivation for 96 h. In all figures staining by Romanowsky–Giemsa method, 400 ×.

Fig. 2. Culture of calf thymus cells at the 11th passage. Here and in Fig. 3, 100 ×.

Fig. 3. Culture of calf embryonic spleen cells at the 10th passage.

tissue. The morphology of the cell cultures was studied over a period of time in nine experiments. For this purpose the original suspension was added (in doses of 2 ml) to penicillin flasks containing coverslips. The coverslips with the cells were fixed in methyl alcohol and stained by the Romanowsky–Giemsa method 0, 3, 6, 9, 12, 18, and 24 h after the beginning of cultivation, and thereafter daily for 4-5 days of cultivation.

Similar procedures were carried out at the same times to investigate the cultures of spleen cells (ten experiments).

EXPERIMENTAL RESULTS

Characteristics of Guinea-Pig Thymus Cultures at Various Times. The original cell suspension consisted of darkly stained, round cells. Large cells with a loosely packed nucleus were seen. The cell composition of the suspension consisted mainly of thymocytes and reticulum cells. From 3-9 h after the beginning of cultivation, many cells were adherent to the glass. These were mainly large and small thymocytes, but in addition a certain number of comparatively large cells with a large and palely stained oval or kidney-shaped nucleus (reticulum cells) could also be seen. At this period collections of cells were beginning to form. A moderate number of mitoses could be seen in the culture.

Besides the proliferating cells, some cells died. A few slightly elongated cells with a long nucleus, resembling fibroblasts, and also cells of the macrophage type, with small thymocytes around them, could be seen.

By 12-18 h the cell composition of the culture showed a marked change: the number of small round cells was sharply reduced, and the predominant cells were large, with palely stained cytoplasm and with a large oval or irregular (segmented) nucleus. The number of fibroblast-like cells was sharply increased. After 24-72 h the morphology of the culture was changed even more sharply: large fibroblast-like cells with a large nucleus were predominant. There were many mitoses. A very few small round cells were still present. Occasionally cells with a kidney-shaped nucleus (of the monocyte type) and multinuclear cells were seen. After 96-120 h (Fig. 1) the fibroblast-like cells formed a continuous monolayer. These cells had a long, oval nucleus with two or three nucleoli, and their cytoplasm was vacuolated. Large round cells with basophilic cytoplasm and with a dense nucleus, resembling lymphoblast-like cells, were uniformly distributed throughout the monolayer. The morphological picture was unchanged during 8-10 days of cultivation without a change of nutrient medium.

Characteristics of Spleen Cell Culture from Calf Embryo at Different Periods. The initial cell suspension consisted of darkly stained round cells of different sizes. After cultivation for 6 h many cells with a kidney-shaped nucleus, many mature lymphocytes and reticulum cells, and also solitary fibroblast-like cells could be seen. From 16-24 h after the beginning of cultivation the number of fibroblast-like cells was appreciably increased. These cells were joined together by their processes to form a syncytium. Together with these cells there were small, medium-sized, and large lymphocytes and macrophages. In some areas the cells had disintegrated. In the 48-h culture a loose layer of fibroblast-like cells was formed, on which lymphoblast-like cells lay singly and in groups. By 72-96 h a continuous monolayer consisting mainly of fibroblast-like cells had formed in the cultures. By this time giant multinuclear cells (16-20 nuclei) had appeared.

Subculture of the Primary Cultures. The primary trypsinized thymus and spleen cell cultures were subjected to prolonged subculture in medium No. 199 and Eagle's medium with 10% bovine serum. The subcultures were carried out in large flasks. To obtain cells for seeding the cultures a 0.02% solution of versene with 5% trypsin in 0.25% solution was used. The seeding ratio of the cultures was 1:2-1:4. The primary culture of guinea-pig thymus cells went through 11 passages, the culture of calf cells through 16 passages, calf embryo cells – 9 passages, calf embryonic spleen cells – 12 passages, and rabbit spleen cells – 5 passages. In each passage a monolayer was formed within 4-5 days. After 2 or 3 passages of the primary thymus culture the large round cells with basophilic cytoplasm and dense nucleus disappeared completely. The culture consisted almost entirely of fibroblast-like cells, elongated or polygonal in shape, forming a loose monolayer (Fig. 2). Solitary mitoses, and here and there giant cells with a large nucleus, were found, and a few cells contained oxyphilic cytoplasmic inclusions.

The spleen subculture also consisted entirely of fibroblast-like cells, although they were all elongated in shape and arranged in regular bands in close contact with each other (Fig. 3). No other morphological differences were found between the subcultures of thymus and spleen, except that the cells in the thymus subculture were much larger.

As a result of these investigations a method of cultivating cells as monolayers from lymphoid tissue was developed. The thymus from embryos and young animals and the spleen from embryos were used for these purposes. In the first few hours of cultivation, fibroblast-like cells appeared and these gradually became the principal cellular element of the culture. The monolayer of the thymus culture consisted of two types of cells: fibroblast-like and lymphoblast-like, their ancestral cells presumably being reticulum cells and thymocytes, respectively. The culture obtained was very stable and preserved its morphological, cultural, and biological properties for 8-10 days without a change of medium. The possibility of prolonged subcultivation of primary cell cultures obtained from lymphoid tissues was demonstrated. A great advantage of the cultures is their high sensitivity to various RNA- and DNA-containing viruses. The well-marked cytopathic action of the viruses of foot-and-mouth disease, vesicular stomatitis, Aujeszky's disease, bovine rhinotracheitis, rabies, vaccinia, and equine rhinopneumonia in primary cultures and subcultures of the lymphoid tissue was established.

On the basis of these experimental results the monolayer cultivation of lymphoid tissue cells from the thymus and spleen of animals can be recommended for use in virological research.

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