DET KGL. DANSKE VIDENSKABERNES SELSKAB MATEMATISK-FYSISKE MEDDELELSER, BIND XXIV, NR. 14

THE INACTIVATION VELOCITY OF PENICILLIN G BY ACIDS AS A FUNCTION OF THE SALT CONCENTRATION

BY

ROLF BRODERSEN



KØBENHAVN I KOMMISSION HOS EJNAR MUNKSGAARD 1948 In previous publications (BRODERSEN, 1 and 2, 1947) the rate of I inactivation of G-penicillin was examined with regard to its dependence on the hydrogen ion concentration and the temperature. The present investigation deals with the variation of the mactivation velocity with the salt concentration.

I. The Mechanism of the Salt Effect.

According to the previously propounded theory of the mechanism of the reaction the course of the inactivation is represented by the following diagram:

$$Pn^{-}H^{+} \rightarrow P' \qquad I$$

$$Pn^{-} + H^{+} \rightleftharpoons HPn \rightarrow P' \qquad II$$

$$+$$

$$H$$

$$\downarrow\uparrow$$

$$HPnH^{+} \rightarrow P' \qquad III$$

The irreversible reactions denoted by single arrows are ssumed to be velocity determining, while the protolytic equibrium reactions denoted by the double arrows are assumed to poceed at such high velocities that equilibrium is established nomentarily. In case of great hydrogen ion concentrations rection III will become dominating. It is not possible on the has of any previous investigations to ascertain whether rection I or reaction II is dominating in case of small hydrogen in concentrations.

Printed in Denmark. Bianco Lunos Bogtrykkeri

1*

We shall return to this question later on. Meanwhile we shall, however, omit consideration of the possibility of reaction I and at the same time assume that the concentration of Pn^-H^+ is small when compared to the concentration of HPn.

If h_0 denotes the velocity of reaction II, and if the other designations are the same as those used previously, the velocities of reactions II and III respectively will be:

$$h_0 = k_0 c_0; \qquad h_+ = k_+ c_+.$$

The total velocity will be equal to the sum of these two figures

$h = h_0 + h_+ = k_0 c_0 + k_+ c_+$

For the equilibriums of the reaction scheme we have:

$$f_{H^{+}} \frac{f_{-}}{f_{0}} c_{H^{+}} \frac{c_{-}}{c_{0}} = K_{(0)}$$
$$f_{H^{+}} \frac{f_{0}}{f_{+}} c_{H^{+}} \frac{c_{0}}{c_{+}} = K_{(+)}$$

Nr. 14

where f denotes the activity coefficients, while $K_{(0)}$ and $K_{(+)}$ are the thermo-dynamic dissociation constants which are independent of the salt concentration.

For stoichiometric reasons we have

$$c = c_0 + c_- + c_+$$
.

As c+ is small compared to c, we have with approximation

$$\mathbf{c} = \mathbf{c}_0 + \mathbf{c}_-.$$

From this we can find c_{-} and substitute in (3), so that we have:

$$c_{0} = \frac{f_{H^{+}} \frac{f_{-}}{f_{0}} c}{K_{0} + f_{H^{+}} \frac{f_{-}}{f_{0}} c_{H^{+}}} c_{H^{+}}$$

 c_+ may now be found by substituting (7) in (4)

$$c_{+} = \frac{(f_{H^{+}})^{2} \frac{f_{-}}{f_{+}} c}{K_{(+)} \left(K_{(0)} + f_{H^{+}} \frac{f_{-}}{f_{0}} c_{H^{+}} \right)} (c_{H^{+}})^{2}$$
(8)

5

(7) and (8) are substituted in (2), and the velocity constant of the whole reaction is found to be:

$$\mathbf{k} = \frac{\mathbf{h}}{\mathbf{c}} = \left(\mathbf{k}_{0} + \frac{\mathbf{k}_{+}\mathbf{f}_{0}}{\mathbf{K}_{(+)}\mathbf{f}_{+}}\mathbf{f}_{\mathbf{H}^{+}}\mathbf{c}_{\mathbf{H}^{+}}\right) \frac{\mathbf{c}_{\mathbf{H}^{+}}}{\frac{\mathbf{f}_{0}}{\mathbf{f}_{-}\mathbf{f}_{\mathbf{H}^{+}}}\mathbf{K}_{(0)} + \mathbf{c}_{\mathbf{H}^{+}}}.$$
 (9)

It is possible to calculate the salt effect from this equation if the variation with the salt concentration of the quantities on the right side of the equation is known.

If in stead of assuming reaction II to take place, we consider the inactivation to proceed according to reaction I, i. e. through the ampho-ion Pn^-H^+ , we find by a deduction analogous to the one particularized above, the following equation:

$$=\frac{\mathbf{h}}{\mathbf{c}} = \left(\frac{\mathbf{k}_{\pm}}{\mathbf{K}_{(\pm)}} \cdot \frac{\mathbf{f}_{0}}{\mathbf{f}_{\pm}} \mathbf{K}_{(0)} + \frac{\mathbf{k}_{+}}{\mathbf{K}_{(+)}} \cdot \frac{\mathbf{f}_{0}}{\mathbf{f}_{+}} \mathbf{f}_{\mathbf{H}^{+}} \mathbf{c}_{\mathbf{H}^{+}}\right) - \frac{\mathbf{c}_{\mathbf{H}^{+}}}{\frac{\mathbf{f}_{0}}{\mathbf{f}_{-} \mathbf{f}_{\mathbf{H}^{+}}} \mathbf{K}_{(0)} + \mathbf{c}_{\mathbf{H}^{+}}} \cdot (10)$$

II. The Salt Effect in Case of Small Salt Concentrations.

Theory.

In the equations (9) and (10) k_0 , k_+ , and k_{\pm} denote the elocity constants for the velocity determining reactions. Such eactions must be supposed to be brought about by collisions even water molecules and penicillin molecules.

As the water molecules are without electric charge the velocity f the reaction will be independent of the salt concentration at mall values of the ionic strength (BRØNSTED (1924) and CHRI-TIANSEN (1924)), for which reason k_0 , k_+ , and k_{\pm} may here econsidered to be constant. The values of these quantities at in ionic strength of 0 we shall denote $k_{(0)}$, $k_{(+)}$, and $k_{(\pm)}$. It ippears from Kirkwood's equation (1924) for the activity coef6

ficient of an ion with both positive and negative charges that the activity of an ion in a diluted salt solution is approximately constant even if an equally large number of positive and nega tive charges are added to the charge of the ion. The activity coefficient is thus chiefly determined by the surplus number of positive and negative charges so that it is possible to consider $f_0 = f_+$.

As $K_{(0)}$ and $K_{(+)}$ are independent of the salt concentration, it is seen that for diluted solutions the salt effect on the inactivation of penicillin must be found to be the same whether we use equation (9) or equation (10). We shall in the following distribution f_1 can be calculated from Poisson-Boltzmann's equation cussion apply equation (10).

We have hitherto assumed that at hydrogen ion concentrations about 10^{-7} m penicillin exists as a monovalent, negative ion, Pn which in acid solution combines with a hydrogen ion and forms the uncharged penicillin molecule HPn. For the purpose of the previous investigations which dealt with the rate of inactivation at constant salt concentration it is, however, of no importance whether HPn is assumed to be without charge or having a positive or negative charge or charges, as long as Pn⁻ is considered to have one negative charge more than HPn. The results will be independent of these assumptions, and it is therefore also impossible to draw any conclusions from these experiments with regard to the total charge of penicillin.

It is quite otherwise when the investigation deals with the effect of the salt concentration on the reaction. In this case the magnitude of the total charge will be of decisive importance as the calculated salt effect will vary according to the assumed num ber of charges of HPn.

In sufficiently diluted solutions it is possible to calculate the salt effect on the velocity of the reaction corresponding to different charges of HPn in the following manner.

If f_n denotes the activity coefficient of an ion of the valency i we have:

$$\mathbf{f}_0 = \mathbf{f}_{\pm}; \qquad \mathbf{f}_{\mathbf{H}^+} = \mathbf{f}_1.$$

If the valency of HPn is referred to as z, we have:

$$f_+ = f_{z+1};$$
 $f_- = f_{z-1}.$

 f_0 in (10) is substituted by f.

Nr. 14

Nr. 14

$$= \left(\frac{\mathbf{k}_{(\pm)}}{\mathbf{K}_{(\pm)}} \mathbf{K}_{(0)} + \frac{\mathbf{k}_{(+)}}{\mathbf{K}_{(+)}} \cdot \frac{\mathbf{f}_{z}}{\mathbf{f}_{z+1}} \mathbf{f}_{1} \mathbf{c}_{\mathbf{H}^{+}}\right) - \frac{\mathbf{c}_{\mathbf{H}^{+}}}{\frac{\mathbf{f}_{z}}{\mathbf{f}_{z-1}} \mathbf{f}_{1}} \mathbf{K}_{(0)} + \mathbf{c}_{\mathbf{H}^{+}}}$$
(13)

If we now substitute $f_n = f_1^{(n^2)}$, we have:

$$= \left(\frac{\mathbf{k}_{(\pm)}}{\mathbf{K}_{(\pm)}} \mathbf{K}_{(0)} + \frac{\mathbf{k}_{(+)}}{\mathbf{K}_{(+)}} \mathbf{f_1^{-2z}} \mathbf{c_{H^+}}\right) \frac{\mathbf{c_{H^+}}}{\mathbf{f_1^{2z-2}} \mathbf{K}_{(0)} + \mathbf{c_{H^+}}}.$$
 (14)

$$-\log f_{z} = \frac{z^{2} A \sqrt{J}}{(DT)^{\frac{3}{2}}}$$
(15)

where A = 1.82 and D = 76 at 30°C, which for z = 1 gives

 $-\log f_1 = 0.52 \, \text{J}$ (16)

where J is the total ionic strength of the solution.

Equations (14) and (16) make it possible to calculate the dependence of the rate of inactivation on the salt concentration for different values of z if the hydrogen ion concentrations and the other quantities of equation (14) are known.

Experiments and Calculation.

The velocity constants of the inactivation of penicillin G were etermined at 30° C at different concentrations of sodium chloride, me measurements being performed according to previously described methods.

Three series of experiments were carried out-at the hydrogen on concentrations 0.024, 0.0024 and 0.0004 m respectively. aluted hydrochloric acid was used as buffer medium during the ist two series of experiments and diluted acetic acid during e last one. Ordinary glycine and acetate buffers, the buffering apacity of which is greater, were not convenient as the experiments were to be carried out at low values of the ionic strength. he hydrogen ion concentrations of the solutions used could be

Nr. 14 Nr. 14

reproduced with an accuracy of 0.01-0.02 p_H-units and did not change measurably during the reaction.

In diluted hydrochloric acid solutions the hydrogen ion concentrations were observed to remain unchanged when penicillinhad been added and to be independent of addition of salt. This was verified by electrometric measurement by means of a glass electrode, the solutions being compared to hydrochloric acid solutions of known concentrations with the same sodium chloride concentrations.

During the third series of experiments, which was performed in 0.0095 m acetic acid, it was observed that the hydrogen ion concentration decreased somewhat when penicillin was added and it is likewise necessary here to allow for the fact that dis sociation equilibrium of acetic acid is sensitive to salt. The hydrogen ion concentration must therefore be determined for each salt concentration during these experiments. This may be done by calculation or experimentally by comparison with hydro chloric acid-sodium chloride solutions. As it is impossible however, to use the experimental method at very small ionic strengths the hydrogen ion concentration has been determined at various larger salt concentrations both by calculation and experimentally. The calculated figures have been found to conform accurately to the experimentally determined results, and all other hydrogen ion concentrations have subsequently been determined by calculation in the following manner.

By addition of penicillin, which in all the experiments deal with here was used in an initial concentration of 10^{-5} m, a reduction of the hydrogen ion concentration is caused which, on the basis of the law af mass action, may be calculated to correspond to a consumption of about $17 \cdot 10^{-5}$ m hydrogen ion. This consumption is constant at different salt concentrations and may be imagined to be caused by the fact that the penicillin employed contains a little bicarbonate. The law of mass action for acetic acid

$$K_{(EH)} = \frac{f_{H^+}f_{E^-}}{f_{EH}} \cdot \frac{c_{H^+}c_{E^-}}{c_{EH}}$$

may now be used for calculating the hydrogen ion concentration as we have

$$c_{E} + c_{EH} = 0.0095;$$
 $c_{E} - - c_{H} = 0.00017$

 $K_{(EH)} = 1.750 \cdot 10^{-5}$ at 30° C. (see HARNED & OWEN, 1934), when, as above, we substitute

$$f_{\rm EH} = 1;$$
 $f_{\rm E} - = f_{\rm H^+} = f_1$

where f_1 is calculated from (16).

The quantities $\frac{k_{(+)}}{K_{(+)}}$, $\frac{k_{(\pm)}}{K_{(\pm)}}$, and $K_{(0)}$ may be found in the following way:

The velocity constant k was determined for an ionic strength of 0 in the three experimental series by extrapolation. The following values were found:

By substituting these three pairs of figures in (10) we get hree equations in which the three quantities which we are trying to determine are the only unknown quantities as all the activity





Nr. 14

(18)

coefficients are unity. These three equations can be solved by an a solution of higher acidity equilibrates a molecule without trial and error, whereby we obtain:

$$\frac{k_{(\pm)}}{K_{(\pm)}} = 6.0$$
 $\frac{k_{(\pm)}}{K_{(\pm)}} = 25.3$ $K_{(0)} = 0.00100$

It is now possible by means of equations (14) and (16) to calculate the relationship between the velocity constant and the ionic strength for different charges of the molecule HPn. The results appear from figs. 1-3 in which the fully drawn curves represent these relations. The points marked with circles represent the values determined experimentally.



Fig. 2. The salt effect on the inactivation process in hydrochloric acid. The abscissa is the square root of the ionic strength. $C_{\mu+} = 0.0024$.

Fig. 3. The salt effect on the inac tivation process in 0.0095 m acet acid. The hydrogen ion concentration varies with the ionic strength.

Conclusion.

The positions of the experimentally determined points as compared to the calculated lines show that z is equal to 0, i.e. that at hydrogen ion concentrations about 10^{-5} the G-penicilia preferably occurs as a single ion with one negative charge, when any charge.

In fig. 2 it is, however, impossible to distinguish with certainty between the two possibilities z = 0 and z = 1. The mean error If the experimental determination of log k in these experiments amounts to 0.02-0.03, so that only the point representing J = 0.3can be said to show a greater deviation from the line z = 0 than may be explained by accidental variations. The theory on which the calculation of the curves is based is, however, not so accurate or this ionic strength that any importance can be attached to he deviation.

This result with regard to the charge of the penicillin molethe is in conformity with the results which we should have expected to obtain according to the structural formula for peni-Illin propounded by British and American scientists (Committee Medical Research and the Medical Research Council, 1945). The partition coefficient of penicillin in water and ether is about unity for $c_{H^+} = about 10^{-4}$, decreases at higher hydrogen on concentrations and increases at lower hydrogen ion concentrations. From this one might be tempted to conclude that penicillin carries the number of charges indicated by the above experiments on the salt effect. It is, however, impossible to draw my definite conclusions without a more detailed knowledge of e numerical variation of the partition coefficient.

By means of the formulae stated above it is possible to callate the dependence of the rate of inactivation of G-penicillin the concentration of salts in the solution corresponding to alues of the ionic strength below about 0.1.

The Salt Effect in Case of Higher Salt Concentrations.

Theory.

The equations (9) and (10), which apply to each of the two ossible inactivation mechanisms, have been deduced irrespective the ionic strength. We may therefor use these equations as basis for the calculation of the effect of salt in solutions of gher concentrations as well. In the following we shall

Nr. 14 Nr. 14

(19)

(20)

(21)

discuss the possibility of an approximate calculation of the quantities in question at higher salt concentrations.

Suppose the ion PnH⁺ is activated by collision with water molecules whereby a complex is formed which may either be subjected to an irreversible decomposition or return to the original condition: a positive penicillin ion and a water molecule.

$$PnH^+ + H_2O \rightleftharpoons Pn^*H^+H_2O \rightarrow Pn'.$$

If we further suppose the irreversible action to proceed slowly when compared to the reversible action so that the complex is in equilibrium with penicillin ion and water, we have:

$$\frac{c_{+}c_{H_{2}O}}{c_{+}^{*}}\cdot\frac{f_{+}f_{H_{2}O}}{f_{+}^{*}}=K.$$

As the velocity of the irreversible conversion of the buffer complex is equal to the velocity at which PnH^+ is consumed we have:

$$c_{+}k_{+} = c_{+}^{*}k_{+}^{*}$$

where k_{+}^{*} represents the probability of the irreversible transformation of a molecule $Pn*H^+H_2O$ within unit of time. We will assume that this quantity is independent of the salt concentration It is difficult to obtain any idea as to the accuracy of this as sumption. It is based on the hypothesis that a substance the molecules of which are transformed spontaneously through an irreversible reaction will exhibit a velocity of reaction independent of the salt concentration.

In equation (20) we shall further substitute

$$f_{+} = f_{+}^{*}$$

the buffer complex and the penicillin ion having the same charge and almost the same molecular weight, since the difference s merely the relatively small water molecule.

By now applying equations (20) any (21) at the salt concentration 0 and at some other salt concentration, we get

$$k_{+} = k_{(+)} \frac{f_{H_{i}O} c_{H_{i}O}}{c_{(H_{i}0)}}$$

where $k_{(+)}$ is k_{+} for a salt concentration of 0 and $c_{(H,O)}$ is the concentration of water for a salt concentration of 0. The fraction in equation (23) is very nearly equal to the ratio of the water vapour pressure of the salt solution to that of pure water at the same temperature. For a 4-molar sodium chloride solution this ratio is 0.87. If we make the further approximation of assuming this fraction to be unity, we have

 $\mathbf{k}_{+} = \mathbf{k}_{(+)} \tag{24}$

13

which means that the velocity constant of the irreversible transformation of the positive penicillin ion is independent of the salt concentration.

The investigations performed by HAMMET & MARTIN (1934) with regard to the influence of the potassium chloride concentration on the hydrolysis of ethyl acetate by means of hydrochloric acid support the view that the approximation represented by the assumptions made here is not more rough than warranted by the purpose.

By a quite analogous deduction we obtain

 $k_0 = k_{(0)}$ and $k_{\pm} = k_{(\pm)}$ (25)

We shall now consider the dependence on the salt concenration of the activity coefficients of the equations (9) and (10). $\frac{f_0 f_{H^+}}{f_+}$ can only with a rough approximation be considered to be constantly independent of the salt concentration. As this quantity, however, only occurs in the second term of the parenthesis of (9) and (10), which in case of the hydrogen ion concentrations used here only amounts to about one tenth of the sum of the two terms, we shall consider it to be unity for all salt concentrations.

LARSSON & ADELL (1931) have determined the dependence in the salt concentration of the activity factor $\frac{f_{H^+}}{f_0}$ for 24 different acids which, as regards charge, are of the same type as asetic acid. On the basis of these measurements in connection with the determinations performed for α -dinitro phenol by HAL-

an & Kortüm (1934), HARNED & OWEN (1943) propounded the

٠Ť

Nr. 14 Nr. 14

rule that the variation of the activity factor with the ionic strength we consider f_{H+} in this equation to be independent of the acid is approximately independent of the acid used, consequently the used, we have activity factor for an acid at a certain salt concentration ma be taken to be the same as that of another acid for which the quantity has been determined at the salt concentration in question Moreover the activity factor is, according to HARNED & OWER equal to $f_{H+}f_{CI}$, a quantity for which they give numerical values in a number of excellent tables. However, the law one applies when the neutral salt added is the same. If f. inst potassium chloride is used in stead of sodium chloride, the value of the activity factor will change. We shall consider this law hold also with regard to penicillin. The approximation thus made should, according to the statements by LARSSON & ADELL and HARNED & OWEN, cause an error in log k which is smaller than 0.1.

Finally we shall consider the calculation of f_{+}/f_{0} .

KIRKWOOD (1934) has devised a formula for the calculation of the activity coefficient f_+ of ampho-ions. The formula hold on the following conditions:

1) The ampho-ions must be spherical.

2) The concentration of the ampho-ions must be so small that the inter-ionic forces between these ions may be neglected

3) The dipole moment of the ampho-ions must be very large compared to that of the solution.

4) No deviations, except of electric nature, from the law applying to diluted solutions must occur.

5) Corrections of the type stated by GRONWALL-LA ME (GRONWALL-LA MER & SANDVED (1928)) originating from no linear terms of the Poisson-Boltzmann equation must be small

Out of these conditions only those listed under 2) and 3) early be assumed to be fulfilled in this case. To overcome this difficult the following consideration is set forth:

Let us consider the above-mentioned rule propounded HARNED & OWEN:

$$\frac{f_{-}f_{H^{+}}}{f_{0}} = \text{constant}$$

which holds only in case the composition of the salt used is same, but which applies irrespective of the acid employed

 $f_{-} = f_0 \cdot constant$ (27)

e the activity coefficient of any monovalent anion may be considered to be a product of two factors: the activity coefficient of the corresponding uncharged molecule and a constant which is independent of the nature of the anion, but dependent on the salt employed in the solution. This constant may be said to represent the share contributed by the negative charge to the activity coefficient of the ion.

An analogous relation may also be deduced for monovalent positive ions. HAMMET & MARTIN (1934) have shown that the reaction velocity of the hydrolysis of ethyl acetate with acids varies as the equilibrium concentration of a positively charged ndicator of the type ammonia-ammonium ion, irrespectively of he nature and concentration of the salt added. The hydrolysis of ethyl acetate is assumed to proceed via the formation of a monovalent cation consisting of an ethyl acetate molecule and hydrogen ion. If we assume the velocity to be proportional to he concentration of this cation, it will be seen that the dissociation equilibrium of the esters as well as of the indicators employed must be independent of the latter, which means that a relation nalogous to equation (26) will apply, hence

$$\hat{z}_{+} = f_0 \cdot \text{constant.}$$
 (28)

In the basis of the equations (27) and (28) it would now be atural analogically to conclude that

$$f_{\pm} = f_0 \cdot \text{constant.}$$
 (29)

The constant of this equation represents the effect of the tharges upon the activity coefficient, and it is seen to be equal If if we assume f_0 to be unity, i. e. if we neglect non-electrical intributions towards the activity coefficient. With a certain apeximation we should be able to assume that it is this electrical ntribution which we obtain from Kirkwood's formula, for which cason we shall replace f_+ by f_+/f_0 in this formula, the more as the latter quantity forms part of equation (10).

Nr. II Nr. 14

These considerations cannot, of course, lay claim to any exact In (30) we further have validity. As, however, exact methods for the calculation of the activity coefficient are lacking, it is the author's intention to attempt to use the results obtained according to the described method, and by comparison with experimentally determined values form an idea of the reliability of the procedure.

Kirkwood's formula is as follows:

$$-\frac{D}{D_0} \ln f = -\frac{Q_0}{2 D k T} \cdot \frac{\varkappa}{1 + \varkappa a} \\ -\frac{\varkappa^2}{2 D k T} \sum_{n=1}^{\infty} \frac{(2n-1) Q_n}{(2n-1) (n+1)^2 a^{2n-1}} \cdot \frac{K_{n-1}(\varkappa a)}{K_{n+1}(\varkappa a) + \frac{n b^{2n+1} \varkappa^2 K_{n-1}(\varkappa a)}{(n+1) (2n-1) (2n+1) a^{2n-1}}}.$$

 Q_n is found from the equation:

$$\mathrm{Q}_n = \sum\limits_{k=1}^{M} \; \sum\limits_{l=1}^{M} \; e_k e_l r_k^n r_e^n \mathrm{P}_n \; \left(\cos v_{kl}\right)$$

P_n being the ordinary Legendre functions which for low values of n have the following configurations:

$$P_{0}(x) = 1,$$

$$P_{1}(x) = x.$$

$$P_{2}(x) = \frac{3x^{2}-1}{2}$$

$$P_{3}(x) = \frac{5x^{3}-3x}{2}$$

$$P_{4}(x) = \frac{1}{8} (35x^{4}-30x^{2}+3)$$

$$P_{5}(x) = \frac{1}{8} (63x^{5}-70x^{3}+15x)$$

 v_{kl} is the angle between the distances represented by r_k and r_e from the centre of the molecule to the charges e_k and e_k is the total number of charges.

$$\varkappa^2 = \frac{8\pi \,\mathrm{N}\,\varepsilon^2 \,\mathrm{J}}{1000\,\mathrm{Dk}\,\mathrm{T}} \qquad \mathrm{where}$$

N is Avogadro's number, 6.06 · 10²³

 ε is the charge of the electron, $4.774 \cdot 10^{-10}$ E.S.U.

k is Boltzmann's constant, $1.375 \cdot 10^{-16}$

J is the ionic strength of the solution.

The functions $K_n(x)$ can be found from

$$K_{n}(x) = \sum_{s=0}^{n} \frac{2^{s} n! (2n-s)!}{s! (2n)! (n-s)!} x^{s}.$$
 (33)

17

D is the dielectric constant of the solution.

 D_0 is the dielectric constant of the solvent.

- b is the radius of the molecule with the activity coefficient f.
- is the sum of b and the mean value of the radii of the ions of the salt solution.

It is improbable that the penicillin molecule is spherical. he value chosen for b must consequently to a certain extent be based on an estimate. If a spatial model of the molecule of openicillin is drawn, it will be seen that the sphere which approximates the penicillin molecule most closely has a radius of 4.7 Å. On the basis of the figure 0.268 cm²/24 hours at 10° C for he diffusion constant of penicillin determined by KLENOW (1947), is calculated from Riecke's equation to be 4.7 Å and from Noke's equation to be 5.2 Å (BRODERSEN & KLENOW, 1947). The pecific gravity of the molecule is in this case taken to be 1.3. from the formula for G-penicillin advanced by British and merican scientists a molecular weight of 334 is obtained. On his basis b is calculated to be 4.7 Å for this molecular specific ravity.

We shall consider the figure 4.7 Å to be the most probable alue for b.

From roentgeno-crystallographic data the mean radius of the dium and the chloride ion is calculated to be 1.0 Å. HARNED WOWEN are of the opinion that at the moment of collision the stance between the centre of the sodium or chloride ion and Kgl. Danske Vidensk. Selskab, Mat.-fvs. Medd. XXIV, 14. $\mathbf{2}$

18

measurements performed by GRONWALL, LA MER & SANDVER Section, these experiments were performed in a 0.0095 m acetic (1928) this distance is about 2 Å. According to HARNED & Owen acid, the hydrogen ion concentration of which is dependent on the difference between these values is due to hydration of the ions. KIRKWOOD, whose investigations are of a more recent date than those performed by GRONWALL, LA MER & SANDVED, is of the opinion that the figure 1 Å should be used. With regard to KIRKWOOD'S own experiments the best conformity between the figures for the activity coefficient of glycine obtained by call culation from the formula and those determined experimentally is obtained at a value of 0.7 Å, although the figure 1.0 Å also gives fairly good conformity. GRONWALL, LA MER & SANDVED'S figures are determined by means of conductivity measurements, and it therefore seems desirable, to use the figures found by these authors, when the purpose is to calculate the conductivity. In our case, where the purpose is to calculate an activity coeff ficient by means of Kirkwood's formula, it appears natural to use a value which has proved statisfactory in connection with this formula. Here we shall therefore consider the "effective mean radius" of the sodium and chloride ion to be 1.0 A This figure is thus, to a certain extent, empirically determined By choosing this value instead of 0.7 Å we have avoided an inconsistency with the crystallographic figures. a is thus 5.7 Å.

We shall assume the distance between the charges of the penicillin ampho-ion to be the same as the distance between the charges in an α -amino, since the configuration of the part α the molecule to which the charges are attached is the same in both cases. WYMAN (1934) has determined the dipole moment of a-amino acids to be 20×10^{-18} E.S.U. From this we find R = 4.2 Å.

With regard to r, the distance from the centre of the molecule to the charges, we shall take it to equal b, assuming the charges to be located on the surface of the molecule.

Moreover we shall assume that D equals D_0 , irrespective of the salt concentration.

We are now able to calculate f_+/f_0 . From the quantities formulae (9) and (10) we have as yet no knowledge of the hydrogen ion concentration. Like some of the experiments of

Nr. 储

the surface of the other ion is greater than 1 Å. According the effect of small salt concentrations dealt with in the preceding the salt concentration. The calculation of the interdependence in the case of small salt concentrations has been dealt with in the section in question. The formulae set forth may, by approximation, be extended to solutions of a somewhat higher concentration by subjecting the activity coefficient to a treatment malogous to that described above. The hydrogen ion concentrations calculated in this manner are found in table 1.

Results.

The values of the velocity constant for different concentrations sodium chloride determined by means of formulae (9) and (0) are to be found in table 1. These two relations are represented y curves in fig. 4 in which the experimental results are plotted. With regard to experimental method reference should be made the section on weak salt solutions. The measurements dealt with here are a direct continuation of the experimental series erformed for low concentrations.

Table 1. (Referring to fig. 4).

	c _{H+} calculate	$\frac{f_{\pm}}{f_0}$ from Kirkwood's formula (30)	— log k from eq. (9)	— log k from eq. (10)
0	0.0003204	1.00	2 18	2.18
°∼. 1	0.0003608	0.98	2.10	2.20
2	0.0003755	0.97	2.21 2.22	2.20
3	0.0003857	0.96	2.22	2.20
6	0.0004060	0.93	2.23	2.20
1	0.0004170	0.89	2,25	2.20
1	0.0004489	0.82	2.25	2.17
51	0.0004626	0.67	2.26	2.10
1	0.0004466	0.52	2.26	1.99
)1	0.0003766	0.38	2.22	1.82
)1	0.0003003	0.29	2.17	1.65
40		· . · ·		·

Nr. 4



20

Fig. 4. The salt effect on the inactivation process in 0.0095 m acetic acid at great salt concentrations. The curves are plotted from the equations (9) and (10); it first of which is based on the assumption that the molecule HPn is labile, and the second on the assumption that PN^-H^+ is labile. The point \bigcirc represent has been determined as the mean value of a great number of determinations

the experimentally determined figures and those calculated from smaller. formula (9), while the conformity with the course calculated It is thus on the whole not very probable that the error to according to (10) is quite good. As appears from the manner which the calculation of log k from equation (9) is subject is which these two equations were deduced formula (9) is base more than 0.4, if the hypothesis with regard to the mechanism on the condition that a penicillin molecule without charge reaction on which (9) is based is correct. As the deviation unstable, due to the prescence of a hydrogen atom in the car determined experimentally in some cases is as great as 0.7, it boxyl group. Formula (10) is on the other hand based on the as may be said that most probably the assumptions on which formula sumption that it is only possible to inactivate the uncharge of is based are incorrect. penicillin molecule by converting it into an ampho-ion, the instance of the we now consider the calculations based on equation (10), bility being caused by the combination of a hydrogen ion and will at once be seen that the difference between the experimenthe nitrogen of the amido group. The question is now whether any determined and the calculated values is smaller than the is warrantable, on the basis of these calculations and experiments wheeted maximum error. This good conformity must, however, to accept as correct the theory on which (10) is based.

1) the assumption that k_0 is constant, 2) the calculation bterm of the parenthesis, at the hydrogen ion concentrations de

in idea of the variation of k₀ with the salt concentration by con-Idering the dependence of the rate of transformation of another incharged molecule on the salt concentration. HARNED & PFAN-TIEL (1922) have examined the dependence of the hydrolysis fethyl acetate by 0.1 m hydrochloric acid on the concentration potassium chloride, which was added in amounts varying from to 3 m. They found a variation in log k of 0.02. Apart from his investigation the literature seems to present no examples of terminations of this type of dependence which appear reliable mough to warrant an application in this connection.

According to the statements in the theoretical section on the alculation of $f_0/f_1f_{H^+}$ the error to which this calculation is ubject cannot be supposed to cause en error greater than 0.1 in gk. As the calculation of the hydrogen ion concentration is ased on the same theoretical suppositions as the calculation of he activity coefficient, the error originating from this calculation may be supposed to be of the same order of magnitude. As, lowever, the conformity between calculated and experimentally etermined hydrogen ion concentrations is even better than might It will be seen that there are considerable discrepancies between inve been expected on this basis, the error is probably even

some extent be ascribed to incidental circumstances, as the The inaccuracy of the calculation performed with equation which the application of Kirkwood's equation is (9) as a basis is essentially due to the inaccuracy attached and are not completely fulfilled, and some of the assumptions whave made in order to be able to calculate the individual $f_0/f_f_{H^+}$, and finally, 3) the calculation of c_{H^+} , hence the second disc constants are rather uncertain. The conformity found shows, wever, that the deviation from the course calculated according with here, is rather small compared to k_0 . It is possible to obtain a seguration (9) as regards direction and order of magnitude Nr. 14 **Mr.** 14

corresponds to the deviation which should be expected to occur the penicillin ion present in a neutral solution has a single negaif the instability of penicillin is assumed to be due to the taking live charge. up of a hydrogen ion at the nitrogen atom in the four-membered III. An attempt is made to calculate the salt effect at high ring.

that the inactivation must be assumed to consist in an opening atom in the four-membered ring. of the four-membered ring and that inactivation in strong An estimate of the errors to which the calculations are subject acid solution, as shown in the above quoted work, is due to shown to confirm this theory about the reaction mechanism. the taking up of a hydrogen ion at this point of the molecule ratheory which is also supported by previous investigations.

Summary.

I. The result of the calculation of the salt effect on the m activation of G penicillin in acid solution is dependent on the electric charge of the penicillin molecule and on whether w assume the inactivation in slightly acid solution to be due to the taking up of a hydrogen ion at the nitrogen atom in the four membered ring or to the taking up of a hydrogen ion in the COO-group.

The location of the hydrogen ion is of no importance in weak salt solutions.

II. A formula (14) from which the salt effect may be culated at small ionic strengths is deduced. This equation coll tains the electric charge of the acid penicillin molecule.

From this formula the salt effect on the inactivation in 0.02 and 0.0024 m hydrochloric acid and in 0.0095 m acetic acid which sodium chloride is added is calculated for different value of the electric charge. In the case of the last mentioned solution allowance is made for the fact that the hydrogen ion concentration varies with the ionic strength.

By comparison with the experimentally determined salt it appears that the acid penicillin molecule is uncharged,

ionic strengths on the basis of the two theories on the reaction It is thus highly probable that the factor which decide mechanism indicated above. To be able to carry out these calwhether a penicillin molecule is stable or unstable in a slightly culations it is necessary to make a fairly large number of simpliacid solution is the existence of a hydrogen ion linked to the wing assumptions, which, of course, renders the results less above mentioned nitrogen atom, while the degree of dissociation reliable. A comparison with the experimentally determined figures of the carboxyl group only influences to a certain extent the shows, however, good conformity with the calculation method velocity at which the inactivation takes place (BRODERSEN, 1947), which is based on the assumption that the instability is due to This theory becomes even more probable when one consider the fact that a hydrogen ion has been taken up at the nitrogen

The expenses of the experiments dealt with in this paper have ken covered by grants from "Medicinalfabrikantforeningen". the penicillin used was kindly placed at my disposal by Proessor K. A. Jensen, M. D. Helge Brodersen, cand. act. perfirmed the calculations. I am indebted to the late Professor N. BRØNSTED, Ph. D. and Professor J. A. CHRISTIANSEN, Ph. for instructive conversations during the work.

m the Department of General Pathology, University of Copenhagen. Professor K. A. Jensen, M. D.).

References.

- 1) BRODERSEN, R.: 1. Trans. Farad. Soc. 1947. 43, 351.
- 2) : 2. Acta chem. 1947, 1, 403.
- BRODERSEN, R. & H. KLENOW: D. Kgl. Danske Vidensk. Selskab, Biol. Medd. XX Nr. 7.
- 4) BRØNSTED, J. N.: J. phys. Chem. 1924, 28, 579. (Z. physik Chemie, 1922, 102, 169; 1925, 115, 337).
- 5) CHRISTIANSEN, J. A.: Z. physik. Chemie, 1924, 118, 35.
- 6) Committee on Medical Research and the Medical Research Council. Nature, 1945, 156, 766.
- 7) GRONWALL, T. H., V. K. LA MER & K. SANDVED: Physik. Z., 1928. 29, 358.
- 8) HALBAN, H. v. & G. KORTÜM: Z. physik. Chemie, 1934, A 170, 351
- 9) HAMMET, L. P. & A. P. MARTIN: J. Am. chem. Soc., 1934, 56 827 and 830.
- HARNED, H. S. & B. B. OWEN: "The Physical Chemistry of Electrolytic Solutions", New York, 1943.
- 11) HARNED, H. S. & R. PFANSTIEL: J. Am. chem. Soc. 1922, 44, 2193
- 12) KIRKWOOD, J. G.: J. Chem. Phys., 1934, 2, 351.
- 13) KLENOW, H.: Acta chem. Scand. 1947, 1, 328.
- 14) LARSSON, E. & B. ADELL: Z. physik. Chem., 1931, A 157, 342 and 1931, A 156, 381.
- 15) WYMAN, J.: J. Am. chem. Soc., 1934, 56, 536.

Indleveret til Selskabet den 29. November 1947. Færdig fra Trykkerlet den 18. Juni 1948.