

Relation Between Amphibian Water Retention Principle and Oxytocic Activity of Pituitary Extracts.

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Heller,¹ Steggerda and Essex,² Oldham,³ and Boyd and Brown⁴ have found that the injection of oxytocic fractions of posterior pituitary extracts (such as pitocin) cause frogs immersed in water to take up more water than injection of pressor fractions (such as pitressin). These results suggest that the water uptake in frogs may be related more to the oxytocic principle than to the pressor principle. If such were so, it should be possible to demonstrate a quantitative relationship between the amount of frog water uptake principle and oxytocic principle in extracts of the neurohypophysis. Such a relationship was found to exist but determination of the nicety of the correlation was limited by the variations inherent in the methods of assay. The finding of this correlation suggests either that these two principles are the same substance or that they separate proportionately in the same methods of extraction.

The amount of the frog water uptake principle was estimated on leopard frogs (*Rana pipiens*) by a method recently devised in this laboratory.⁵ The method of assay consisted in injecting into the dorsal lymph sac, 5 different increasing doses of extract per 10 g body weight of frog. Each dose was injected into 10 frogs acclimatized to water at 4°C and the dosage range was judged from preliminary assays with one or 2 frogs to a dose. The frogs were carefully dried and weighed before injection and again at 18 hours after injection at which time previous results had shown that they have taken up their maximum load of water. This peak was held for several hours when the assay was performed at the low temperature of 4°C, which was the advantage of using this low temperature, as it permitted 2 weighing assays and consequently permitted a greater number of animals being used at one time.

The mean percentage uptake of water in each group of frogs was

¹ Heller, J., *Arch. exp. Path. u. Pharmacol.*, 1930, **157**, 323.

² Steggerda, F. R., and Essex, H. E., *Proc. Soc. Exp. Biol. and Med.*, 1934 **32**, 425.

³ Oldham, F. K., *Am. J. Physiol.*, 1936, **115**, 275.

⁴ Boyd, E. M., and Brown, G. M., *Am. J. Physiol.*, 1938, **122**, 191.

⁵ Boyd, E. M., and Mack, E. G., *Endocrinology*, in press.

then found and these means were plotted against dosage. To be acceptable, a curve with 3 points along the maximal plateau was required; if such a curve was not obtained, a more suitable series of doses was selected and the assay repeated. From this curve we selected our unit dose which we have defined⁵ as the smallest amount of extract, per 10 g body weight of leopard frogs in water equilibrium at 4°C, required to produce in 18 hours in 10 frogs a mean gain in weight which is within 25% of the greatest mean percentage gain in weight of at least 3 similar groups of frogs yielding distributed, maximal plateau gains. The figure of 25% was semi-arbitrary and was based upon a statistical analysis of the expected variation of maximal responses discussed in our previous communication.⁵

To determine the variation one may expect to get with this method, 12 repeated assays, with a total of at least 50 frogs to an assay, were performed on the same sample of pituitrin. The mean number of mm³ of this pituitrin found to contain one frog unit was 5.7 with a standard deviation of 1.9, giving 33 as the value for Pearson's coefficient of variation.

We have named this principle, assayed as above, the amphibian water retention principle.⁵ We have qualified the title with the word 'amphibian' because the exact relation between this principle and the principle which inhibits water diuresis in mammals is not yet established. We have used the descriptive title 'water retention' rather than 'water uptake' because Boyd and Whyte^{6, 7} have presented evidence which indicates that the uptake of water is probably due to an inhibition of the normal loss of water with little or no effect on water intake. This conception of the mechanism of the pituitrin-uptake reaction has been substantiated by further studies in this laboratory.⁸

Twelve different batches of Solution of Posterior Pituitary, U.S.P. XI, or of Pituitary (Posterior Lobe) Extract, B.P. 1932, obtained from 4 different manufacturers were assayed for amphibian water retention principle. The units per ml were then compared with the international oxytomic units per ml and this comparison is presented in Table I. Since the extracts used were pharmacopoeial products assayed by standard methods and produced by reputable manufacturers, we accepted the assay of the manufacturer for the international oxytomic units at the time that the extract was put upon

⁶ Boyd, E. M., and Whyte, D. W., *Am. J. Physiol.*, 1938, **124**, 759.

⁷ Boyd, E. M., and Whyte, D. W., *Am. J. Physiol.*, 1939, **125**, 415.

⁸ Boyd, E. M., Clark, K. J., and Smith, A. E., unpublished data.

TABLE I.
Water Retention and Oxytocic Units of Posterior Pituitary Extracts.

Manufacturer	Year of Manufacture	Water Retention Units/ml	Oxytocic Units per ml	Water Retention Units	
				Oxytocic Units	
Parke, Davis: Obstretical	1934	40	10	4	
'' '' Surgical	1933	200	20	10	
'' '' ''	1938	100	20	5	
'' '' ''	1937	200	20	10	
'' '' Pitocin	1938	200	20	10	
'' '' Pitressin	1938	<10	<1	10	
Connaught	1937	100	10	10	
Frosst	1932	100	10	10	
''	1933	100	10	10	
''	1938	50	10	5	
Squibb: Surgical	1937	100	20	5	
'' Obstretical	1937	100	10	10	

the market. At the present time we are not prepared in this laboratory to reliably assay for international oxytocic units. We were reasonably certain that the manufacturers' assays were carefully done. This was checked by correspondence and in 2 instances by a personal visit by one of us to the control laboratory in which the extracts were assayed. In the case of each product it appeared that at least 4 comparisons with standard had been made and that Pearson's coefficient of variation of the results would probably average about the same as that found in our assay for amphibian water retention principle.

With the variations inherent in both methods of assay so great, it was obvious that any correlation found could be only approximate at the best. Most of the pituitary extracts were diluted to contain either 10 or 20 international oxytocic units per ml and if a correlation existed, one would expect to find some factor times these numbers, of amphibian water retention units per ml. This factor is given in the 5th column of Table I and is derived from the figures given in columns 3 and 4.

There were found from 4 to 10 times as many amphibian water retention units per ml as international oxytocic units. In 8 of the 12 extracts there were 10 times as many amphibian water retention units as oxytocic units. Considering the variation inherent in both methods of assay, this appeared reasonably significant.

Hence the correlation coefficient of figures in columns 3 and 4 of Table I was calculated after the formulae of Wylie.⁹ The correlation coefficient was found to be +0.8 with a probable error of 0.07. The coefficient being positive and near 1, the correlation between the units

⁹ Wylie, J., Ch. XIV of Currie, J. R., *A Textbook of Hygiene*, Livingstone, Edinburgh, 1930.

per ml was positive and significant. The coefficient was over 11 times its probable error so that the odds were overwhelming that it was significant.

These results indicate that there is a correlation between the content of international oxytocic units and amphibian water retention units per ml of posterior pituitary extracts. The methods of assay were not sufficiently accurate to show just how close this correlation may be. On the other hand, the water diuresis inhibiting principle in mammals is usually considered more closely related to the pressor principle.^{10, 11} With the evidence available, this would appear to be a characteristic difference between the 2 principles. It is possible, however, that the difference in the methods of assay may account for this apparently different distribution and this is now being investigated in this laboratory.

It will be noted in Table I that there were little or no differences in the content of amphibian water retention units per ml of old versus recent extracts. We are not in a position to know if the content of oxytocic units per ml in the same extracts was also unaffected, or seemingly unaffected, by age. The evidence suggests, however, that the amphibian water retention principle is stable in these extracts for periods of up to 7 years at least.

Summary. Twelve posterior pituitary extracts were found to contain 4 to 10 times as many amphibian water retention units per ml as international oxytocic units. Variation in the technics of assay prevented an estimation of the nicety of this correlation.

¹⁰ Hemingway, A., and Peterson, J. M., *J. Physiol.*, 1929, **67**, 24.

¹¹ Gargle, S. L., Gilligan, D. R., and Blumgart, H. M., *New Eng. J. Med.*, 1928, **198**, 169.