assume, therefore, that a complementary substance is furnished by the pituitary which cooperates with A.P.L. in its effect on the ovary. Whether this substance is identical with any of the known pituitary hormones or not remains to be proved, although experiments now under way in this laboratory seem to indicate that this complementary substance is not identical with the known pituitary hormones.

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Automatism of Anuran Lymph Hearts as Obtained by Transplantation.

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Although the normal lymph hearts of Anura are typically neurogenic (Brücke and Umrath[1]) even to being homolaterally synchronized (Pratt and Reid[2]) the beating of these organs *in situ* after interruption of the spinal nerve impulses has often been observed. Such preparations, even if intrinsically ganglion-free, do not altogether eliminate the possibility of some peripheral nervous influence. Moreover, the full, regular beats of excised lymph hearts in isotonic salt solutions originally observed by Moore[3] were not obtained by Brücke,[4] whose results by the same method (irregular contractions like the fibrillations of the blood heart) have since been confirmed by the writer. The method here described, permitting indefinitely continued observation with isolation in a favorable environment, has made possible the recognition and detailed study of a fully developed automatic rhythm.

An anterior lymph heart exposed by the dorsal route is cut away from the surrounding muscles, the hooked transverse process of the third vertebra, and the vertebral vein. The tongue is everted and a small incision made in the basihyoid (retrolingual) membrane near the posterior border of the underlying lymph sac (sinus basi-

[1] Brücke, E. T., and Umrath, K., *Pflüger's Arch.*, 1930, **224**, 631.

[2] Pratt, F. H., and Reid, M. A., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 1019.

[3] Moore, A., *Am. J. Physiol.*, 1901, **5**, 87, 196.

[4] Brücke, E. T., *Pflüger's Arch.*, 1906, **115**, 334.

hyoideus). The extirpated lymph heart, a collapsed oval sac, is inserted through this opening and moved forward to the anterior margin of the sinus, where it usually becomes fixed by the connective tissue. The transparency of the membrane covering this lymph sinus makes it possible to observe the implant at any time by simply everting the tongue.

The observations were made on etherized or spinal animals, usually 10 days or more after operation. In every instance the transplanted tissue had become a firm, spherical mass. In all but 2 of the 13 active cases the tissue was well vascularized by connections with the vessels of the tongue. However, the 2 bloodless organs which floated freely in the lymph of the sinus contracted as spontaneously and coordinately as those that were attached. The rate of beat of the individual transplants varied considerably. A tendency to rapid periodic grouping was characteristic of the younger transplants, but all were eventually capable of long continued regular rhythm.

Not only was the transplanted tissue favorably placed for microscopic observation, but its activity could easily be recorded mechanically or electrically when large frogs *(R. catesbeiana)* or toads *(B. marinus)* were the experimental animals. Kymographic records were made by placing on the transplant a vertical straw supporting the writing-arm of a light heart lever. The responses to artificial stimuli showed that the automatic lymph heart had acquired cardiac-muscular properties. Single shocks produced extra-contractions followed by a pause longer than the normal intersystolic interval; these stimuli were ineffective during systole; a faradic current did not tetanize, although it sometimes increased the rate; and the organ responded maximally at threshold. Curare usually failed to stop an active transplant, while in 2 cases it produced a temporary increase in rate. Adrenalin chloride (1:100,000 and 1:10,000) introduced into the lymph sac had, however, apparently no effect upon either the amplitude or the rate. Throughout the above observations the organ was protected from drying by the thin overlying membrane.

The isolation of the transplants from systemic heart and larger muscle currents is particularly advantageous for electrical recording. By using a liquid-contact lead to an amplifier and bifilar oscillograph an uncomplicated diphasic curve was obtained. The character of this action potential sustains the histological findings that nerve cells are absent from the transplanted tissue, since multiple oscillatory discharges, common alike to the normal lymph heart mechan-

ism[1] and to that of invertebrate hearts (Garrey[5]), seem completely wanting.

The transition to intrinsic automacity on the part of lymphatic hearts is thus shown to involve a radical functional change from skeletal to cardiac muscular properties. Results in detail will appear in a later report.

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Studies on the Adrenal. II. Extraction of Cortical Hormone from Urine.

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It is probable from many physiological observations that the active principle of the adrenal cortex is of the nature of a general tissue hormone. If this be the case it should be present, albeit in small quantities, throughout the body and possibly in various secretions. Its presence was therefore sought in the urine.. Perla and Gottesman[1] claimed to have extracted the hormone from urine. From each liter of urine they obtained a quantity of hormone comparable to that obtained from about 225 gm. of the fresh beef adrenal glands by the method of Hartman.[2] Such a high yield would speak either for the excellency of urine as a source of the hormone or for the poor yield obtainable from glands by the method utilized. Unfortunately, Perla and Gottesman used as evidence for the presence of cortical hormone in urine the increased resistance of rats to histamine when the extract was simultaneously injected. The indirect nature of this method of test renders their conclusion open to question.

Our urinary extracts were prepared by extracting the fresh urine with benzene. When other than freshly voided urine was used, the products were less active, having apparently undergone decomposition, as might be readily expected. After washing the benzene with water, it was removed *in vacuo* at 40°C., after adding an

[5] Garrey, W. E., *J. Cell. and Comp. Physiol.*, 1932, **1**, 209.

* Aided by a grant from the Hartley Corporation.

[1] Perla, D., and Gottesman, J. M., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 1024.

[2] Hartman, F. A., *Endocrinol.*, 1930, **14**, 229.