TABLE II. Serum Phosphatide Values Measured In 7 Normal Subjects After Chromatographic Separation on Silicic Acid Plates.*

| | μg P per ml | | | | |
|---|---|---|---|---|---|
| Subject | Le | LLe | Sph | PTE | TP-P† |
| 1 | 60.8 | 9.7 | 19.1 | 1.4 | 91.0 |
| 2 | 70.9 | 11.6 | 21.4 | 2.1 | 106.0 |
| 3 | 82.3 | 12.6 | 28.1 | 3.2 | 126.2 |
| 4 | 78.5 | 13.0 | 22.9 | 3.7 | 118.1 |
| 5 | 68.6 | 15.3 | 27.7 | 4.8 | 116.4 |
| 6 | 81.8 | 12.0 | 28.8 | 3.2 | 125.8 |
| 7 | 79.8 | 12.6 | 29.2 | 2.3 | 123.9 |
| Avg | 74.7 | 12.4 | 25.3 | 3.0 | 115.3 |

* 4 sq cm areas used.
† Total phosphatide phosphorus.

TABLE III. Comparison of Average Normal Serum Phosphatide Values Obtained by TLC and Impregnated Paper Chromatography.

| | μg P/ml | | % of TP-P | |
|---|---|---|---|---|
| Compound | TLC | IPC* | TLC | IPC* |
| Le | 74.7 | 73.3 | 64.8 | 66.4 |
| LLe | 12.4 | 10.5 | 10.8 | 9.5 |
| Sph | 25.3 | 24.9 | 21.9 | 22.6 |
| PTE | 3.0 | 3.0 | 2.4 | 2.7 |
| TP-P | 115.3 | 111.7 | 99.9 | 101.2 |

* Impregnated paper chromatography.

values previously reported by several workers.

GPC determinations run on 2 normal serums and 2 pancreatitis serums did not reveal any GPC in these instances. On the other hand, significant amounts of GPC were present in 2 duodenal drainages aspirated from patients with normal pancreatic function.

*Summary.* The use of thin layer chromatography for analyzing phosphatides and their hydrolysis products quantitatively is described and applied to the analysis of serum phosphatides. The results are similar to those obtained with silicic acid impregnated paper chromatography and column techniques, though the time for the analysis is markedly shortened.

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## Effect of Corticotropin and Growth Hormone on Survival in Hypophysectomized Toads. (28286)

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Toads of the species *Bufo bufo* L. die within a few weeks or months after extirpation of the pars distalis of the hypophysis, length of survival depending mainly upon the temperature. Corticotropin (ACTH) and growth hormone (GH) have both been found very potent in increasing length of survival in *B. arenarum* with extirpated pars distalis (1). We have compared the potency of ACTH and GH in prolonging life in *B. bufo* with extirpated pars distalis.

*Methods.* Two series of experiments were performed: in the first series, started in May, only females weighing 60-140 g were used; in the second series, started in September-October, equal numbers of females, 50-70 g,

TABLE I. Effect of GH and ACTH on Survival of Toads *(B. bufo)* with Extirpated Pars Distalis.

| Groups | Date of operation | No. of animals | Treatment | Survival, days |
|---|---|---|---|---|
| 1 | May 10 | 7 | .1 ml $H_2O$ | 17.0 ± 1.1* |
| 2 | " 10–12 | 8 | .1 mg GH | 27.0 ± 1.3 |
| 3 | " 30 | 8 | .01 mU ACTH | 21.3 ± .9 |
| 4 | " 10 | 8 | 10 " " | 126 to >166 |
| 5 | Oct. 8 | 7 | None | 22.9 ± 1.0 |
| 6 | Sept. 28 | 8 | .1 mU ACTH | 34.6 ± 3.0 |
| 7 | " 27 | 7 | 1 " " | 61 to >66 |
| 8 | " 26 | 8 | 10 " " | 37 to >67 |

Groups 1-4 injected 3 times per wk. Groups 6-8 injected daily or 6 days per wk.
* ± S.E.

and males, 30-40 g, were used. The toads were kept in glass jars with a little water at 21 ± 1°C; they were fed heart meat or ground beef. ACTH (Orthana) was injected in doses ranging from 0.01-10 mU. A purified bovine GH preparation (NIH-GH-B3) was obtained as a gift from the Endocrinology Study Section, National Institutes of Health; 1 mg of the preparation was stated to contain 0.1 mU ACTH. GH was administered in doses of 0.1 mg, containing 0.01 mU ACTH. The hormones were injected subcutaneously in 0.1 ml water 3 times a week in the series started in May, and daily for the first 3 weeks followed by 6 days per week in the series started in Sept.-Oct.

*Results.* After extirpation of the pars distalis, the control toads survived for 2-3 weeks (Table I). The toads operated on in Sept.-Oct. lived about 6 days longer than those operated on in May; this difference in length of survival was statistically significant as shown by the t test ($0.001<P<0.01$). Injections of 0.1 mg GH increased survival by 10 days. Injections of 0.01 mU ACTH also increased survival, but only by about 4 days. This increase is significant at the 2% level ($0.01<P<0.02$), but is significantly smaller than the increase in survival caused by the GH injections ($0.001<P<0.01$). Daily injections of 0.1 mU ACTH were only a little more effective than injections of 0.01 mU ACTH 3 times weekly, increasing survival from ca. 23 to ca. 35 days ($0.001<P<0.01$). However, 1 or 10 mU ACTH injected daily, or 10 mU 3 times per week, sustained life for several weeks or months. The treatment maintained the toads in a healthy condition for a time, but eventually they developed symptoms typical of severe ACTH deficiency, such as increased torpidity, capillary dilatation, and finally death. There was no difference in results between daily injections of 1 and 10 mU ACTH; at the end of the experiment Groups 7 and 8 (Table I) had 5 surviving toads of which one in each group was in poor condition.

There was no difference in length of survival between males and females either in the untreated group or in the group receiving 0.1 mU ACTH daily, but of the toads receiving larger doses of ACTH, the females lived longer. None of the toads in Group 4 which consisted exclusively of females, died during the first 126 days. At the end of the experiment or 166 days after the operation, 3 toads still survived, though they showed definite symptoms of ACTH deficiency. Of the toads in Groups 7 and 8, only 1 female out of 7 had died at the end of the experiment, 66-67 days after the operation, but 4 out of 8 males died. Two of the surviving males were weak, whereas all the surviving females appeared healthy.

*Discussion.* ACTH in amounts as low as 0.01 mU, injected 3 times a week, significantly lengthened survival in the toad *B. bufo* with extirpated pars distalis. Increased survival was also obtained with injections of 0.1 mg GH, and the effect was significantly greater than that of 0.01 mU ACTH stated to be contained in 0.1 mg of the GH preparation. Apparently the large dose of bovine GH itself had a life maintaining activity in

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