CIV. THE STABILITY OF WATERY SOLUTIONS OF THE OXYTOMIC PRINCIPLE OF THE PITUITARY GLAND.

By JOHN HENRY GADDUM.

From the National Institute for Medical Research, Hampstead, N.W. 3.

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It has long been known that the substance in the posterior lobe of the pituitary gland which produces contractions of the uterus is destroyed by alkalis in the cold [Guggenheim, 1914], is stable in weakly acid watery solution, and is destroyed by boiling in strong acids [Abel and Nagayama, 1920; Dale and Dudley, 1921].

Adams [1917] showed that when weakly acid extracts were heated the oxytomic activity, measured on the guinea-pig's uterus, disappeared from the solution in such a way that its rate of disappearance at any moment was proportional to the activity present at that moment. The solutions were much more stable at p_H 3 than at p_H 5.

This paper deals with a number of determinations which have been carried out, under my direction, by my assistant, L. S. Drewell, with the object of obtaining more complete quantitative knowledge of the stability of this substance. Buffered extracts were prepared from the British standard preparation of the posterior lobe, heated for different times and assayed. The tests were carried out on the virgin guinea-pig's uterus by the method described by Dale and Laidlaw [1912], the details of the apparatus being those described by Burn and Dale [1922]. The results are comparable with, and in many ways similar to, the results obtained by Krogh and Hemmingsen [1928] in their study of the action of heat on insulin. For the sake of uniformity the notation of these authors has been adopted in the present paper.

METHODS.

Heating was carried out in ampoules of hard glass, sealed and placed in a water-bath, the temperature of which was maintained constant. This was effected either by means of a toluene regulator, or when, as in most cases, temperatures near to 100° were required, by boiling the water and maintaining its level with an overflow tube—a procedure which produced a constant temperature of about 99° .

Over a wide range of p_H it would be inconvenient to study the rate of destruction at low temperatures, since it would be necessary to keep the

extracts for many months in order to get any considerable destruction. The effect of p_H on the stability has, therefore, been most completely studied at 99°. The application of these results to the stability at lower temperatures is discussed below.

The hydrogen ion concentration of the solutions was buffered where possible with NaOH and NaH_2PO_4 in different proportions [Prideaux, 1911]. Potassium was excluded from the solutions because it would have interfered with the final assay. Between p_H 3 and p_H 5.5, where the buffering of phosphates is slight, Walpole's HCl-acetate mixtures were used. For one measurement at p_H 8.5 Palitsch's boric acid-borate mixture was used [Clark, 1928].

The total concentration of phosphate, acetate or borate was $N/7$, so that these solutions were approximately isotonic with blood. The p_H of the solutions was determined colorimetrically, and in most cases electrometrically, and was found not to differ widely from the p_H calculated from the weights of the salts used in preparing the buffer. Solutions of p_H 1 and p_H 2.1 were obtained by diluting a standard extract in 0.25 % acetic acid with HCl, so that the final concentration of HCl was $N/10$ and $N/100$. Similar solutions in N HCl, N NaOH and $N/10$ NaOH were taken to represent p_H 0, p_H 14, p_H 13. In these very acid and alkaline solutions the initial concentration of the pituitary extract was 1 unit per cc. In all the other experiments it was 2 units per cc.

The measurements of p_H were made at room temperature, whilst most of the experiments were carried out at about 100°. It is improbable that this circumstance introduced any considerable error since the p_H of phosphate buffers [Walbum, 1920], acetate buffers [McIntosh and Smart, 1920; Clark, 1928], and strong acids and bases are said to be practically unaffected by changes of temperature. In all cases the solutions were made faintly acid for testing.

In the case of the observations between p_H 3 and p_H 7.25 extracts were made by boiling the standard powder in the buffer solution for 10 minutes, cooling and filtering. The filtrate was heated to a known temperature for a known time and its activity then determined by comparison with a standard extract in 0.25 % acetic acid. The assumption was made that the extraction was complete, and a small correction was applied for the loss of activity during the initial 10 minutes' boiling. In the case of the observations at p_H 11.1 it was possible in the light of more complete knowledge to adopt a more satisfactory procedure. The extraction was carried out in a solution of acid sodium phosphate. A portion of this extract was kept as a standard solution and another portion was treated with a suitable quantity of NaOH, maintained at a constant temperature for a definite time and then faintly acidified with HCl, cooled, made up to a known volume and tested.

The course of the reaction.

If Adams's [1917] conclusions as to the course of the reaction are correct, the velocity of the change may be measured, like that of the destruction of

insulin, in terms of a constant k which is equal to the rate of fall per hour of $\log_{10} C$ (where C is the concentration of the active principle).

The fact that k remains constant has been confirmed by carrying out two or more experiments at each of seven different values of the p_H in which the time of heating was varied. These results are shown with others in Fig. 1. The values of k showed no regular tendency either to increase or decrease and the variations which occurred were no larger than might have been expected.

Fig. 1. Abscissae— p_H . Ordinates— $\log k$ —where k is rate of destruction at 99° . Dots represent direct observations. Crosses represent results calculated from observations at 25° .

In order to determine k under any particular circumstances it is desirable that the reaction should continue until destruction is nearly complete, since in this case the error of the assay has less effect on the result. The experiments have therefore been planned so that the percentage destruction was always over 50 and usually about 90, but it was, of course, not possible to ensure uniformity in this respect.

The effect of temperature.

The variation of the velocity of the reaction with the absolute temperature T is defined with sufficient accuracy by Arrhenius's equation

$$k_1 = k_0 e^{\frac{\mu}{R} \left(\frac{T_1 - T_0}{T_1 T_0} \right)},$$

where k_1 and k_0 are the rates of destruction at absolute temperatures T_1 and T_0 . Measurements have been carried out at p_H 6.55 and at p_H 11.1 and the results are recorded in Table I. In each case three estimates of the constant μ in the above equation have been obtained by combining the result of the experiment at 99° with the results of those at lower temperatures.

Table I.

Temp. °	Time of heating (hrs.)	Activity left %	k (corrected)	μ
p_H 6.55				
99	1.33	17.6	0.503	—
80	16.0	1.39	0.111	20,900
60	42.0	11.6	0.02	20,500
50	69.2	33.8	0.0056	22,100
p_H 11.1				
99	0.1	8.0	10.97	—
74.5	0.783	3.0	1.94	18,200
50	10.68	3.3	0.142	21,300
25	47.67	33.3	0.01	21,000

The variations in μ are irregular and are not large considering the methods used. For the purpose of calculation it is assumed that μ has a constant value of 21,300—an estimate of its value which was obtained graphically. These observations have also been taken to justify the assumption that the value of μ is independent of the p_H . The absolute value of μ is low compared with the values obtained for the spontaneous disintegration of various more complex substances [Arrhenius, 1915]. It is lower than the value obtained for the destruction of insulin—28,300 [Krogh and Hemmingsen, 1928], but it is high compared with the values met with in most chemical reactions.

The effect of hydrogen ion concentration.

The variation of k with the p_H is large and in order to get all the readings on the same curve the p_H has been plotted in Fig. 1 not against k but against its logarithm. The readings shown by dots were obtained in the boiling water-bath, the temperature of which was 99°. The readings shown by crosses were obtained at 25° and the values which would have been obtained at 99° were calculated from the data given above and plotted.

In the region of strong acidity the results fall on a straight line the slope of which indicates that the rate of destruction is directly proportional to the hydrogen ion concentration.

In the region of strong basicity the results also fall on a straight line, but in this region the rate of destruction is proportional to $[C_{OH}]^{0.73}$.

The effect of variation of the p_H on the stability is at a minimum between p_H 7 and 8.

For the sake of comparison similar curves for insulin and for acetylcholine are shown. The former is taken from the paper by Krogh and Hemmingsen [1928]. The latter was obtained by combining some observations published by Hofmann [1930] with some unpublished observations which Dr K. Mathes has kindly allowed me to use.

One experiment was carried out to test the possibility that the rate of destruction was considerably affected by the amount of salt in the solution. It was found that when the concentration of phosphate at p_H 7.2 was divided by ten, the other factors remaining constant, the value of $\log k$ fell by 0.071.

It was concluded that if any salt effect is present it is small compared with the other factors and it has been neglected.

All the results are summarised in Fig. 2.

Fig. 2. $\log k$ is determined on scale B by aligning the p_H on scale A with the temperature on scale D. The percentage destruction is obtained on scale E by aligning $\log k$ on scale B with the time on scale C.

The use of this nomogram is most readily explained by taking an example. The p_H of the standard extract in 0.25 % acetic acid is about 4 [Kamm *et al.*, 1928]. If a thread be stretched from p_H 4 on scale *A* to 100° on scale *D* it will be found that when this solution is boiled $\log k = \bar{2}.9$. By stretching the thread through $\bar{2}.9$ on scale *B* and 10 minutes on scale *C* it is found that when this solution is boiled for 10 minutes about 3 % of the oxytocic principle is destroyed. Similarly, if this solution is kept for a year at 0° the loss of activity is about 5 % and would probably not be detected. This last conclusion has been confirmed.

It should be emphasised that these results were obtained with extracts of a powder which contained all the substances present in the posterior lobe of the pituitary which are soluble in water, but not in acetone. It is possible that preparations that are either more or less pure may give different results. It has, however, been found that the stability of the preparation known as "pitocin" or "oxytocin" [Kamm *et al.*, 1928], at p_H 7 and 99° , is identical, within the error of the method, with that of the oxytocic substance in the comparatively crude extracts used in the present experiments.

SUMMARY.

The rate of destruction of the substance in watery extracts of the standard preparation of the posterior lobe of the pituitary gland, which causes contraction of the guinea-pig's uterus, has been measured at different hydrogen ion concentrations and at different temperatures. The results are summarised in a nomogram (Fig. 2) which gives the amount of destruction to be expected when a solution of any given p_H is heated for any given time at any given temperature.

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