cytes is severely depleted over a relatively long period of time and this with possible impairment of phagocytic function(11,12) may account for the difference in effectiveness of antibodies under the 2 conditions studied.

Summary. Immunization with *Proteus* prior to LD_5 irradiation increased resistance of mice and rats to challenge with the living organism. Immunization with mixtures of the bacteria which occur most frequently in postmortem blood increased resistance to challenge in mice. Immunization with these mixtures did not increase survival in mice exposed to LD_{65-95} radiation.

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Effect of Xenoplastic Adrenal Transplants upon Limb Regeneration in Normal and Hypophysectomized Newts (*Triturus viridescens*). (21274)

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The following experiments arose from investigations concerned with the role of the pituitary as a requisite for regeneration in newts (1,2,3,4,5). When, in addition, it was recently shown that the administration of ACTH and also of several cortical extracts to hypophysectomized newts, otherwise incapable of regeneration, reinstated in these animals the capacity for regeneration(6), it became probable that the adrenal glands might be an important or even essential hormonal factor in limb regeneration. Unfortunately, the participation of the adrenals in regeneration is not demonstrable by adrenalectomy because of the diffuseness of these glands in urodeles. As one of the methods to explore the premise that purely adrenal products may replace the missing pituitary in limb regeneration, this research has investigated the effects of xenoplastically transplanted frog adrenals into previously hypophysectomized newts.

Materials and methods. All the experiments were performed upon normal adult or upon hypophysectomized newts (*Triturus viridescens*) and the adrenal transplants were obtained from adult *Rana pipiens* and *R. palustris* kidneys by dissection immediately before transplantation. For control purposes muscle transplants have been used. The frog adrenal or muscle transplants were introduced under a skin pocket of the heavily vascularized lower jaw. Using this technique several series of transplantations were made. In a first group the hypophysectomized newts received a single adrenal transplant; in another group one adrenal was implanted at the time of amputation and additional frog adrenals were implanted at the fifth and also at the tenth day following amputation. In a final group the transplantation, limited to one adrenal only, was delayed until the sixteenth day after amputation at which time the second limb of the newt was freshly amputated. All limbs were studied histologically and the heads of

TABLE I. Effect of Frog Adrenal Transplants upon Limb Regeneration in Hypophysectomized Newts.

No. of animals	1 adrenal simultaneous with amputation	1 adrenal with first amputation, 1 or 2 additional transplants 5 or 10 days later	1 adrenal 15 or 16 days after amputation
2	++		
4	0		
8		++	
3		0	
3		*	
20			++

++ Advanced, typical regeneration of the limb. * Arrested regeneration. 0 Typical non-regeneration.

the animals which regenerated were sectioned to verify the completeness of pituitary removal.

Results. Effects of adrenal transplants on the regeneration of normal animals. Single adrenal transplants to control series of normal animals had no observable effect either upon the usual pattern of regeneration or upon the general behavior of these animals. As might be expected the endocrine system with intact pituitary is far more competent than the hypophysectomized system to compensate for abnormal hormone balances.

Regeneration of limbs of hypophysectomized newts with adrenal transplants. As may be seen in Table I, only 2 hypophysectomized animals were observed to regenerate under the influence of single adrenal transplants and many cases of typical non-regeneration were recorded in this group. Somewhat better success was obtained with increased doses of adrenal tissue, particularly when additional adrenal transplants were performed 5 or 10 days later. When viewed in the context that hypophysectomized animals never regenerate, regeneration in more than one-half of the animals which received adrenal transplants on the tenth day following amputation, appears to demonstrate that purely adrenal products are competent to replace the pituitary in regeneration in histologically verified absence of this gland. A final series involving transplantation delayed after amputation, however, provides a more consistent picture. A single adrenal was transplanted into these hypophysectomized newts 15 or 16 days after the first amputation at which time a second limb was

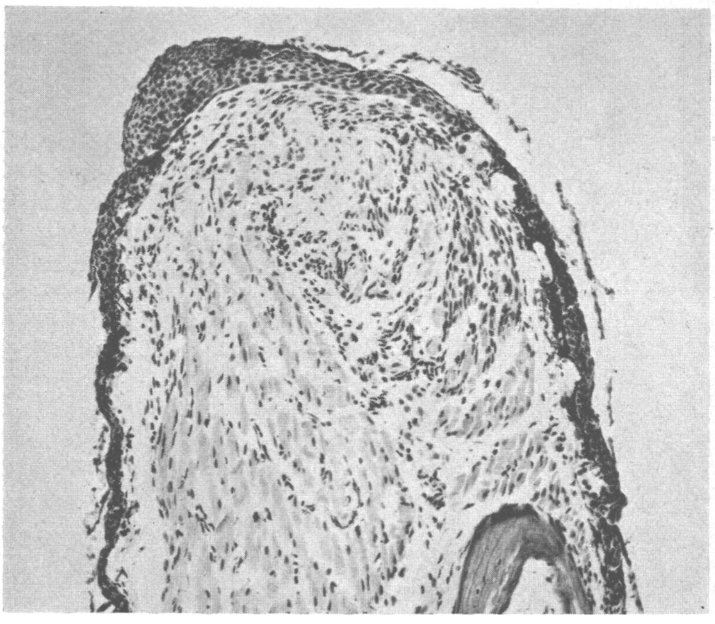

FIG. 1. Section through right forelimb of a hypophysectomized newt, the limb pictured having been amputated at the time of adrenal transplantation. Limb fixed 23 days after amputation exhibits typical non-regeneration indicated by the dermis with darkly staining chromatophores invading the area and separating the cut tissues from the epidermis.

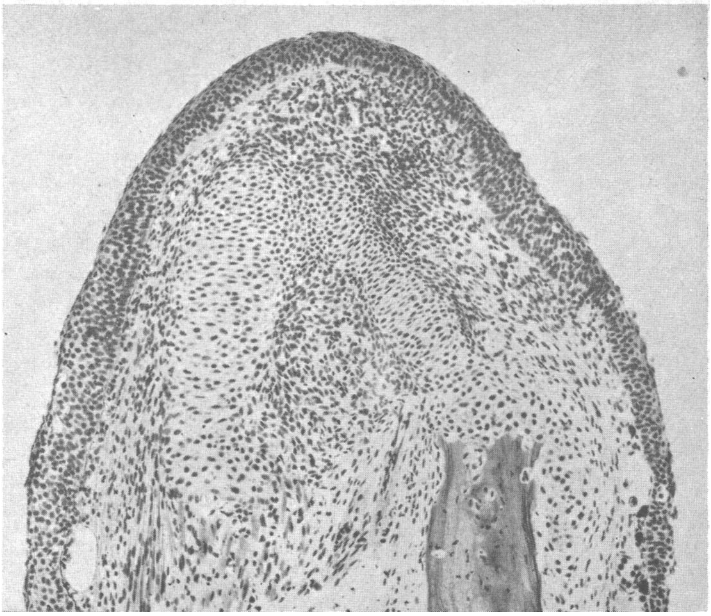

FIG. 2. Photomicrograph of a section through the regenerating left forelimb of the same animal as represented in Fig. 1, but which was amputated sixteen days previous to introduction of a single frog adrenal transplant. Limb was fixed 39 days after amputation and shows advanced regeneration with differentiation of skeletal formations.

amputated. In all 20 cases in which transplantation was performed on the fifteenth or sixteenth day after amputation, regeneration was initiated in the limb amputated previous to the transplantation, whereas typical non-regeneration was observed in the opposite limb of each animal, amputated at the time the adrenal transplant was introduced. The photomicrograph of Fig. 1 from a hypophysectomized newt represents a section through the right forelimb which was amputated simultaneously with the introduction of an adrenal transplant. Sixteen days earlier, the left limb (Fig. 2) of the same animal was amputated simultaneously with the extirpation of the pituitary and was fixed after 23 days of regeneration. These figures illustrate well the results of the experiment while recapitulating, as it were, the general procedure followed. Fig. 1 shows that a limb from a hypophysectomized newt may not regenerate when a frog adrenal is transplanted simultaneously with the amputation. However, the same adrenal transplant brings about typical regeneration in the other limb of this animal which has been allowed to "regenerate" previous to transplantation for 16 days in histologically proved absence of the pituitary, a result which has been confirmed in all the cases of this series.

Observations on hypophysectomized animals with muscle transplants. Frog muscle (generally *M. sartorius*) transplanted into the lower jaw under conditions identical with those described for adrenal transplants produced no regeneration whatsoever in these hypophysectomized newts. Clearly then, the introduction of pieces of foreign tissue other than adrenal, at whatever time, does not reinstate the capacity for regeneration in hypophysectomized newts, an observation which as this is written has been confirmed by identical results with other tissues.

Discussion. The above results provide an affirmative answer to the question whether purely adrenal products are capable of acting in the same way as exogenous ACTH, cortisone or experimental stress (unpublished results from this laboratory) to reinstate in hypophysectomized animals the ability to regenerate. This investigation, therefore, is interpreted as supporting the hypothesis that the stress of amputation in naturally regenerating animals is mediated by the anterior pituitary which elicits a hyperadrenal response, comparable to that which has been observed in mammalian endocrinology under conditions of stress(7). That the previous work with injected cortisone(6) produced regeneration because of the hormone supplied and not because of contaminants is shown by the success of the above experiments in which adrenal tissue may induce regeneration of limbs in hypophysectomized newts. That the effect of the operation of transplantation itself is negligible is demonstrated by the fact that single or plural muscle transplants and lately also other tissue introduced simultaneously or successively in respect to amputation never result in regeneration within limbs of hypophysectomized newts. While, then, frog adrenal transplants in hypophysectomized newts may perhaps not function normally over extensive periods of time even when administered in larger amounts, they will nonetheless induce regeneration if introduced during a period when the amputated limb of a hypophysectomized newt has become susceptible to adrenal therapy. The unexpected success of delayed single adrenal transplants in initiating good regeneration in amputated limbs of animals deprived for two weeks of their pituitary deserves special attention. This observation implies a relatively short period of effective function for the adrenal transplant and it also suggests that the balance between regeneration and non-regeneration is a labile one, a fact which tallies with other observations in the field of regeneration.

Summary. 1. Our previous experiments showed that recuperation of regeneration in limbs of hypophysectomized newts may be obtained by replacement therapy with ACTH and with adreno-cortical hormones. We tested the hypothesis that an hyperadrenal state is necessary for regeneration. The study of effect of xenoplastically transplanted single or multiple adrenal frog glands upon regeneration of the fore limb in previously hypophysectomized newts was the method used. Control experiments showing that frog muscle tissue and also other tissues transplanted under similar conditions had no effect upon the non-regeneration typical for hypophysectomized newts indicated that xenoplastic transplants *per se* produced no observable effects upon regeneration. Frog adrenals, however, while not affecting regeneration in normal animals were found capable of restoring to hypophysectomized newts the ability to regenerate amputated limbs otherwise lost after extirpation of the pituitary. 2. Hypophysectomized animals are more susceptible to adrenal therapy 10 to 20 days after amputation than during earlier periods, since it is when transplanted at this time that frog adrenals most consistently produced regeneration. These results while not clearly understood, point to the existence of a critical stage within the post-amputational phases of regeneration when regeneration is particularly susceptible to adrenal hormones, endogenous or experimentally supplied. 3. Heretofore unsuspected involvement of adrenal secretions in limb regeneration of newts is thus demonstrated and the pituitary-adrenal synergism discovered for conditions of "stress" in mammals acquires a new importance for regenerative processes in amphibia.

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