focusing. Fig. 2 is a schematic diagram of the power supply used to operate the flash tube. Synchronization was achieved with the E. Leitz photomicrographic equipment.

Using Kodachrome 1 or 2 daylight film, normally exposed photographs of microcirculatory events were obtained. The shutter was set at 1/125 second, during which the electronic flash was fired, emitting a daylight quality, high intensity light for about 1/1000 second. The high shutter speed and slow film rating prevented exposure of the film from the viewing light, so that the exposure was the result of the electronic flash alone. It is estimated that the light from the flash tube is 500 times more efficient than the incandescent source previously employed. An example of the hepatic microcirculation *in vivo* is shown in Fig. 3.

Summary. An electronic light source for *in vivo* transillumination and photography of microcirculatory events within solid organs is described. This high intensity light source was balanced for daylight color film and was placed directly below the organ in the direct optical path of the microscope. To minimize detrimental effects of normal organ function, the temperature of the light carrier and flash tube at its tip did not exceed normal body temperature. This optical system was combined with a constant light source for viewing and focusing.

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Cultivation of Mouse Thymus in Organ Culture.* (29157)

DONALD PINKEL
Laboratory of Virology, St. Jude Hospital, and Department of Pediatrics, University of Tennessee, Memphis

Recent discoveries regarding the role of the thymus gland in immunologic phenomena and leukemogenesis suggest the need for a method of *in vitro* cultivation of this organ that allows preservation of its architecture and cytology.

Trowell(1) placed thymus tissue of adult mice and rats on lens paper suspended by a tantalum wire grid over synthetic medium and observed, in some instances, survival of tissue for as long as 6 days. Ball and Auerbach(2) used a plexiglas-millipore filter assembly to cultivate epithelial thymus rudiments of 12-day old mouse embryos. They demonstrated development of lymphoidal thymuses from these rudiments over periods of 14 to 20 days.

In this publication is described the morphology of intact thymus of 2-week old mice cultivated for prolonged periods by a modification of an organ culture technique described previously(3).

Materials and methods. Fourteen-day-old Balb/C mice were anesthetized by intraperitoneal administration of sodium pentobarbital, their thymus glands were removed aseptically with care not to break their capsules, and the paired glands were separated with minimal trauma in a dish containing growth medium.

Five tetrafluorethylene (Teflon®) rings, described previously(3), were placed in each sterile Petri dish and 5.5 ml of growth medium was added to each dish. The medium consisted of 90% Eagle's minimum essential medium(4) as modified by Goldstein(5) and 10% horse serum with antibiotics at concentrations of 100 units penicillin, 50 μg streptomycin, and 2 μg amphotericin per ml. Each

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Petri dish was tipped to alternate sides as sterile 1.8 cm diameter circular pieces of lens paper were placed on the Teflon rings in such a way that no air was trapped within the rings. A thymus gland was placed intact, dorsal surface down, on each lens paper, the tops replaced on the Petri dishes, and the cultures incubated at 37.5°C in a water-saturated atmosphere containing 4% CO_2 in air. Medium was changed weekly by removing the fluid outside of the rings and replacing it with equal amounts of fresh medium. Fig. 1 portrays the culture system.

At intervals cultured glands were removed, fixed in 10% buffered formalin, sectioned, and stained with H & E.

Results. Thymus glands cultured by this technique maintained their architecture. The interlobular septa of the thymus glands remained intact and cortical-medullary rela-

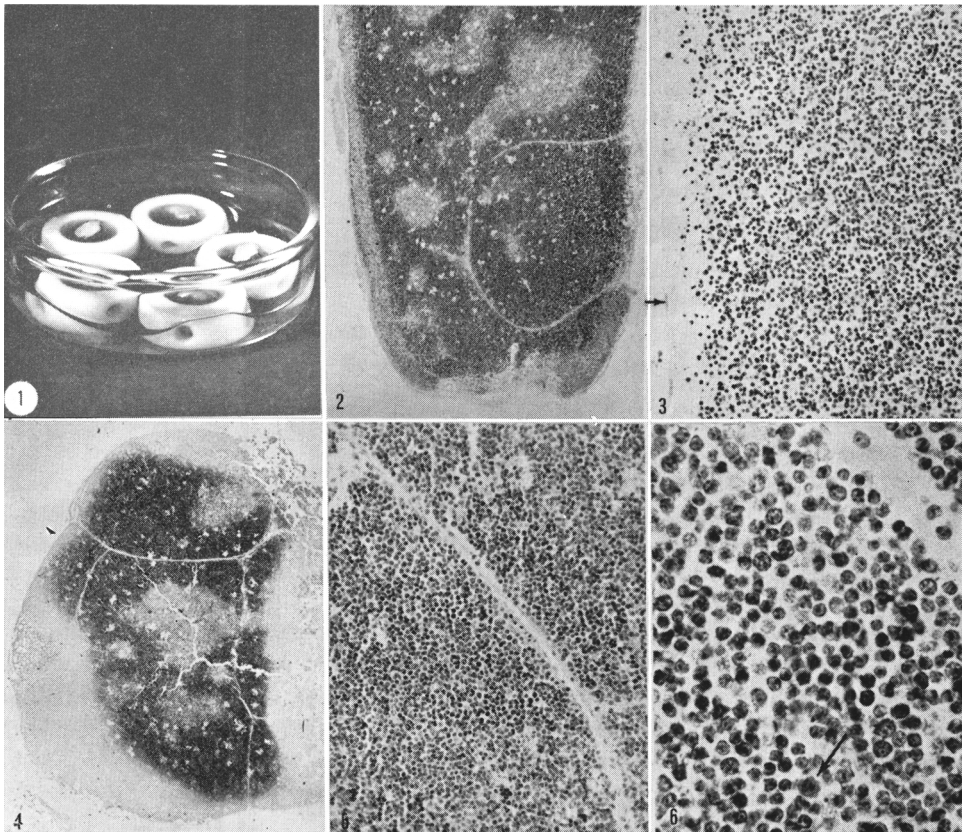

FIG. 1. Organ cultures of mouse thymus glands, cover of dish removed.

FIG. 2. Thymus, after 29 days cultivation. Note intact septa and cortical medullary relations, subcapsular zone of necrosis, and small foci of necrosis within cortical tissue. H & E stain. 15×.

FIG. 3. Thymus cortex, after 29 days cultivation. Note fibrous capsule (arrow), subcapsular zone of necrosis, predominance of medium sized lymphocytes with scattered reticular cells. H & E stain. 75×.

FIG. 4. Thymus gland, after 83 days cultivation. Note loss of capsule, considerable peripheral necrosis, focal cortical necrosis, and some breaking up of fragment, yet general preservation of architectural features such as interlobular septa and cortical medullary relations. H & E stain. 15×.

FIG. 5. Thymus cortex, after 83 days cultivation. Note intact interlobular septum, sheets of lymphocytes, predominantly medium sized. H & E stain. 125×.

FIG. 6. Thymus cortex, after 83 days cultivation. Note the many medium and large lymphocytes with prominent nucleoli, occasional reticular cell, intact fibrocytes of interlobular septum. There is one cell in mitosis (arrow); the mitotic figure was apparent when depth of focus was alternated. H & E stain. 490×.

tions were preserved during the periods of cultivation, which ranged from 4 days to 83 days (Fig. 2, 4). Of 77 glands cultured, 67 appeared to have viable lymphoid tissue in appreciable amounts when taken for section, while the remaining 10 were almost totally necrotic.

The capsule of the gland degenerated during the first week of cultivation but by the end of the second week it underwent a fibrous proliferation, and by day 20 was restored to its initial appearance (Fig. 3). In succeeding weeks it became progressively thinned and by 12 weeks was lost (Fig. 4).

During the second week focal necrosis of subcapsular lymphoid tissue developed, which became uniform and increased with time (Fig. 2, 3, 4). Increased numbers of reticulum cells, macrophages and a few giant cells appeared in this area during the second week and persisted until about the eighth week.

The medulla of the gland began to degenerate during the first week of culture. The reduction in cell population caused the reticular framework to appear more prominent. Lymphocytes disappeared first but after 2 weeks the reticular-epithelial cells began to die, so that after 3 weeks of culture the medulla was for the most part necrotic. The epithelial cell collections corresponding to Hassall's corpuscles appeared intact for the first 5 days of culture but thereafter they degenerated and disappeared by day 14.

Thymic blood vessels showed endothelial thickening by the 5th day of culture and by the second week this thickening produced obliteration of most vessels.

Cortical lymphoid tissue persisted in an apparently healthy state throughout the periods of cultivation (Fig. 2-6). The ratio of large and medium lymphocytes to small lymphocytes appeared to increase with increasing age of the cultures (Fig. 5). Prominent nucleoli became common and mitotic figures were seen (Fig. 6). The ratio of viable appearing lymphoid tissue to the medullary area and the subcapsular zone of necrosis decreased as cultures aged. After 4 weeks small areas of focal necrosis appeared within the lymphoid tissue and persisted (Fig. 2, 4).

Discussion. The lymphoid tissue of thymus glands behaved differently on organ culture than that of spleen fragments. Except for its margins, the tissue persisted for many weeks while its blood vessels disappeared early. On the other hand, spleen fragment cultures, as described previously(3), exhibited necrosis and reticulum cell hyperplasia early and then underwent spontaneous lymphoid follicle regeneration around pairs of blood vessels, which remained patent. This difference may be related to two factors: first, the thymus glands were cultured intact rather than in fragments, and second, thymus lymphoid tissue does not grow in follicles around blood vessels as do the lymphatic elements of spleen and lymph nodes.

The important feature of the thymus gland cultures described here is the persistence of relatively large amounts of apparently healthy lymphoid tissue *in situ* for many weeks, which suggests that this technique may be useful for the study of immunological phenomena and lymphatic leukemogenesis.

Summary. The cultivation of thymus glands of 2-week-old mice by an organ culture technique is described. The architecture of the gland was preserved, and, although gradual degeneration of most elements developed, large amounts of healthy lymphoid tissue persisted for periods of up to 12 weeks.

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