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# THE EFFECT OF THE HYDROGEN-ION CONCENTRATION ON THE STABILITY OF THE ANTIDIURETIC AND VASOPRESSOR ACTIVITIES OF POSTERIOR PITUITARY EXTRACTS

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Guggenheim [1914], Dudley [1919], Abel & Nagayama [1920] and Dale & Dudley [1921] showed that strong acids and alkalies destroy the oxytocic hormone of the post-pituitary gland. The loss of oxytocic activity proceeds in a manner characteristic of a single substance decomposing according to the law for monomolecular reactions [Adams, 1917]. The destruction of the oxytocic hormone is not peculiarly due to the nature of the acid used but a function of the hydrogen-ion concentration of the medium [Stasiak, 1926]. Investigations on the stability of the oxytocic principle covering a much wider range of *p*H values and temperatures were published by Gerlough [1930], Gaddum [1930] and Gerlough & Bates [1930]. Gerlough determined the rate of decomposition at 100° C. between *p*H 2·0 and 5·0. Using solutions of different oxytocic strength he showed the rate of destruction to be independent of the initial concentration of the oxytocic principle. This conforms to the requirements of a first order reaction. Gaddum's observations cover the whole range of *p*H and temperature between 25 and 99° C. His nomogram enables us to calculate the amount of destruction of the oxytocic principle in a solution of any given *p*H heated for any given time at any given temperature. Values obtained from Gaddum's nomogram conform reasonably well with Gerlough's figures.

Data on the influence of changes in *p*H on the stability of the vasopressor and antidiuretic activity of post-pituitary extracts are surprisingly scanty. Guggenheim [1914] states that 2*N* NaOH inactivates both the oxytocic and the blood pressure raising action of post-pituitary

extracts if applied at room temperature for 2 hr. Abel & Nagayama [1920] investigated the influence of various concentrations of hydrochloric acid on the pressor activity and found no appreciable loss of pressor activity if post-pituitary extracts are boiled in 0·25% HCl (*p*H 1·16 calculated) for 30 min. Boiling for 30 min. with 0·5% HCl (*p*H 0·96 calculated) almost if not completely annulled the pressor response. 1·0% HCl (*p*H 0·57 calculated) abolished every trace of the specific action. Dale & Dudley [1921] and Stasiak [1926] repeated and on the whole confirmed these experiments. Abel & Nagayama and Dale & Dudley were concerned with the content of histamine or a histamine-like substance of impure post-pituitary preparations, and performed their blood pressure experiments on animals anaesthetized with ether. Cats in ether anaesthesia are optimally sensitive for the depressor action of histamine [Gaddum, 1936]. Since Abel & Nagayama and Dale & Dudley published their results it has been demonstrated repeatedly [Kolls & Geiling, 1924; Gruber, 1929; Swanson, 1929; Heller & Kusunoki, 1933] that the circulatory effects of pituitrin and pitressin are impaired by anaesthetics and hypnotics. Cats under ether anaesthesia are 10–20 times less sensitive to small doses of the vasopressor principle than spinal animals. It is clear, therefore, that animals under ether anaesthesia are unsuitable for the accurate assay of the post-pituitary pressor principle. Smith & McClosky [1924] investigated the influence of various temperatures on the stability of the oxytocic and pressor principles presumably at the hydrogen-ion concentration resulting from the preparation of their standard extract. The rate of deterioration of the pressor activity ran parallel to that of the oxytocic potency, and this was taken as favouring the chemical identity of the oxytocic and pressor principle. Their method of assay for the pressor substance is not stated. Kamm, Aldrich, Grote & Bugbee [1928] mention that non-acidified extracts of the post-pituitary gland lose more of the pressor than of the oxytocic activity when boiled for a short time. The stability of the two principles in acidified extracts appeared to be the same. There seems to have been no systematic investigation of the effects of the hydrogen-ion concentration on the stability of the antidiuretic activity of post-pituitary extracts.

Our present information about the influence of *p*H changes on the stability of the post-pituitary principles is thus fairly extensive for the oxytocic substance, but exceedingly meagre for the other principles. The present series of experiments was, therefore, performed to collect data on the stability of the vasopressor and antidiuretic fraction of post-pituitary extracts.

## Methods

The post-pituitary extracts employed in this investigation were Messrs Parke, Davis and Co.'s pituitrin, pitressin and pitocin. The term milliunit is used to express the activity of 0·1 c.mm. of pituitary (posterior lobe) B.P. extract. Clark & Lubs' series of buffers was used for the *p*H values between 2·0 and 10·0 and analytically pure hydrochloric acid and sodium hydroxide solutions for *p*H values beyond that range. The hormone was mixed with the appropriate buffer solution to obtain a concentration of 500 milliunits per c.c. Part of this mixture was put into a refrigerator and used as standard solution, another part was filled into glass ampoules. The sealed ampoules were heated in a water bath at about 99° C. and withdrawn at the times shown in the tables and quickly cooled. The contents were suitably diluted with 0·9% saline and tested as quickly as possible.

The hydrogen-ion concentrations were measured colorimetrically using B.D.H. capillators and were found to be the same before and after heating.

*Estimation of pressor potency.* Spinal cats were used, those not giving a good pressor response to doses below 10 milliunits per kg. being rejected. With such doses complete recovery of sensitivity to the pressor principle occurs in 40–45 min. Injections of an arbitrary dose of the standard were repeated until the responses were sufficiently constant. A dose of the unknown extract which, judged from preliminary tests, could be expected to cause approximately the same rise of blood pressure as that due to the standard was then injected. The following order of injections was observed: *a*, *a*, *b*, *a*, *b*, *a*, *a* (standard = *a*, unknown extract = *b*).

*Estimation of antidiuretic potency.* Burn's [1931] rat method was used with slight modifications. The rats received water up to 2 hr. before the subcutaneous injection of the post-pituitary extract. A curve relating the dose of antidiuretic principle to the time elapsing before maximal rate of excretions occurs was constructed by injecting 15–20 groups of rats with a series of doses of the standard hormone solution (pitressin). Results obtained with the unknown solutions are related to this curve. For every estimation of an extract of unknown strength one or two control groups of rats were injected with appropriate dilutions of the standard. If gross deviation from the standard curve occurred, all groups of rats in actual use were restandardized. Several dilutions of an unknown extract were used for the estimation of its potency, to ensure

their coming within the range of the maximum sensitivity of rats to subcutaneous injections of the antidiuretic factor, i.e. between 1 and 6 milliunits per 100 g. rat [Heller & Urban, 1935].

*Estimation of oxytocic potency.* The guinea-pig uterus method was used [Dale & Laidlaw, 1912]. The order of injection was *a*, *a*, *b*, *b*, *a* (standard solution = *a*; unknown solution = *b*).

## Results

The destruction of the antidiuretic and the vasopressor activities of post-pituitary extracts at 99° C. and various values of *p*H are shown in Table I.

Table I. The destruction of the antidiuretic and the vasopressor activities of post-pituitary extracts at 99° C. and various values of *p*H

| | Antidiuretic principle (*A*) | | Vasopressor principle (*V*) | | | |
|---|---|---|---|---|---|---|
| *p*H | Time of heating, min. | Activity left % | Time of heating, min. | Activity left % | Ratio *A*/*V* | Ratio *A*/*V*. Activity of *V* left expressed as percentage of *A* left |
| 0·57 | 120 | 6·1 ± 0·43* | 90 | 3·0 ± 0·13 | — | — |
| 0·96 | 60 | 60·0 ± 22·40 | — | — | — | — |
| | 180 | 11·3 ± 2·90 | 180 | 4·3 ± 1·20 | 2·66 ± 0·096† | 100/37·6 |
| 2·0 | 180 | 43·7 ± 5·30 | 200 | 30·0 ± 12·00 | — | — |
| | 300 | 29·6 ± 4·95 | 300 | 6·8 ± 1·85 | 4·35 ± 0·230 | 100/23·0 |
| 3·0 | 270 | 38·5 ± 4·55 | 270 | 15·0 ± 7·10 | 2·57 ± 0·216 | 100/39·5 |
| 5·0 | 210 | 17·2 ± 1·10 | 140 | 12·0 ± 2·80 | — | — |
| 6·4 | 240 | 18·1 ± 5·4 | 240 | 4·0 ± 2·70 | 4·58 ± 0·234 | 100/21·8 |
| 8·0 | 180 | 19·1 ± 0·50 | 180 | 12·7 ± 0·37 | 1·55 ± 0·001 | 100/66·6 |
| | 300 | 9·7 ± 0·43 | 300 | 1·1 ± 0·21 | 9·25 ± 0·044 | 100/10·8 |
| 10·0 | 60 | 17·8 ± 3·10 | 60 | 1·8 ± 0·43 | 9·90 ± 0·307 | 100/10·1 |
| | 90 | 11·5 ± 0·85 | 90 | 0·9 ± 0·57 | 12·62 ± 0·342 | 100/ 7·9 |
| | 125 | 4·3 | — | — | — | — |

* Standard error.

† Standard error of ratio.

Adams [1917], Gerlough [1930] and Gaddum [1930] showed that the thermal decomposition of the oxytocic principle can be conveniently expressed in terms of a first order reaction, i.e. $k = [1/t\ 2{\cdot}3 \log (a/a-x)]$, where $a$ = initial concentration of hormone, and $x$ = amount destroyed in the time $t$. The equation requires the independence of the constant $k$ from $t$ at any given value of *p*H. The velocity constant $k$ for the antidiuretic and the vasopressor activity was, therefore, determined in several instances for different times at the same *p*H. Since the differences between the values of $k$ after different periods of heating are not larger than can be expected from the error of the methods of assay,

and since they are distributed in an irregular manner the use of the equation was adopted for the present investigation.

The values for $k$ are significantly different for the antidiuretic and the vasopressor activity at all the $p$H values used. Moreover, the values of $k$ for the antidiuretic factor are consistently lower than those for the vasopressor factor. $k$ in Figs. 1 and 2 is represented by log $1/k$ so that

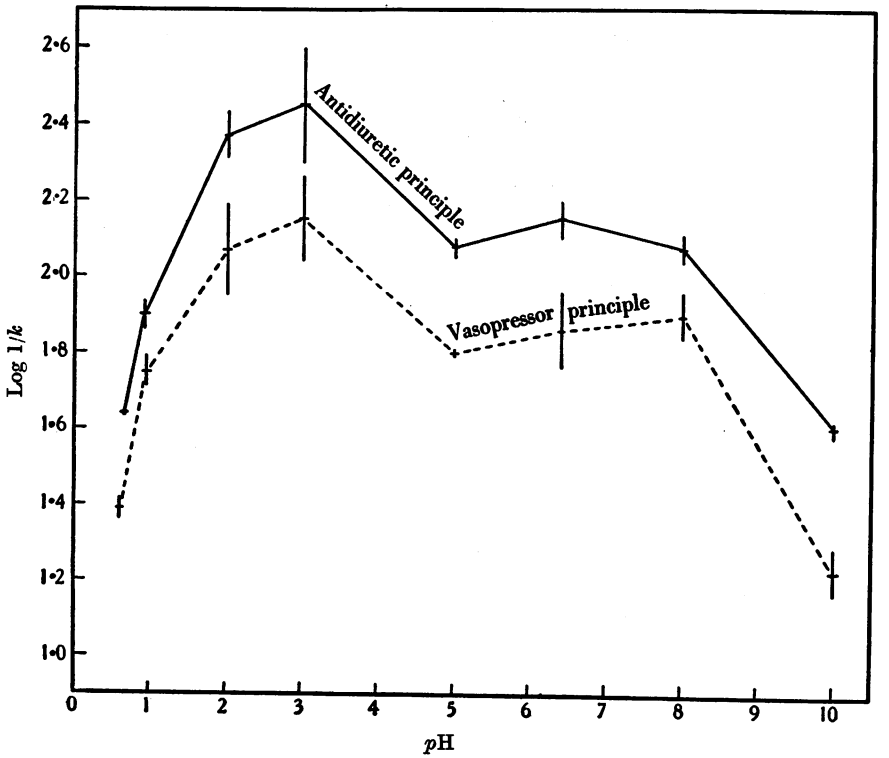


Fig. 1. Comparison between the rate of thermal decomposition of the antidiuretic and vasopressor principles of the posterior pituitary gland between $p$H 0·57 and 10·0. Abscissa, $p$H. Ordinate, log $1/k$, where $k$ is the rate of destruction at 99° C. An increase of log $1/k$ signifies an increase of stability. The duration of heating does not affect the value of $k$. The values of $k$ shown at $p$H 2·0, 8·0, and 10·0 include observations obtained at various periods of heating. —— Antidiuretic principle. ---- Vasopressor principle. The vertical lines indicate the standard error.

an increase of log $1/k$ signifies an increase in stability. Fig. 1 shows the stability curves of the antidiuretic and the vasopressor activities between $p$H 0·57 and 10·0, the antidiuretic principle being more stable over the whole range. The rate of destruction of both principles is not

much altered by shifts of the hydrogen-ion concentration between *p*H 5·0 and 8·0. The maximum stability for the antidiuretic as well as for the vasopressor principle is in the region of *p*H 3·0, i.e. in the same region as that of the oxytocic hormone. The general shape of the stability curves for the oxytocic and the antidiuretic vasopressor fraction is, however, widely different (Fig. 2). The antidiuretic and vasopressor factors are much less sensitive to shifts of the hydrogen-ion concentration

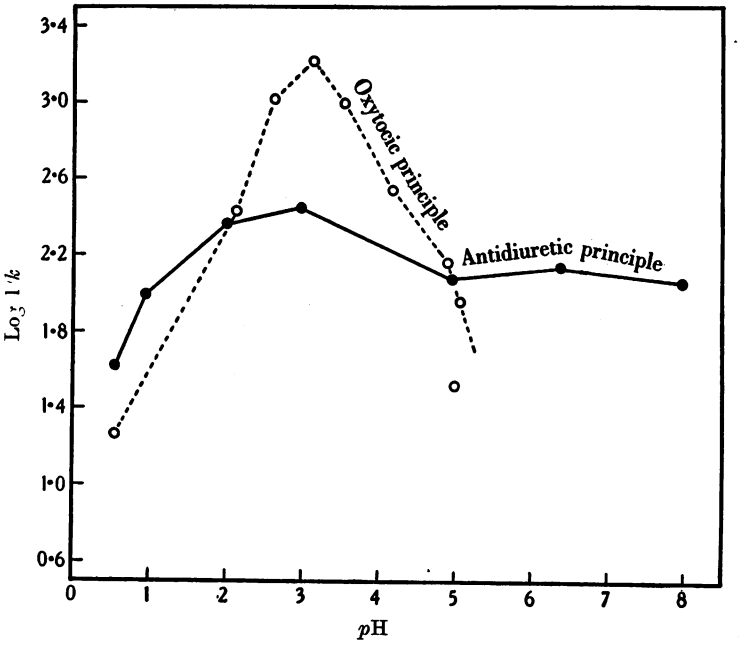


Fig. 2. Comparison between the rate of thermal decomposition of the antidiuretic principle and the oxytocic principle of post-pituitary extracts. Abscissa and ordinate have the same significance as in Fig. 1. The curve for the oxytocic principle was taken from a paper by Gerlough [1930].

than the oxytocic hormone. Judging from the stability curves the oxytocic principle should be more stable in the region of *p*H 2·0 to 4·5, but less stable than the other principles at very high and very low hydrogen-ion concentrations. The rate of decomposition of the oxytocic factor at three values of *p*H was determined to check this conclusion. The determinations (Table II, last column) were only approximate, as sufficient experimental data of other authors [Gaddum, 1930; Gerlough, 1930] are extant. The values for $a-x$ (=percentage of activity left) for the antidiuretic and the vasopressor factors in Table II are the

same as in Table I. The corresponding figures for the oxytocic principles were calculated with the help of Gaddum's nomogram (Table II, penultimate column). Direct observations were made for more convenient times (Table II, last column). The results leave no doubt about

TABLE II. A comparison between the rates of destruction of the antidiuretic, the vasopressor and the oxytocic activities of post-pituitary extracts at various hydrogen-ion concentrations. (Temperature = 99° C.)

| | | Anti-diuretic principle. Activity left %. Direct observations | Vaso-pressor principle. Activity left %. Direct observations | Oxytocic principle. Activity left %. Calculated | Oxytocic principle. Direct observations | | |
|---|---|---|---|---|---|---|---|
| *p*H | Time of heating, min. | | | | Time of heating, min. | Activity left % | Author |
| 0·96 | 180 | 11·5 | 3·1 | 0·01 | 30 | 20·0 | Abel & Nagayama [1920] |
| | | | | | 30 | 22·5 | Heller [1939] |
| 3·0 | 270 | 38·5 | 15·0 | 66·0 | 240 | 86·0 | Gerlough [1930] |
| | | | | | 270 | 90·0 | Heller [1939] |
| 6·4 | 240 | 16·3 | 3·9 | 2·7 | 80 | 17·6 | Gaddum [1930] |
| | | | | | 120 | 10·0 | Heller [1939] |

the above-mentioned differences in the stability of the oxytocic and the other post-pituitary factors.

The difference in the stability of the antidiuretic and the vasopressor principle is much the same at all values of *p*H between *p*H 0·57 and 10·0, but probably somewhat greater at the alkaline end of the scale. The differences in the thermal stability of the two principles can be used to procure solutions of the antidiuretic principle which are practically free of vasopressor activity. By boiling pitressin for 90 min. at *p*H 10·0 solutions can be obtained which contain only about 8 parts of the vasopressor principle to 100 parts of the antidiuretic factor (see Table I).

Such solutions were assayed on normal human beings as well as by the standard test on rats. The subject drank 100 c.c. of water per sq. m. body surface every 15 min. until a "plateau" of excretion was established. The freshly prepared solution of the antidiuretic principle was then injected subcutaneously. Urinary volume, specific gravity and urinary chlorides were determined at intervals of 15 min. The subject had previously been standardized with series of doses of "pitressin", i.e. with a post-pituitary preparation containing equal proportions of antidiuretic and vasopressor activity. The values of $a-x$ (=percentage activity left) for the experiments on human subjects amounted to about 12·5 % as compared with 11·9 % in the rat experiments. Fig. 3 (I) shows the effect of a dose of the "purified" antidiuretic factor where $a-x$ was assumed to be 10 %. A comparison with the effects of the corresponding

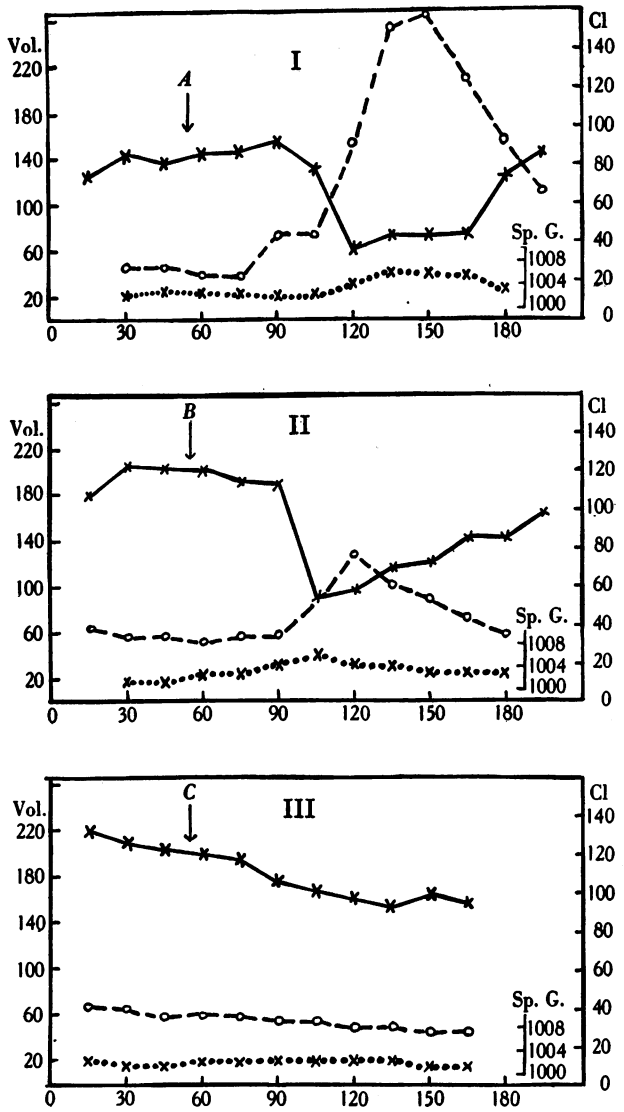


Fig. 3. The effect of a preparation of the "purified" antidiuretic principle on the water diuresis and the urinary chlorides of a normal human subject. Abscissa, time in min. *Vol.* urine flow in c.c. ( × —— × ). Cl, chloride concentration as mg./100 c.c. (o---o). *Sp. G.* specific gravity of urine ( × ··· × ). The subject was a healthy male (33) with a body surface of 1·9 sq. m.

I (3. iv. 39). At *A*, 0·29 c.c. of a preparation of the "purified" antidiuretic principle containing the equivalent of about 10 milliunits pitressin in 0·1 c.c. was injected subcutaneously.

II (5. iv. 39). At *B*, 0·29 c.c. of a solution of pitressin containing 10 milliunits per 0·1 c.c. were injected subcutaneously into the same subject as in Exp. I.

III (7. iv. 39). At *C*, 0·29 c.c. of a solution of pitressin containing 1 milliunit per 0·1 c.c. were injected subcutaneously into the same subject as in Exps. I and II.

dose of pitressin (Fig. 3 (II)) shows that the "purified" antidiuretic factor preparation contains rather more than 10% of the original antidiuretic activity of "pitressin". Previous experiments on cats have shown that such preparations contain less than 1% of the original pressor potency (Table I). The ratio of antidiuretic to vasopressor potency is therefore roughly 10 to 1. Preparations of the "purified" antidiuretic principle have the typical action of crude post-pituitary extracts on water diuresis and on the concentration and the total excretion of urinary chlorides. These actions are not due to the "contamination" by the vasopressor principle. An injection of the amount of pitressin corresponding to the "contaminating" amount of vasopressor principle has no effect on water diuresis or chloride excretion (Fig. 3 (III)).

## Discussion

Different rates of thermal inactivation for the oxytocic and for the antidiuretic-vasopressor fraction of post-pituitary extracts were to be expected. The comparative ease with which these principles can be separated by fractional precipitation [Kamm *et al.* 1928; Stehle, 1933] and by differential adsorption indicates a different chemical constitution of the active principles as do the results of chemical analyses of highly purified preparations of the two fractions [Stehle & Fraser, 1935; Du Vigneaud, Sealock, Sifferd, Kamm & Grote, 1933]. The data presented in this paper show, as expected, marked differences in the stability of the oxytocin and vasopressin fractions, the former being the more stable between *p*H 2·0 and 4·5, but less stable outside this range. The *p*H must, therefore, be specified if a statement about the relative stabilities of these active principles is to be significant.

Several hints have appeared suggesting the existence of separate antidiuretic and pressor hormones in the pituitary gland. Bylsma, Burn & Gaddum [1928], for example, found the relative activities of the antidiuretic, pressor and oxytocic principles to be different in four commercial extracts. Kamm, Grote & Rowe [1931] report the preparation of a "derived" antidiuretic, practically non-pressor substance from post-pituitary extracts. The details of their process of preparation are, however, not given. Several authors [Zondek, 1935; Sulzberger, 1933; Turner, 1936; Böttger, 1936; Fraser, 1936] claimed for their preparation of the melanophore (erythrophore) hormone an antidiuretic effect without the corresponding pressor activity. Several explanations of these findings seem to be possible: (1) The vasopressor substance was originally present

in the anterior and the intermediate lobes (from which they prepared their extracts) but was destroyed or separated from the antidiuretic factor by the chemical processes employed for the isolation of the melanophore hormone. (2) The antidiuretic but not the vasopressor principle is formed by cells of the intermediate and the anterior lobe. (3) Post-pituitary hormones diffuse into other parts of the gland and the antidiuretic factor is preferentially bound. An accumulation of an antidiuretic non-pressor substance in the anterior lobe is also suggested by the work of Richards & Downes [1935]. The data obtained by Noble, Rinderknecht & Williams [1938] in a case of suggested hyperfunction of the posterior pituitary show that an antidiuretic substance is excreted in the urine much in excess of a pressor principle. In view of these findings both the antidiuretic and the pressor assay were used in the present series of experiments to test the stability of the antidiuretic-vasopressor fraction. The results show a different measure of stability for both activities at all values of *p*H investigated. The distribution of the differences throughout the whole range of hydrogen-ion concentrations was uniformly in one direction, i.e. the vasopressor activity was always more quickly destroyed than the antidiuretic factor. Extracts can thus be obtained which exhibit approximately 8 parts of pressor to 100 parts of antidiuretic activity. Such preparations have the typical action of post-pituitary extracts on the water diuresis and on the concentration and total excretion of urinary chlorides. These results suggest the presence in the vasopressin fraction of two chemically very similar principles which, however, hydrolyse at different rates. The difference is possibly due to the blocking of hydrolysis by the different constitution of a group in the molecule of the antidiuretic factor.

## Summary

1. The stability of the antidiuretic and vasopressor activities of post-pituitary gland extracts at hydrogen-ion concentrations ranging from *p*H 0·57 to 10·0 has been determined. The stability of the oxytocic principle was investigated within a more limited range of *p*H.

2. The oxytocic principle is less stable than the antidiuretic or vasopressor activity in the region of strong acidity and strong alkalinity. It is, however, more stable than those activities in the region of *p*H 2·0 to 4·5 (Fig. 2).

3. The antidiuretic factor is more stable than the vasopressor factor at all *p*H values between 0·57 and 10·0 (Fig. 1).

4. By making use of the different stability of the antidiuretic and vasopressor principle, preparations were obtained which contained only about 8 parts of pressor activity to 100 parts antidiuretic activity (Table I).

5. Such preparations have the typical action of post-pituitary extracts on the water diuresis and on the urinary chlorides of normal human subjects (Fig. 3).

6. It is suggested that the antidiuretic and the pressor actions of post-pituitary extracts are due to two chemically different principles.

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