Thus, we conclude that the rather small differences evidenced in the hydrolysis of acetylcholine and a non-ionic ester such as methyl acetate fit in with what we know about the slow steps and activated complexes in these reactions.

SUMMARY

1. The kinetics of hydrolysis of acetylcholine bromide by hydrogen ion and hydroxyl ion have been followed by conventional methods. The

bimolecular velocity constants respectively are, in $\text{l.mole}^{-1}\text{sec.}^{-1}$,

$$k_{\text{H}^+} = 4.7 \times 10^7 e^{-16570/RT}$$

and

$$k_{\text{OH}^-} = 1.0 \times 10^9 e^{-12180/RT}.$$

2. These data are very similar to those for non-ionic esters, such as methyl acetate. The A factor for k_{H^+} is about 5 times smaller than for such esters, while for k_{OH^-} it is about 20 times larger. These differences find an explanation in the ionic charge of the activated complex.

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Acetylcholine

2. THE HEAT OF HYDROLYSIS

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A recent hypothesis of nerve conduction (Nachmansohn, 1945, 1950) supposes that the depolarization of the nerve membrane is brought about by acetylcholine, and that the polarized state is renewed by the hydrolysis of the acetylcholine by its esterase. The synthesis of acetylcholine is supposed to involve the high-energy phosphate bond in acetyl phosphate, in turn synthesized from creatine phosphate. In support of these views, there is the evidence that in *Electrophorus electricus* the energy release calculated for the observed creatine phosphate hydrolysis approximately equals the energy of electrical discharge. Nachmansohn has, however, pointed to a discrepancy in the energy balance of the postulated recovery process. Meyerhof & Schulz (1935) found $\Delta H_1^0 = -10700$ cal./mole for the neutral hydrolysis of creatine phosphate, from which Lipmann (1941) has calculated $\Delta G_1^0 = -10000$ cal. Lipmann (1946) subsequently corrected his calculated value for the free energy of hydrolysis of phosphopyruvate by 4700 cal. and since his creatine phosphate figure was based on the phosphopyruvate figure, it is appropriate to apply this correction to creatine phosphate, which now

brings its free energy of hydrolysis to -14700 cal. Within the accuracy of the calculations this may be taken as 15000 cal. If the ΔG_1^0 for acetylcholine synthesis is similar to the values for other esters, it will be close to zero cal. and there will, therefore, be a loss of 15000 cal./mole in the recovery process. As a first step in an investigation of ΔG_1^0 , we have measured the heat of alkaline hydrolysis $-\Delta H_2^0$, from which we can calculate the value for hydrolysis by neutral molecules $-\Delta H_1^0$, and thus obtain an estimate of ΔG_1^0 . Later we hope to obtain the equilibrium constant K , and thus $\Delta G_1^0 = -RT \ln K$, using hydrogen ion as a catalyst. Since this work was completed, Hestrin (1950) has published a value for K and ΔG_1^0 , using the enzyme-catalysed reaction. We shall consider this work in the discussion.

Thermochemistry of the hydrolysis

The hydrolysis of acetylcholine by cholinesterase at physiological pH may be written:

The enzyme-catalysed reaction, with the enzyme preparations available to us, was rather too slow for the vacuum-jacket calorimeter used in this paper. We have accordingly measured the heat of hydrolysis by alkali,

If we have the quantity ΔH_3^0 defined by the equation

$$\begin{aligned} \text{then} \quad \Delta H_1^0 &= \Delta H_2^0 + \Delta H_3^0, \\ &= \Delta H_2^0 + 13\,490 \text{ cal.} \end{aligned}$$

The ΔH_3^0 value is taken from the compilation of Everett & Wynne-Jones (1939).

Since choline is a strong base, no correction is necessary for the formation of undissociated $\text{HOCH}_2\text{CH}_2\text{N}(\text{CH}_3)_3\text{OH}$. The dissociation constant for choline, reported by Price & Lewis (1933) as 8.71×10^{-6} at room temperature, has been found to be in error, in agreement with the general rule that all quaternary nitrogen hydroxides are completely dissociated.

EXPERIMENTAL

Method. The calorimeter used has been described in another paper (Dilke, Eley & Sheppard, 1950). A Dewar vessel was used, temperature being measured by the resistance change in a thermistor, and the water equivalent obtained by an electrical resistance coil. A quantity of acetylcholine was weighed out and dissolved in 60 ml. water in the calorimeter. The delivery pipette contained 5 ml. of 1.3 N-NaOH, which always sufficed to give an excess of alkali over acetylcholine. The calorimeter was immersed in a 25° thermostat, and when temperature equilibrium of the contents had been obtained the NaOH was blown over from the pipette into the acetylcholine and the resistance-time record taken with the thermistor. Under these conditions, the hydrolysis reaction went virtually to completion. Finally, the water equivalent was determined. The cooling corrections were made by White's method as outlined in the paper quoted.

The procedure was to hydrolyse different weights of acetylcholine w , so as to give heats varying from 5 to 35 cal., which could be measured to an accuracy of 0.1 cal. If M is the molecular weight of acetylcholine, Q its heat of hydrolysis per mole (i.e. $-\Delta H_2^0$), then the actual heat x evolved in any experiment will be

$$x = D + (Q/M)w,$$

where D should be the heat of dilution of the NaOH solution in 60 ml. water. Thus, from the slope of the straight line connecting x and w , we determine $Q = -\Delta H_2^0$.

Materials. Acetylcholine bromide. The B.D.H. (British Drug Houses Ltd.) product recrystallized from ethanol as colourless needles, m.p. 142–143°.

Acetyl- β -methylcholine chloride. The Savory and Moore product (Amechol) was washed with ether, m.p. 172°.

Benzoylcholine chloride. This was prepared from β -chloroethyl benzoate and trimethylamine by the method recommended by Easson & Steadman (1936). The yield was 50%, m.p. 204°.

Choline bromide. The B.D.H. product was recrystallized from ethanol.

Fig. 1. Heat evolved, as a function of weight of choline derivative hydrolysed. ○, acetyl- β -methylcholine chloride; □, benzoylcholine chloride; △, acetylcholine bromide.

RESULTS

The heat of dilution, D , was found to be negligible in a separate experiment. The results of experiments with acetylcholine bromide, Amechol and benzoylcholine chloride are shown in Fig. 1. In each case the best straight line has been drawn through the points by the method of least squares, and the probable error has been calculated for the value of the heat Q . In the case of benzoylcholine chloride the points lie very well on a straight line and the error is less than 0.1 k.cal./mole and has been omitted. In the case of choline bromide the heat of mixing with sodium hydroxide was found to be negligible.

Hydrolysis of choline chloride. A salt of a strong acid with a weak base (basic constant K_b) gives an acid solution. If C be the concentration of salt, K_w the dissociation constant of water, a measurement of the pH will give a value of K_b , using the equation

$$\text{p}K_b = \text{p}K_w - \log C - 2\text{pH}.$$

Thus, Price & Lewis (1933) found a choline chloride solution of $C = 0.038$ mole/l. to have a pH of 5.13, leading to $\text{p}K_b = 5.16$. These results were obtained with both gold-quinhydrone and glass electrodes, and it is not easy to see where the error lies. In our case, we found the initial pH to be quite

near to neutrality, 0.043N solution having a pH of 6.88. However, there is a difficulty in that the pH of choline chloride solutions from which carbon dioxide is excluded tends to drift down to 5.7. A hydrolysis reaction may be responsible, as we have noticed trimethylamine on exposing wet crystals to the air, thus possibly

DISCUSSION

The results are collected in Table 1.

Table 1. *Heats of alkaline ($-\Delta H_2^0$) and neutral ($-\Delta H_1^0$) hydrolysis*

Ester	$-\Delta H_2^0$ (kg.cal./mole)	$+\Delta H_1^0$ (kg.cal./mole)
Acetylcholine bromide	12.4 ± 0.2	1.09 ± 0.2
Amechol	12.8 ± 0.7	0.69 ± 0.7
Benzoylcholine bromide	12.2	1.29
Choline bromide	0.0	—

The decrease in experimental error over the series Amechol, acetylcholine, benzoylcholine is noteworthy. This order is, in fact, the order of decreasing hygroscopicity, so the error probably arises in weighing out the substances for hydrolysis. In all cases, the time of reaction is 1–1.5 min., and so the error cannot arise from differences in cooling corrections. It is hoped at some later date to obtain values of ΔH_1^0 from enzyme-catalysed hydrolyses in an adiabatic calorimeter. Both the heat of mixing of choline bromide and sodium hydroxide and the pH measurements on choline chloride solution agree in classifying choline as a strong base, so no correction to ΔH_2^0 is necessary for incomplete dissociation of the choline hydroxide.

The Gibbs free energy change ΔG_1^0 (Hestrin writes it ΔF) for neutral hydrolysis is given by

$$\Delta G_1^0 = -RT \ln K,$$

where $K = \frac{[\text{CH}_3\text{COOH}][\text{ROH}]}{[\text{CH}_3\text{COOR}][\text{H}_2\text{O}]}$. Since Hestrin

(1950) finds $K = 4$ at $T = \text{about } 296^\circ$, $\Delta G_1^0 = -820$ cal./mole, and hence the entropy change ΔS_1^0 is

$$\Delta S_1^0 = \frac{\Delta H_1^0 - \Delta G_1^0}{T} = 6.4 \text{ cal./deg.mole.}$$

This is a not unreasonable value. The value of -820 cal./mole for ΔG_1^0 corresponds to the same standard state for all reactants, e.g. 1 molal or 1 molar. The actual figure given by Hestrin is for a standard state for the water (55.5M), and for the other reactants at 1M concentration.

The neutral hydrolysis of ethyl acetate, classically investigated by Berthelot and St Gilles, does not go as far as that of acetylcholine. Thus, for this case, $K = 0.25$ (cf. Parks & Huffman, 1932) and thus $\Delta G_1^0 = 820$ cal./mole. The heat of this hydrolysis does not seem to have been measured, so that we cannot calculate ΔS_1^0 for comparison with the above value. The error involved in heats of combustion is too large to make it worth while to use these data to calculate ΔH_1^0 for comparison with acetylcholine.

To summarize, with a reasonable value for the entropy of hydrolysis, our values of ΔH_1^0 will lead to a value for ΔG_1^0 , the free energy change of hydrolysis, in the neighbourhood of zero, in agreement with the value recently published by Hestrin (1950). This work thus confirms the suggestion made by Nachmansohn (1945) that a synthesis of acetylcholine, starting with creatine phosphate, will involve a free energy loss of about 14000 cal./mole.

We hope to give further data on the free energy and entropy of the acetylcholine hydrolysis when we have completed our investigations of the equilibrium constant of the acid-catalysed reaction.

SUMMARY

1. Values of ΔH_{298}^0 have been measured for the alkaline hydrolysis of acetylcholine bromide and related substances. These lead to small values for the neutral hydrolysis, similar to those found for ordinary esters, in contrast to the high values characteristic of creatine phosphate hydrolysis.

2. There will be a loss of free energy of about 14000 cal. in a postulated synthesis of acetylcholine starting from creatine phosphate.

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