onstrated that this respiratory stimulating effect of the cardiac glycosides in heart slices occurred only if lactic acid or glucose was present in the incubation media. Furthermore, this effect of cardiac glycosides could not be elicited if the heart tissue was homogenized. Thus, the presence of a functional cell membrane was necessary for the actions of cardiac glycosides.

Our experiments indicate that the increase in rate of metabolism of glucose to carbon dioxide (as observed in our earlier experiments) by the myocardium, subsequent to administration of the cardiac glycoside, is a function of an increase in transport of glucose (galactose) across the membrane of the myocardial cell.

Summary. Galactose transport into the myocardium of the anesthetized intact dog was measured as function of time following administration of either digoxin (0.065 mg/kg) or insulin (1 unit/kg). Administration of these drugs resulted in an increase in galactose entry and accumulation in the myocardium, thus indicating an increase in rate of glucose transport into this tissue. The data support the conclusion that digoxin increases myocardial glucose metabolism by facilitating entry of glucose into the myocardial cell.

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Effect of Neurohypophyseal Principles on Adenohypophyseal Activity in Toads. (25635)

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It was shown that lysine-vasopressin injected into the common toad *Bufo bufo* (L.) was able to release hormone(s) from the pars distalis of the hypophysis(1), supporting the theory that vasopressin is chemically related to natural pars distalis stimulating factors(2). The present paper reports further investigation of the effect of neurohypophyseal principles on pars distalis function in toads. We were especially interested in determining minimal effective dose of vasopressin and in seeing whether the oxytocic principle was also able to stimulate the pars distalis.

Methods. In the toad, extirpation or inactivation of the pars distalis inhibits shedding of the slough, but not keratinization of the epidermis. Eventually, therefore, the skin becomes thickly cornified. The pars distalis stimulating activity of the preparations used could be evaluated from their effect on the skin. A preparation was judged capable of stimulating the pars distalis if it caused total or partial shedding of the slough within 24 hours after injection into toads with inactivated pars distalis, but not after injection into toads with extirpated pars distalis. Inacti-

TABLE I. Effect of Neurohypophyseal Principles on Shedding of Slough in Toads with Extirpated or Transplanted Pars Distalis of the Hypophysis.

	Dose (I.U.)	No. of cases of: Sloughing	No sloughing	Pars distalis
Lysine-vasopressin, highly purified	.001	0	6	Transplanted
	.01	7	4	
	.1	14	1	
" synthetic	.1	8	0	"
" highly purified	.01	0	6	Extirpated
	2.0	0	3	
Insipidin AB (pressor preparation)	.001	3	2	"
	.01	4	0	
Syntocinon (synthetic oxytocin)	.1	0	6	Transplanted
	1.0	14	4	
Pitupartin AB (oxytocin preparation)	.1	0	5	"
	1.0	5	0	
Pitupartin AB	1.0	0	8	Extirpated
Extract of pars nervosa of whale*		3	0	Extirpated
Idem toad†		4	7	"
" "		2	7	Inactivated‡

* Extract of ½ mg acetone dried isolated pars nervosa of blue whale *(Balaenoptera musculus)* was inj. into each toad. Material was boiled with frog's Ringer for 5 min.

† Acetic acid extract of 1/10 of neuro-intermediate lobe of toad *(Bufo bufo)* inj. into each toad.

‡ Hypothalamic-hypophyseal portal circulation interrupted by extirpation of median eminence(1).

vation of the pars distalis was performed either by transplanting the gland into one of the eye muscles or by extirpation of the median eminence(3,1). The toads used were mostly males weighing 30 to 40 g. Injections were made of synthetic or highly purified lysine-vasopressin, of synthetic oxytocin (Syntocinon Sandoz), and of the pressor preparation Insipidin AB and the oxytocic preparation Pitupartin AB. Moreover some experiments were made with crude extracts of the pars nervosa of the blue whale (*Balaenoptera musculus* L.) and of the neuro-intermediate lobe of the common toad.* Generally the toads never shed the slough spontaneously after extirpation or inactivation of the pars distalis. However, some toads did slough once within a week after transplantation of pars distalis or after extirpation of the median eminence. As the transplanted or otherwise inactivated pars distalis is revascularized within a week of operation, this single shedding of the slough may be caused by substances leaking out from necrotic pars distalis tissue upon reestablishment of circulation. Each toad received from one to 4 injections, first about a week after operation. If sloughing followed injection, the next injection was not given until the skin was once more abnormally keratinized. Toads with extirpated or inactivated pars distalis survive for only a few weeks or months depending upon temperature and time of year. Before death the toads become increasingly weaker and cease to shed the slough in response to injections of pars distalis stimulating factors. The toads were therefore used only as long as they appeared healthy.

Results. Minimal effective dose of lysine-vasopressin is close to 0.01 international unit (I.U.) (Table I). Injection of this dose induced sloughing in 7 of 11 toads, whereas 0.001 I.U. was without effect. 0.1 I.U. caused shedding of epidermal cornification in all but one of the toads with transplanted pars distalis. As might have been expected, no significant difference was observed in effect of

* We thank Dr. Vincent du Vigneaud, Cornell University, for a gift of synthetic lysine-vasopressin, and Dr. Aa. Theil Nielsen, Royal Danish School of Pharmacy, for a gift of highly purified lysine-vasopressin.

synthetic and highly purified lysine-vasopressin. 2 I.U. of purified lysine-vasopressin was without effect on the skin in the hypophysectomized toads confirming previous results that synthetic lysine-vasopressin has no direct effect on sloughing(1).

Injection of the commercial preparation Insipidin caused sloughing even in hypophysectomized toads. The factor causing sloughing must have been present in high concentration, since amounts of Insipidin containing as little as 0.001 pressor unit were effective. The pars distalis stimulating effect of the vasopressin component of Insipidin could therefore not be observed.

A substance eliciting sloughing was likewise present in the pars nervosa of the blue whale and the neuro-intermediate lobe of the toad, since extracts of these glands provoked sloughing in hypophysectomized toads.

It is reasonable to assume that the "sloughing factor" that has been demonstrated in crude or partially purified neurohypophyseal extract is identical with the factor released from the pars distalis in response to vasopressin. This factor may be identical with adrenocorticotropic hormone (ACTH)(4) which is known to be present in significant amounts in the pars nervosa of the rat(5,6).

Injection of pars nervosa extract corresponding to 1/10 of a toad pars nervosa produced sloughing at least as often in the toads with extirpated pars distalis (in 4 out of 11) as in toads with inactivated pars distalis (in 2 out of 9). Thus, no additional effect on sloughing due to release of the sloughing factor from the pars distalis could be observed in the group with inactivated pars distalis. In separate experiments it was ascertained that inactivated pars distalis responded normally to injection of lysine-vasopressin. It can therefore be concluded that amounts of specific pars nervosa hormone(s) contained in 1/10 of a toad pars nervosa are not sufficient to cause sloughing by release of the sloughing factor from the pars distalis.†

Synthetic oxytocin and Pitupartin were capable of releasing the sloughing factor from the transplanted pars distalis, but larger doses than those of lysin-vasopressin were required to obtain an effect. Minimal effective dose was about 1 I.U., 0.1 I.U. being ineffective. Since Pitupartin is guaranteed to possess an activity of less than 0.02 pressor units for each oxytocic unit, the sloughing caused by injection of one oxytocic unit of Pitupartin may be due in part to presence of vasopressin in the preparation. Pitupartin apparently contained little or no sloughing factor as injection of 1 I.U. was without effect on the skin in hypophysectomized toads.

Discussion. In mammals, vasopressin is generally more effective than oxytocin in stimulating the inactivated pars distalis. In the rat minimal effective dose of synthetic vasopressin in releasing ACTH is about 0.1 I.U.(8). The rats weighed 300 g, or about 10 times as much as the toads used in the present experiments. Consequently, when compared on the basis of unit body weight the ACTH releasing mechanism in the rat and the sloughing factor releasing mechanism in the toad are about equally sensitive towards vasopressin. In the rat, vasopressin has been under consideration as the natural ACTH releasing factor(9,10). However, a closely related, but more potent peptide has been isolated from neurohypophyseal and hypothalamic tissue, so the ACTH releasing factor in mammals is probably not identical with the pars nervosa hormones, vasopressin and oxytocin(11,12,13). It would therefore be of interest to compare the effects of vasopressin and of the specific mammalian ACTH releasing factor on adenohypophyseal activity in toads.

Summary. In the toad *Bufo bufo* (L.) the pars distalis was inactivated by transplantation to one of the eye muscles or by extirpation of the median eminence. Minimal dose effective in releasing a sloughing factor from inactivated pars distalis was 0.01 I.U. of highly purified or synthetic vasopressin and 0.1 I.U. of synthetic oxytocin. Insipidin, a commercial preparation of vasopressin, and crude extracts of the pars nervosa of whale and toad contained large amounts of the sloughing factor which is probably identical with ACTH.

† Injection of extract of 1/1000 or less of toad pars nervosa suffices to produce antidiuresis in the toad(7).

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Biological Synthesis of a Growth Factor for Mammalian Cells in Tissue Culture.* (25636)

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The search for simplified or chemically defined media has long occupied the efforts of those interested in growth of mammalian cells *in vitro*. Much of the recent reported work in this area has been done with the L strain of mouse skin fibroblasts isolated by Earle(1). Merchant and Rahn(2), Kuchler and Merchant (unpublished), and Waymouth(3) have shown the effects of peptone components on growth of L strain fibroblasts, and Gerarde and Jones(4) reported that bacto-peptone substituted for dialyzed embryo extract promoted collagen formation in chick embryo lung cultures. The chief difficulty encountered in preparation of defined media is that of replacing the serum which is usually present to the extent of 10-40% of volume. Lieberman and Ove(5) reported that a protein factor isolated from bovine serum stimulated growth *in vitro* of human appendix cells for short periods of time in presence of peptone-albumin, but in absence of whole serum. Recent experiments by Kutsky(6) have shown that a nucleoprotein isolated from embryo extract stimulated growth of chick embryo heart fibroblasts in cell cultures. Human cell culture lines have not as yet been adapted to growth in serum-free media, either chemically defined or containing peptone as a serum replacement. Preliminary experiments in our laboratory have shown that a stable cell line of mouse lung fibroblasts adapted to a serum-free medium by Pumper(7) release into the medium proteinaceous substance(s) capable of replacing the serum requirement of a human liver cell line isolated by Chang(8). Human liver cells were incapable of growing on the peptone-containing medium unless serum or protein from mouse lung cell growth fluid was added. Following this initial finding experiments were designed to separate the material responsible for stimulation of human liver cells from the dialyzable components of the growth fluid, and to study the physiological activity of the unpurified factor.

Methods. A. *Cell lines.* 1. Mouse lung cells adapted to a serum-free medium(7): Mouse lung cells were grown in milk dilution bottles on a medium consisting of 99% medium 199, 1 per cent Difco Bacto-peptone, 100 mg % dextrose, and 50 units each of penicillin and streptomycin per ml. The peptone was prepared in a 10% solution, and

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