GROWTH OF ORGAN CULTURES OF GUINEA PIG LYMPH GLANDS IN A MEDIUM CONTAINING HOMOLOGOUS SERUM

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Until recently no method of prolonged cultivation of lymphoid tissue in vitro was possible in which the cells did not undergo dedifferentiation, but the tissue structures were preserved or developed de novo. Yet in order to study the immunologic functions of lymphoid tissue it is extremely important to have an in vitro model in which structurally organized lymphoid tissue would be preserved.

This paper describes a new method of multiple organ cultivation of whole lymph glands and describes the main results of its application.

EXPERIMENTAL

The inguinal and auxillary lymph gland of adult guinea pigs were cultivated in medium No. 99, containing 10-15% warm homologous serum, 4 mg glucose, and 100 units each of penicillin and streptomycin per ml of medium. The lymph glands were placed on Millipore filters, held by plastic supports with holes at the boundary between two phases—the liquid phase of nutrient medium and the gaseous phase consisting of 95% oxygen and 5% carbon dioxide, saturated with water vapor. The plastic support with 14 round holes, 5-7 mm in diameter, was placed in a Conway dish (Fig. 1) into which the nutrient medium was poured up to the level of the filters placed above the holes. Type HA (thickness 150 μ, diameter of pores 0.45 μ) and AUFS (thickness 100 μ, diameter of pores 0.6-0.9 μ) Millipore filters were used. Physiological saline was poured into the inner cylinder of the dish to maintain the humidity. After explantation of the lymph glands, the gaseous mixture was blown into the dish and the lid was fixed securely to the rim of the Conway dish which had first been smeared with a mixture of mineral oil and melted wax. The nutrient medium and the gaseous phase were changed every 72 h.

The cultures were fixed with alcohol-formol after cultivation for 2, 5, 6, 7, 8, 9, 11, and 30 days. The pieces of tissue were taken from the filters, embedded in paraffin wax, and cut into serial sections which were stained with methyl green-pyronine and hematoxylin-eosin. Total preparations were made from the filters and stained with alum-hematoxylin. Altogether 50 cultures were studied.

EXPERIMENTAL RESULTS

After 2 days in vitro degeneration of the lymphocytes was observed in the medulla and cortex of the lymph glands, with preservation of small groups of lymphocytes in the peripheral zone beneath the capsule in the loops of the network of reticular cells, and in the dilated peripheral sinuses (Fig. 2A). Most of the lymphocytes were small, and sometimes lymphoblasts and solitary plasma cells were seen. The central part of the explant had degenerated.

In total preparations of cultures at this time three zones of cell growth could be distinguished: 1) beneath the explant were individual fibroblasts; 2) around the fragment was a zone of actively proliferating stroma, containing small and larger groups of lymphocytes lying between and above the stroma cells; 3) the border of the growth zone was formed by a thin layer of fibroblasts-like cells. Lymphocytes were seen on the surface of many of the fibroblasts in this area. Such fibroblasts, packed round with lymphocytes, are named "feeding-trough" cells (Fig. 2B). The number of lymphocytes in contact with one reticular cell sometimes exceeded 10. They were mainly small lymphocytes but occasionally pycnotic forms and, in isolated cases, lymphoblasts were observed.

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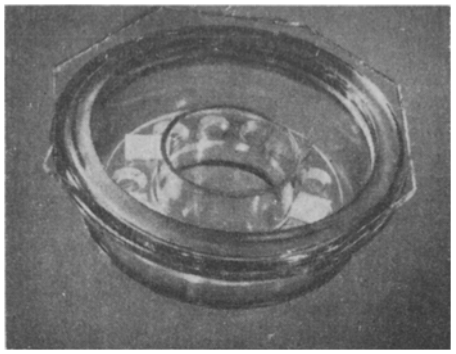

Fig. 1. Cultivation vessel for the multiple organ culture method.

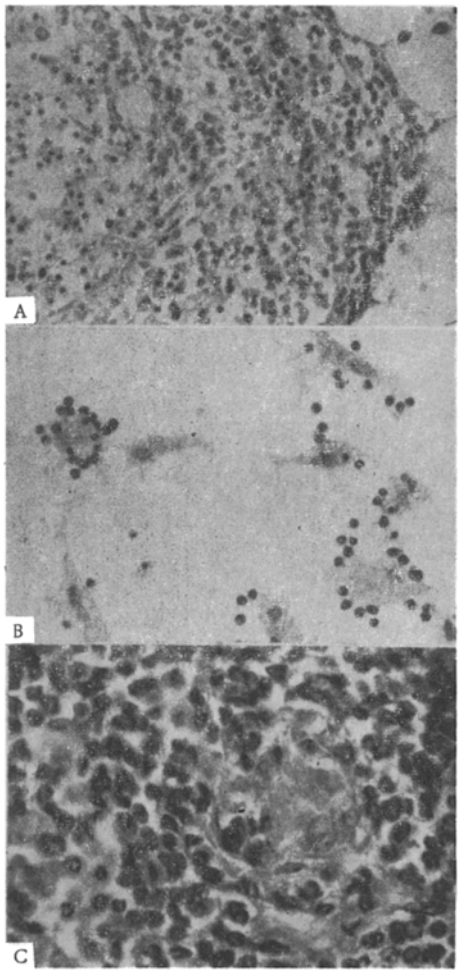

Fig. 2. Culture of a lymph gland. A) Section, time 2 days; lymphocytes are preserved in the peripheral zone beneath the capsule; B) total preparation, time 2 days; reticular "feeding trough" cells with lymphocytes on them; C) section, time 9 days; a secondary follicle with a spherical structure in its center.

In sections of the 5- and 6-day cultures proliferation of the lymphoid tissue was observed at the periphery of the explant. In the zone of proliferation of the lymphocytes spherical structures were seen, consisting of elements of the stroma which by this time had attained a considerable size and included several tens of cells. Small, large, and medium-sized lymphocytes were compactly arranged around them. On the 7th-9th day of cultivation complete regeneration of the cortical layer of the lymph gland had taken place, with the formation of normal lymphoid follicles with proliferating cells (Fig. 2C). In the total preparations of the lymph glands at these times of cultivation signs of maturation of the stroma were seen: vacuolation of the cells and accumulation of brown pigment granules in them. After longer cultivation of the lymph glands some of the explants on the HA filters died. For instance, in one total preparation of the five fixed on the 11th day, a compact layer of connective-tissue cells was preserved beneath the explant. In the sections of the cultures at this time the same picture was found as on the preceding days—the formation of secondary follicles at the periphery of the explant.

By using the AUFS filters, with larger pores than the HA filters, lymph gland cultures of longer life could be obtained. In these conditions regeneration took place not only of the cortex, but also of part of the medulla of the lymph glands. After 30 days in vitro the lymphoid tissue still remained viable.

The results obtained show that organ cultivation of whole lymph glands creates conditions enabling not only proliferation of the lymphocytes, but also the formation of the complex structures of lymphoid tissue—the secondary follicles. The use of homologous serum instead of heterologous had a beneficial effect on proliferation of the lymphocytes and stroma [1, 2]. The pore size of the filters also had a marked effect on the survival time of the explant: cultivation of lymph gland on filters with large pores (0.6-0.9 μ) gave cultures with a longer life. Unlike the methods of organ cultivation of lymphoid tissue suggested recently [3, 4], in which tiny fragments of lymphoid organs were explanted, in this case whole lymph glands were cultivated. Preservation of the capsule of the glands probably aided the survival and subsequent proliferation of the lymphocytes, and also the formation of secondary follicles. Besides the complex lymphoid structures, elementary structures consisting of a "feeding trough" cell surrounded by lymphocytes were also observed in the cultures. Examination of the living cultures showed the appearance of long processes on the lymphocytes, penetrating into the reticular cell. The transfer of substances and particles takes place along these processes [5]. Probably the formation of these reticular cell—lymphocyte structures may be important also in the development of immunity [6].

In the organ cultures of whole lymph glands a primary immunologic response could be obtained to horse γ-globulin antigen. Synthesis of specific antibodies, as demonstrated by the increase of radioactivity on an immunosorbent, took place on only a small scale. The induction of the immune response in such cultures was probably associated with the presence of lymphoid tissue in their complex structures, and the low level

of antibody synthesis was due to the small number of plasma cells. Regeneration of the medulla is essential for the differentiation of plasma cells, and as mentioned above, in the cultures this was slight. For that reason a method of cultivation must be developed which provides the conditions enabling differentiation of plasma cells.

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