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Successful Cultivation of Spleen Fragments in Organ Culture.* (28005)

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Since the description of an organ culture system by Strangeways and Fell(1) a number of investigators have described methods for short term cultivation of hematopoietic and other tissues. With many of these procedures the specimen is placed at the gas-liquid interphase in suitable culture medium so that it has maximum contact with the gas phase yet has liquid exchange with the medium.

Parker(2) maintained rabbit spleen fragments for 4 days in a fluid medium with 80% oxygen in the gas phase. Trowell(3) kept rat lymph nodes for 4 days on cotton wool soaked with serum-saline medium in a gas phase of pure oxygen and also cultivated tissues on lens paper suspended by a tantalum wire grid over a synthetic medium(4). His attempts to cultivate thymus, spleen and bone marrow were unsuccessful(4). Chen(5) described the differentiation of rat embryo spleen in 8 days on lens paper floating on medium composed of fowl serum and chick embryo extract.

This report concerns successful culture of spleen fragments for prolonged periods by a modification of the organ culture technic.

Materials and methods. Fourteen- to 28-day-old Swiss mice were anesthetized by intraperitoneal administration of sodium pentobarbital and their abdomens opened aseptically. After exsanguination from the abdominal aorta their spleens were removed, placed in a sterile Petri dish containing several drops of Dulbecco's phosphate buffered saline(6), and cut into approximately 2 mm diameter fragments.

Sterile tetrafluorethylene (Teflon®) rings, 1.6 cm in diameter and 0.6 in thickness with four 2 mm side holes, were placed in sterile 10 ml beakers and 1 ml of medium was added. The medium consisted of 80% 199 solution and 20% calf serum (Difco) with antibiotics at concentrations of 100 units penicillin and 50 μg streptomycin per milliliter. The pH of the medium was adjusted to 7.2 with $NaHCO_3$. Each beaker was tilted and a 1.8 cm diameter circular piece of ultra-violet sterilized lens paper (Fisher No. 11-996) was then quickly soaked in the medium, placed over the top of the ring and the beaker leveled. The tilting during placing of the lens paper was required to prevent air bubble formation. A spleen fragment was placed on the lens paper, cut surface down, and each beaker was sealed with a No. 5 "cafe au lait" rubber stopper and incubated at 37°C. When the

FIG. 1. Organ culture of spleen fragment.

FIG. 2. Spleen fragment, after 6 days cultivation. Note reticulum cell hyperplasia, medium and small lymphocytes with vesicular and pyknotic nuclei. H & E stain. 375 ×.

FIG. 3. Spleen fragment, after 30 days cultivation. Note lymphoid follicles. H & E stain. 60 ×.

FIG. 4. Spleen fragment, after 30 days cultivation. Central area of lymphoid follicle, demonstrating paired vessels, lymphocytes at various stages of maturity, and scattered plasma cells. H & E stain. 180 ×.

FIG. 5. Spleen fragment, after 30 days cultivation. Margin of lymphoid follicle, demonstrating megakaryocyte, reticulum cells, lymphocytes, plasma cells. H & E stain. 900 ×.

FIG. 6. Imprint of spleen fragment, after 16 days cultivation, demonstrating plasma cells. Wright-Giemsa stain. 900 ×.

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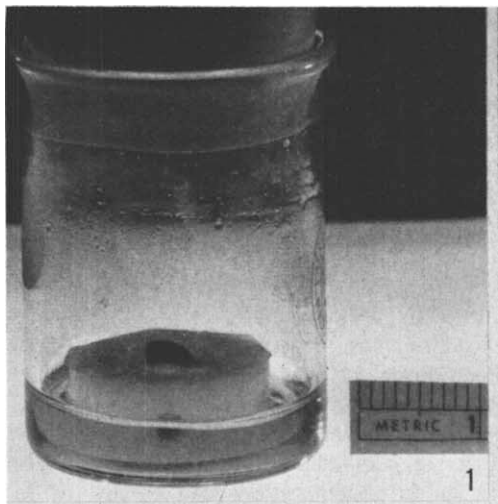

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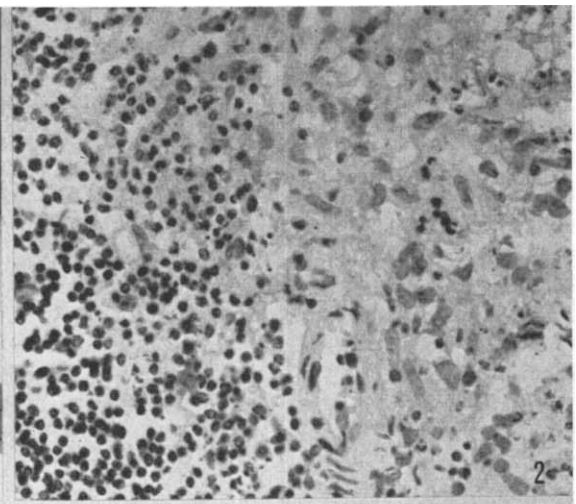

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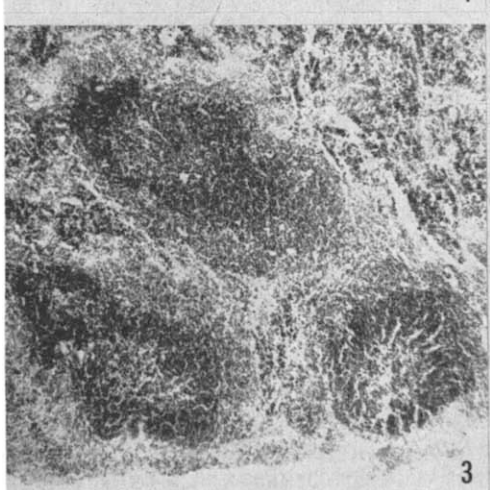

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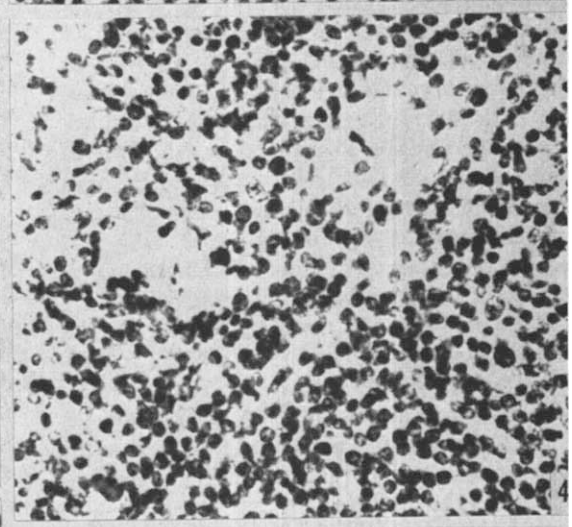

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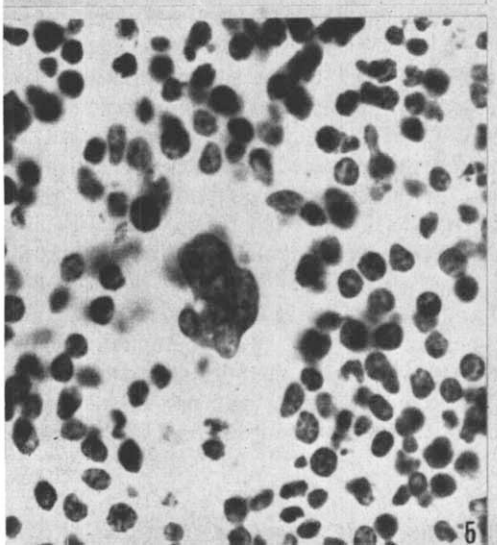

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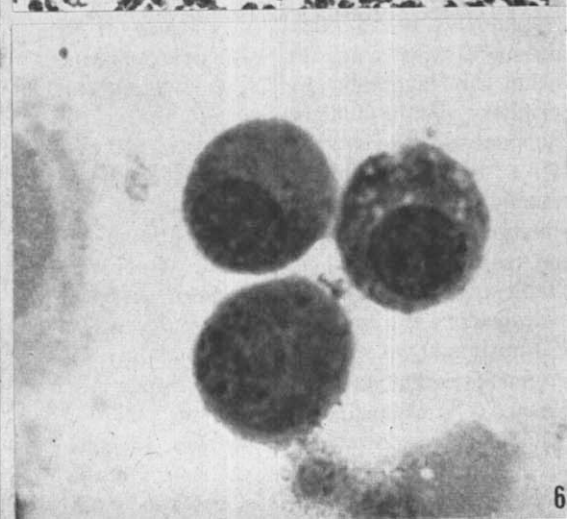

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pH of the medium dropped below 6.8, usually within 3 to 4 days, partial medium changes were made by aspirating liquid outside the Teflon® ring with a Pasteur pipet attached to a vacuum bottle followed by addition of 0.4 ml of fresh medium into the same space. Fig. 1 portrays the culture.

At intervals of 3 days, cultured fragments were removed, fixed in 10% buffered formalin, sectioned and stained with H & E. Some fragments were cut and imprints made on glass slides, air dried and stained by the Wright-Giemsa method.

Results. In the first 10 days of cultivation the fragments contract from about ⅔ to ½ of their original size. Microscopically, follicular architecture is lost and lymphocytes show degenerative changes of pyknosis and loss of staining affinity. Lysed red cells are seen at the periphery and hemoglobin pigments are found in macrophages, which become numerous in the peripheral area. At 6 days reticulum cells are abundant and arranged in large bands and clusters along the outer portion of the fragment as well as within it (Fig. 2). Septal tissue appears more prominent, possibly due to contraction of the fragment.

By 15 days the fragments tend to be rounded and small lymphoid follicles are apparent among the reticulum cells, necrotic lymphocytes and septal tissue. The follicles form about small pairs of arteries and veins that appear patent but free of cells. Subsequently, the follicles enlarge and contain lymphocytes in all stages of maturation so that by 30 days lymphatic tissue predominates within the fragments (Fig. 3, 4). About the periphery of the follicles and of the entire fragment degenerating lymphocytes may be seen as well as healthy appearing plasma cells, reticulum cells, megakaryocytes (Fig. 5) and macrophages. In addition a thin, fibrous tissue capsule partly covers the fragment. Viable-appearing follicles have been observed in fragments cultivated as long as 62 days.

Imprints of cut surfaces of 20- to 30-day-old fragments reveal reticulum cells, lymphocytes, plasma cells (Fig. 6) and fibrocytes.

This technic has been used, also, to cultivate other tissue fragments. A transplantable Wilms's tumor of the W/Fu rat was maintained for 10 days in a healthy appearing state with intact morphology and mitotic activity. These fragments, after 10 to 24 days in culture, gave rise to tumors within 2 to 3 weeks when reimplanted in rats of the W/Fu strain. Attempts to cultivate kidney, liver and brain fragments by the technic described above have been unsuccessful.

Discussion. Although loss of follicular architecture and necrosis of lymphatic elements occurred in the early stages of spleen culture, by 2 weeks evidence of follicular regeneration was present and by 4 weeks the spleen fragment was repopulated by apparently normal lymphoid, reticular and megakaryocytic elements.

The reestablishment of lymphoid follicles after initial degeneration may be due to development of vascular channels in the fragment, through which nutrient fluid flows up into the fragment by capillary action, as well as to the absorptive property of the fragment itself. The fact that the follicles form about pairs of arteries and veins suggests this.

The results described above indicate that this technic may be useful for quantitative studies in virology, immunology and biochemistry. The presence of large numbers of lymphocytes and plasma cells suggests the suitability of this organ culture system for investigation of antibody formation, protein synthesis and leukemogenesis.

Summary. A new modification of the organ culture technic has been described utilizing glass beakers, Teflon® rings and lens paper. It is relatively simple and easily adapted to large-scale use. Mouse spleen fragments cultivated by this method demonstrated early loss of follicular pattern, degeneration of lymphocytes and proliferation of reticulum cells. After 2 weeks of cultivation new lymphoid follicles formed and by 4 weeks follicular pattern was well established, with appearance of lymphocytes in all stages of development. In addition, plasma cells, megakaryocytes and reticulum cells were present and at the periphery, which was partially surrounded by a fibrous capsule, there were present numerous macrophages containing hemoglobin pigment.

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Quantitative Studies on Enzyme Repression in the Developing Chick Embryo and Newly Hatched Chick.* (28006)

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An earlier report from this laboratory described the first known instance of negative feedback control of the level of a biosynthetic enzyme during embryonic development(1). The controlling compound is creatine, or a related metabolite, and the target enzyme is arginine-glycine transamidinase. This control has been operationally termed end-product repression, by analogy with bacterial systems, until its mechanism can be more completely elucidated(2). Since analogous feedback controls may be involved in the establishment and maintenance of tissue-specific enzyme patterns, closer study of the properties of the only known model system appears warranted. In this paper, quantitative and kinetic relationships between the controlling compound, and its precursors, and the target enzyme are examined.

Materials and methods. In general, the procedures employed were as described elsewhere(1). However, by modifying the physical state of creatine, more effective repression and more reproducible results can be obtained. Commercial crystalline creatine hydrate was dissolved in a minimal volume of water, with stirring, and an equal volume of acetone was added as quickly as possible. The suspension was allowed to settle, and the supernatant solution decanted. The residue was filtered with suction on a fritted glass funnel, washed thoroughly with acetone, spread on filter paper, and allowed to dry. The resulting fine powder suspends readily in water and is easily injected into eggs. Its large surface to volume ratio facilitates solution in the egg after injection. Transamidinase activities are reported as μmoles of hydroxyguanidine formed from arginine and hydroxylamine/hr/g liver (wet wt.).

Results. The compounds most effective in lowering the transamidinase level of embryonic chick liver are creatine, and its biosynthetic precursors. If creatine precursors must be converted to creatine before serving as a repressor, it might be expected that precursors of creatine metabolically proximal to the controlled reaction could not repress transamidinase completely, since the enzyme would be needed to form the repressor. Citrulline, 290 μmoles/egg injected on 7th day, represses transamidinase to 50% of control values by the 12th day. When glycine, 530 μmoles/egg, was injected with citrulline, transamidinase was repressed to 20% of control values. Almost identical results were obtained when arginine was substituted for citrulline. Apparently the chick embryo can convert citrulline to arginine, which then must react with glycine before the actual repressor is formed. In contrast to citrulline, ornithine does not repress transamidinase significantly. This is consistent with the fact that chicks lack ornithine transcarbamylase(3).

Fig. 1 shows dose-response curves for both creatine and guanidinoacetate. Efforts to modify the quantitative response to guanidinoacetate by simultaneously injecting potential competing methyl group acceptors have given inconclusive results, although repression can be antagonized significantly by compounds with similar charge distributions, such as betaine and 4-aminobutyrate. Efforts to achieve endogenous repression by encouraging hens to secrete repressors into the egg were unsuccessful: feeding laying hens for 20 days

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