Tissue Culture Studies of Auricular Appendages Obtained at Mitral Commissurotomy.* (24053)

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In some patients clinical and laboratory signs of rheumatic activity persist for many months despite the administration of penicillin to eliminate any hidden foci of streptococci and prophylactic medication to prevent reinfection. Such cases suggest the possibility that some agent other than group A streptococci may be a factor in prolonging activity of the rheumatic process. In recent years the finding of Aschoff bodies in a high percentage of the biopsies obtained at mitral commissurotomy from patients in whom there have been no signs of rheumatic activity for many years and in whom there is no evidence of a recent streptococcal infection, is pathologic evidence that the rheumatic process may remain active for decades(1,2). The persistence of this pathognomonic granuloma suggests the possibility of a viral agent.

In the past attempts to isolate a virus from patients with rheumatic fever have been unsuccessful. The discovery by Rowe and his coworkers(3) that a latent virus, (adenovirus) could be isolated from adenoid tissue obtained from routine adenoidectomies, suggested that other viruses might be demonstrable by similar technics. Tissue cultures of auricular appendages, therefore, were undertaken.

Material and methods. Specimens of auricular appendages were collected in the operating rooms of a number of cooperating hospitals.† The tissue was placed in Hanks balanced salt solution and taken to the laboratory where tissue cultures were prepared. At the same time a small piece of tissue was sent to the pathologic laboratory for preparation of histologic sections. Tissue cultures were also prepared from the foreskin of an 8-year-old boy and from cardiac tissue of 2 human fetuses. Specimens of auricular appendages, foreskin and fetal cardiac tissue were cut into fragments about one square mm in size and chicken embryo extract added. Four to 6 fragments were placed in each of 12 or more tubes which had been coated with chicken plasma. One ml of Eagle's basal medium(4) containing 100 units each of penicillin and streptomycin per ml, 25 units of mycostatin per ml and 10% calf serum by volume, was added to each tube. The tubes were incubated at 37°C without rotation. The medium was changed every 3rd or 4th day. Other specimens of auricular appendages were minced, ground in a mortar with alundum in a small amount of Hanks balanced salt solution. After centrifugation the supernate was added to actively growing fibroblasts derived from foreskin or fetal cardiac tissue.

Results. Specimens of auricular appendages were obtained from 17 patients varying in age from 26 to 57 years. Typical Aschoff bodies were found in 3 of these 17 specimens, (23%). The pathologic report of one specimen was: "rheumatic endocarditis, ? active, with focal myocarditis." No Aschoff bodies were found in the remaining 13 specimens and only hypertrophy of the cardiac muscle was noted in the pathologic reports.‡

Tissue Cultures of auricular appendages. Tissue cultures were prepared from 8 specimens. Cells resembling fibroblasts grew out after 6 to 8 days. These cultures were maintained for periods of 2 to 4 months, and were examined at frequent intervals. No cytopathogenic changes suggesting the presence of a viral agent were observed. None of the 8 specimens used in these experiments showed Aschoff bodies.

Extracts of emulsified auricular appendages.

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Supernates of emulsions of auricular appendages obtained from 2 patients were added to tissue cultures of actively growing fibroblasts derived from the foreskin of a normal 8-year-old boy. One of these auricular appendages showed Aschoff bodies, the other was negative. No inhibition of growth or cytopathogenic changes were observed in the cultures of the normal fibroblasts following addition of either of these supernates to the culture medium.

Supernates of emulsions of 2 auricular appendages, both of which showed Aschoff bodies, were added to actively grown fibroblasts derived from 2 human fetuses. Addition of this material to the culture medium did not impede growth or produce cytopathogenic changes.

Summary. Attempts to demonstrate a viral agent in auricular appendages obtained at commissurotomy from rheumatic patients with mitral stenosis were unsuccessful.

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Effect of Synthetic Lysine-Vasopressin on Adenohypophysial Activity in Toads. (24054)

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In various mammals administration of vasopressin releases adrenocorticotrophin from the pars distalis (anterior lobe) of the hypophysis(1-4). According to Guillemin and coworkers(5,6), the natural ACTH-releasing hypothalamic factor is a peptide that is perhaps chemically related to, but not identical with, the pars nervosa hormone vasopressin itself. Substances related to the mammalian pars nervosa hormones have been found in the neurohypophysis of all vertebrates(7-11).* Further, the function of the amphibian pars distalis is under hypothalamic control(12,13). This investigation was carried out to determine whether mammalian pars nervosa hormones are able to stimulate pars distalis activity in amphibians too.

Methods. The experiments were made on toads (*Bufo bufo* (*L*)) in which hypothalamic control of the pars distalis was prevented by interruption of the connections between hypothalamus and hypophysis. In such animals, as well as in hypophysectomized toads, moulting is inhibited, but not the keratinization of the epidermis. Eventually, therefore, the skin is thickly cornified. However, stimulation of the inactivated pars distalis causes shedding of all old cornification. Shedding of the hyperkeratinized skin was therefore used as a criterion of stimulation of the pars distalis. *Technic.* In 12 toads connections between hypothalamus and hypophysis were interrupted by extirpation of the median eminence, and the pars distalis was widely separated from the hypothalamic wound (Group A). The operation immediately interrupts the circulation in the pars distalis, but within a week or less blood flows again at a normal rate in the capillaries of the gland. The function of the gland, however, remains severely impaired, and moulting is inhibited when the

* In amphibians and amniote vertebrates the neurohypophysis is differentiated into two separate structures, the pars nervosa (neural lobe) and the median eminence, whereas the neurohypophysis is undifferentiated in cyclostomes and most fishes(21,22).