the present experiment; but the activity was weak compared with that of the relatively small doses of 1 or 10 mU ACTH. This result contrasts with the findings(1) that in *B. arenarum* with extirpated pars distalis, GH injections maintained life for a longer period of time than ACTH injections. However, in the experiments on *B. arenarum* a large dose of ACTH, 0.5 mg 3 times a week, was used. In *B. bufo* high levels of ACTH may cause symptoms similar to those resulting from a deficiency of ACTH(3). It would therefore be of interest to know whether in *B. arenarum* a smaller dose of ACTH might result in increased length of survival. Since the ACTH content of the GH preparation used in the experiments on *B. arenarum* was not stated, it seems necessary to check the effect on survival of the amount of ACTH possibly contained in the rather large dose of GH used (0.25 mg 3 times a week), before final conclusions can be drawn concerning the relative potency of ACTH and GH in maintaining life in hypophysectomized *B. arenarum*.

It is unlikely that lack of gonadotropic hormones is of any importance in the death of the hypophysectomized toads. Neither does lack of thyrotropin seem to be the cause of death in *B. bufo*, since injections of thyroxine in pars distalis extirpated individuals of this species slightly reduced the length of survival(2). In hypophysectomized *B. arenarum*, however, thyroxine was found to have some life prolonging activity(1). It thus appears that of the well known pars distalis hormones, ACTH is by far the most potent in sustaining life in hypophysectomized *B. bufo*, and perhaps also in other bufonids.

Summary. *Bufo bufo* kept at 21°C died about 17 days after extirpation of pars distalis of the hypophysis when operated in May, and after about 23 days when operated in September-October. Subcutaneous injections of 1 or 10 mU ACTH daily or 3 times a week increased length of survival by several weeks or months, but eventually the toads died with symptoms similar to those of ACTH deficiency. Injections of 0.1 mg GH 3 times a week had little effect on survival.

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Functionally Active Tadpole Hypophyses Homografted Under the Median Eminence of Adult Toads. (28287)

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In the toad *Bufo bufo*, extirpation of the pars distalis of the hypophysis causes abnormal moulting, and the animals survive only a few weeks or months, depending upon the temperature. Homografts of pars distalis placed under the median eminence of the neurohypophysis may maintain normal moulting and increase survival time. Even the small pars distalis of a newly metamorphosed toad is able temporarily to maintain moulting and life in an adult individual up to a hundred times larger. Eventually, however, the homografts break down and the recipients die(1). The present studies were performed to determine whether the hypophyses of tadpoles or the hypophyseal rudiments of embryos are also capable of sustaining life in adult toads.

Methods. The experimental technique and

TABLE I. Effect of Hypophyseal Homografts on Survival in Toads with Extirpated Pars Distalis.

Stage of donor	Length of survival
	Days
Controls	12,13,13,15,16,18
Tail bud stage h^x	13,14,14,14,15,16
	Weeks
Controls	2,2,2,2,3,3,3,3,3,3,3,3,4
Limb bud stages h^x	3,3,4,6,7
Foot paddle stages h^x	2,2,3,3,5,5,5,5,5,5,6,8,8,8,10,10, 10,11,16
Foot stages h^x	5,6,6,9,19,34

h^x = hypophyseal homografts.

Survivals longer than the control values are underlined.

histological procedures have been described (1). Embryos in the tail bud stage corresponding to about Stage 17 of *Rana pipiens* embryos(2), and tadpoles of various stages served as donors. The most immature tadpoles possessed bud shaped hindleg rudiments, corresponding to Stages III-IV in *Rana pipiens*(3); in the most advanced stages, the hindlegs had toes and were functioning. All the tadpoles were 8 weeks old, and their wide differences in development were due to differences in food supply. Grafting of embryonic hypophyseal rudiments was done in April and of tadpole hypophyses in June. The recipients were about 30 g *Bufo bufo* males with extirpated pars distalis, each of them receiving one homograft. The temperature varied between 19-20°C during summer and 12-15°C during winter.

Results. Neither moulting nor survival of the recipients was influenced by the transplanted hypophyseal rudiments from embryos in the tail bud stage; but transplants obtained from tadpoles significantly increased survival time in 23 of 30 recipients (Table I). Apparently, the functional capacity of the grafts increased with size and development of the donors, the best results being obtained with the grafts from the advanced stages. Most of the experimental animals that survived longer than the controls exhibited periods with normal moulting cycles.

Microscopic examination of the transplants was performed in 21 of the 23 toads in which the transplants had presumably been temporarily functioning. In 20 toads no healthy transplants were found, but only small, if any, remnants of undifferentiated hypophyseal cells infiltrated by lymphocytes and connective tissue. Probably, therefore, death in these toads resulted from deficiency of hypophyseal function, following the breakdown of the transplants. However, the time of onset of the homograft reactions, which eventually lead to the destruction and absorption of the transplants, apparently varied from a few weeks to several months. In the toad that died after 34 weeks, moulting first became slightly abnormal about 28 weeks after the operation, indicating that the onset of the homograft reaction was long delayed in this animal, or that the reaction proceeded very slowly. In one toad, which died 16 weeks after operation, intact pars distalis tissue was still present and no homograft reaction was encountered. Moreover, in this toad, moulting had been normal throughout the period of survival, so the animal probably did not die from deficient hypophyseal function.

Discussion. Previous work showed that pars distalis homografts were functionally active temporarily when placed under the median eminence of the tuber cinereum of the brain of adult toads deprived of their own pars distalis(1). The homografts studied(1) were obtained from newly metamorphosed toads. The present findings of normal moulting and prolonged survival in toads with their own pars distalis replaced by pars distalis transplants of tadpoles confirm and extend previous results.

The only adenohypophyseal hormone known to normalize moulting and effectively to prolong survival in hypophysectomized *Bufo* is corticotropin. Growth hormone and prolactin have little or no effect nor does thyroxine(4,5,6,7). From the effects observed in the present work on *B. bufo,* it therefore seems reasonable to conclude that pars distalis transplants from tadpoles are capable of corticotropin production.

Hypophyseal rudiments obtained from embryonic donors at the tail bud stage failed to exert any noticeable effect in the recipients. Whether this is due merely to the smaller

amounts of tissue or to a failure of the rudiment to differentiate cytologically and to produce corticotropin remains to be elucidated.

Summary. Hypophyseal rudiments from tail bud stage embryos and hypophyses from tadpoles of various stages were homografted under the median eminence of adult *Bufo bufo* males with extirpated pars distalis. The rudiment had no effect on moulting or survival of the recipient, but the tadpole hypophysis increased survival time in 23 of 30 recipients. Most of the toads surviving longer than the controls showed periods with normal moulting. The best results were obtained with transplants from the advanced tadpole stages. Corticotropic activity of the transplant was probably responsible both for normal moulting and increased survival time. Homograft reactions resulted in destruction of the transplants after several weeks or months.

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Formation of Collagen. I. Antigenicity of Collagen Fractions. (28288)

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Extracts of rat and rabbit collagen made with acetic acid or neutral salts have proved antigenic insofar as they induce complement fixing antibodies(1,2). Since these studies were performed with crude and heterogeneous preparations of connective tissue, we have now attempted to determine the antigenicity and immunological specificity of purified soluble and insoluble collagen preparations.

Materials and methods. Leg tendons from old (6-8 months) and young (20-30 days) Leghorn chickens were used. Acetic acid extract of tendon collagen was prepared as previously reported by others(1). The fractionation procedure of Jackson(3,4) was applied to tissues of young chickens in order to obtain larger quantities of the water-soluble protein fraction, neutral salt-soluble fraction and acid-soluble fraction(3,4,5); the insoluble fraction was obtained from the tissues of older chickens. With exception of the portion extracted with acetic acid, collagen fractions were purified by successive washings and reprecipitations(3,4), and finally lyophilized. Proline and hydroxyproline contents were determined by established methods(6, 7).

Two lots (6 animals each) of adult male rabbits, each 6 months old and weighing 3 kg, were immunized, one group with acetic acid extract and the other with insoluble fraction. In each case an homogenate of the antigen in 0.01 N acetic acid (1 mg protein/ml) was administered intraperitoneally, a schedule of 4 injections per week with regularly spaced rest periods being followed. Blood samples were obtained at 4, 5 and 6 months after start of immunization. Antibodies against the soluble fractions were induced by injection of 3 lots (6 animals each)

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