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).

TABLE I. Effect of Synthetic Lysine-Vasopressin and Insipidin AB on Moulting in Toads with Extirpated Median Eminence (Group A) or Median Eminence + Pars Distalis (Group B).

Group		No. of toads in which cornified layer of skin was Shed	Not shed
	U		
A	.1 lysine-vasopressin	12	0*
B	.1 lysine vasopressin	0	12
	1. " "	0	6†
	.1 Insipidin AB	11	1‡

* In one of the toads hyperkeratinization of the epidermis did not occur, probably due to establishment of vascular connections between hypothalamus and pars distalis.

† Only half of the group was used in this exp.

‡ The toad was dying with symptoms of hypophysial deficiency(12).

blood supply is of extra-hypothalamic origin (12). Twelve toads with extirpated pars distalis and median eminence served as controls (Group B). All toads were females weighing 40-100 g. Synthetic lysine-vasopressin (7 U per ml) and the commercial vasopressin preparation Insipidin AB (20 U per ml) were injected subcutaneously.†

Results. 0.1 U lysine-vasopressin caused shedding of cornified layers in all toads of Group A, but in none of Group B (Table I). In Group B even 1 U lysine-vasopressin was without effect. Thus lysine-vasopressin induced moulting by stimulating the inactivated pars distalis. In Group B 0.1 U of commercial vasopressin Insipidin AB caused shedding of cornified layers in all toads but one. The commercial preparation, therefore, apparently contained enough pars distalis activity as an impurity to induce moulting directly in hypophysectomized toads.

Discussion. The present results provide further evidence for a relationship between pars nervosa hormones and pars distalis-controlling hypothalamic factors. In amphibians, birds, and mammals, hypothalamic control is mediated *via* the median eminence(12, 15-17). In dogs, ACTH-releasing activity has been demonstrated in blood drawn from the portal vessels that drain the blood from the median eminence into the pars distalis capillaries(18). However, the pars distalis controlling factors are not necessarily produced in the median eminence. In toads, for example, the factors most likely originate in hypothalamic centers, as do the pars nervosa hormones(20). Common chemical and functional properties of the hypothalamo—pars nervosa and hypothalamo—median eminence systems might have been anticipated because, phylogenetically, the pars nervosa and the median eminence are both derived from an undifferentiated neurohypophysis(21, 22).

The common phylogenetic origin of the two neurohypophysial structures of higher vertebrates raises the question about the sequence of origin of the various neurohypophysial functions. Two facts indicate that the specific functions of the pars nervosa are secondary and that the vertebrate neurohypophysis may primarily have been established as mediator of neural control of adenohypophysial function(24-26). These facts are 1) that all blood from the capillaries of the undifferentiated neurohypophysis of lower vertebrates is drained through the pars distalis, whereas the pars nervosa of higher vertebrates has a separate circulation; and 2) that numerous attempts to demonstrate typical pars nervosa (*e.g.* water balance) effects of neurohypophysial extracts in fish have given negative results(10,23). This theory is not incompatible with the demonstration of typical pars nervosa activities in the fish and even the cyclostome hypophysis when extracts of the gland have been tested on higher vertebrates (7,9,11). That extracts of the lamprey hypophysis, for example, show pressor, antidiuretic, and oxytocic effects in tests on rats may be due to chemical relationship between pars nervosa hormones of the rat and neurohypophysial pars distalis stimulating hormones of the lamprey. Thus functional evolution of the vertebrate neurohypophysis may have been accompanied by only small changes in chemical constitution of neurohypophysial hormones.

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Summary. In toads (*Bufo bufo (L.)*) with deficient pars distalis function due to extirpation of median eminence, subcutaneous injection of synthetic lysine-vasopressin causes shedding of the hyperkeratinized stratum corneum. In hypophysectomized toads lysine-vasopressin is without effect. Lysine-vasopressin is thus able to stimulate pars distalis activity in toads, indicating a chemical relationship between pars nervosa and median eminence (adenohypophysiotropic) hormones.

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Histopathology of Lymphocytic Choriomeningitis in Mice Spared by Amethopterin. (24055)

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Amethopterin, a folic acid antagonist, spares the lives of mice infected with normally fatal lymphocytic choriomeningitis (LCM), but does not prevent virus replication in spared mice. Folic acid-deficient diet produces a similar effect(1,2). These alterations resemble those obtained earlier with whole body X-irradiation; in mice spared by this treatment, decreased tissue reactivity or diminished inflammatory cellular exudate were suggested as the explanation for survival (3). The present report indicates that mice spared by amethopterin tend to develop inflammatory reaction more slowly than untreated mice, most of them showing great modification of the natural disease process, but that a significant number survive even though extensive inflammatory reaction is present.

Materials and methods. The LCM virus used was a murine strain maintained through more than 400 serial intracerebral (i.c.) passages(4). Preparations were mouse brain emulsions (ground by hand in a mortar and uncentrifuged) made to 10% suspensions and decimally diluted in 0.85% buffered saline. The i.c. dose was 0.03 ml for all animals. Mice were N.I.H. Swiss "general purpose," of both sexes, weighing 16-18 g when inoculated. They were fed a stock diet consisting of whole