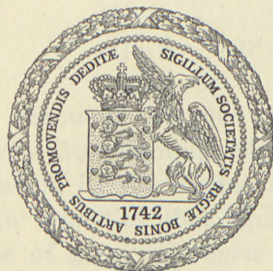


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MATEMATISK-FYSISKE MEDDELELSER, BIND XXIV, NR. 14

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

BY

ROLF BRODERSEN



KØBENHAVN

I KOMMISSION HOS EJNAR MUNKSGAARD

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HOLF BRODERSZEN



KÖRBERG

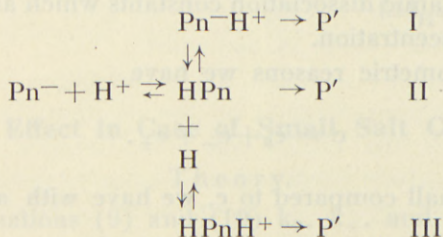
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In previous publications (BRODERSEN, 1 and 2, 1947) the rate of inactivation of G-penicillin was examined with regard to its dependence on the hydrogen ion concentration and the temperature. The present investigation deals with the variation of the inactivation 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:



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

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:

$$h_0 = k_0 c_0; \quad h_+ = k_+ c_+. \quad (1)$$

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

$$h = h_0 + h_+ = k_0 c_0 + k_+ c_+. \quad (2)$$

For the equilibriums of the reaction scheme we have:

$$f_{\text{H}^+} \frac{f_-}{f_0} c_{\text{H}^+} \frac{c_-}{c_0} = K_{(0)} \quad (3)$$

$$f_{\text{H}^+} \frac{f_0}{f_+} c_{\text{H}^+} \frac{c_0}{c_+} = K_{(+)} \quad (4)$$

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

$$c = c_0 + c_- + c_+. \quad (5)$$

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

$$c = c_0 + c_-. \quad (6)$$

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

$$c_0 = \frac{f_{\text{H}^+} \frac{f_-}{f_0} c}{K_0 + f_{\text{H}^+} \frac{f_-}{f_0} c_{\text{H}^+}} c_{\text{H}^+} \quad (7)$$

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

$$c_+ = \frac{(f_{H^+})^2 \frac{f_-}{f_+} c}{K_{(+)} \left(K_{(0)} + f_{H^+} \frac{f_-}{f_0} c_{H^+} \right)} (c_{H^+})^2 \quad (8)$$

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

$$k = \frac{h}{c} = \left(k_0 + \frac{k_+ f_0}{K_{(+)} f_+} f_{H^+} c_{H^+} \right) \frac{c_{H^+}}{\frac{f_0}{f_- f_{H^+}} K_{(0)} + c_{H^+}} \quad (9)$$

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 amphi-ion Pn^-H^+ , we find by a deduction analogous to the one particularized above, the following equation:

$$k = \frac{h}{c} = \left(\frac{k_{\pm}}{K_{(\pm)}} \cdot \frac{f_0}{f_{\pm}} K_{(0)} + \frac{k_+}{K_{(+)}} \cdot \frac{f_0}{f_+} f_{H^+} c_{H^+} \right) \frac{c_{H^+}}{\frac{f_0}{f_- f_{H^+}} K_{(0)} + c_{H^+}} \quad (10)$$

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 velocity constants for the velocity determining reactions. Such reactions must be supposed to be brought about by collisions between water molecules and penicillin molecules.

As the water molecules are without electric charge the velocity of the reaction will be independent of the salt concentration at small values of the ionic strength (BRØNSTED (1924) and CHRISTIANSEN (1924)), for which reason k_0 , k_+ , and k_{\pm} may here be considered to be constant. The values of these quantities at an ionic strength of 0 we shall denote $k_{(0)}$, $k_{(+)}$, and $k_{(\pm)}$. It appears from KIRKWOOD's equation (1924) for the activity coef-

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 negative 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 $f_0 = f_{\pm}$.

As $K_{(0)}$ and $K_{(\pm)}$ 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 discussion 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 number 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 n , we have:

$$f_0 = f_{\pm}; \quad f_{\text{H}^+} = f_1. \quad (11)$$

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

$$f_+ = f_{z+1}; \quad f_- = f_{z-1}. \quad (12)$$

f_0 in (10) is substituted by f_z

$$k = \left(\frac{k_{(\pm)}}{K_{(\pm)}} K_{(0)} + \frac{k_{(+)}}{K_{(+)}} \cdot \frac{f_z}{f_{z+1}} f_1 c_{H^+} \right) \frac{c_{H^+}}{\frac{f_z}{f_{z-1} f_1} K_{(0)} + c_{H^+}} \quad (13)$$

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

$$k = \left(\frac{k_{(\pm)}}{K_{(\pm)}} K_{(0)} + \frac{k_{(+)}}{K_{(+)}} f_1^{-2z} c_{H^+} \right) \frac{c_{H^+}}{f_1^{2z-2} K_{(0)} + c_{H^+}} \quad (14)$$

f_1 can be calculated from Poisson-Boltzmann's equation

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

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

$$-\log f_1 = 0.52 \sqrt{J} \quad (16)$$

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 determined at 30°C at different concentrations of sodium chloride, the measurements being performed according to previously described methods.

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

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 penicillin had 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 dissociation 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 hydrochloric 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 dealt 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 of 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

$$K_{(\text{EH})} = \frac{f_{\text{H}^+} f_{\text{E}^-}}{f_{\text{EH}}} \cdot \frac{c_{\text{H}^+} c_{\text{E}^-}}{c_{\text{EH}}} \quad (17)$$

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

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

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

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

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:

$$\begin{array}{cccc} c_{H+} = & 0.024 & 0.0024 & 0.00031 \\ k = & 0.16 & 0.028 & 0.0065 \quad \text{for } J = 0. \end{array}$$

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

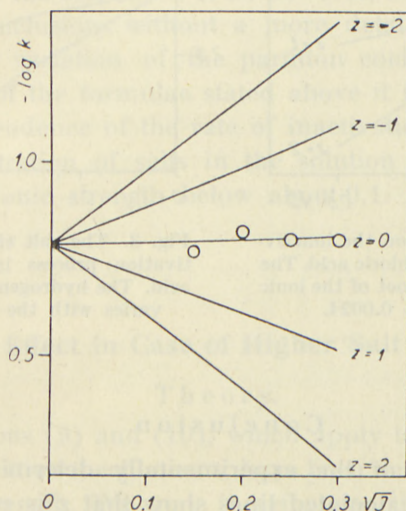


Fig. 1. The salt effect on the inactivation process in hydrochloric acid. The abscissa is the square root of the ionic strength. $c_{H+} = 0.024$.

coefficients are unity. These three equations can be solved by trial and error, whereby we obtain:

$$\frac{k_{(+)} }{K_{(+)} } = 6.0 \quad \frac{k_{(\pm)} }{K_{(\pm)} } = 25.3 \quad K_{(0)} = 0.00100 \quad (18)$$

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.

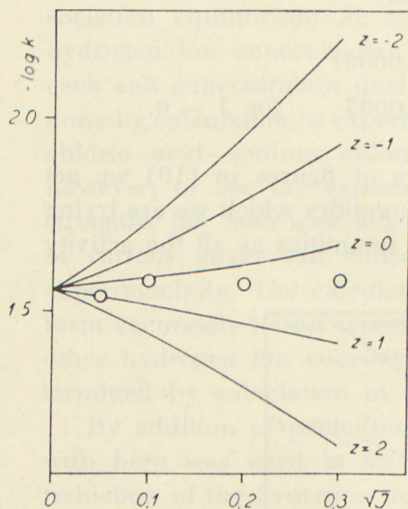


Fig. 2. The salt effect on the inactivation process in hydrochloric acid. The abscissa is the square root of the ionic strength. $C_{H^+} = 0.0024$.

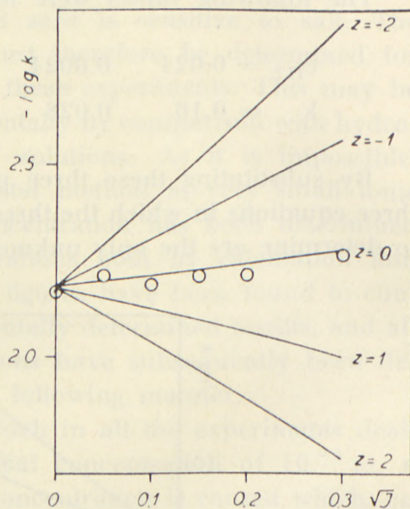


Fig. 3. The salt effect on the inactivation process in 0.0095 m acetic acid. The hydrogen ion concentration varies with the ionic strength.

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-penicillin preferably occurs as a single ion with one negative charge, which

in a solution of higher acidity equilibrates a molecule without 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 of the experimental determination of $\log k$ in these experiments amounts to 0.02—0.03, so that only the point representing $J = 0.3$ can 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 for this ionic strength that any importance can be attached to the deviation.

This result with regard to the charge of the penicillin molecule is in conformity with the results which we should have expected to obtain according to the structural formula for penicillin propounded by British and American scientists (Committee on 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 ion 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 any definite conclusions without a more detailed knowledge of the numerical variation of the partition coefficient.

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

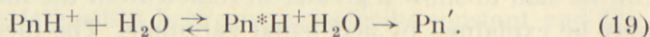
III. The Salt Effect in Case of Higher Salt Concentrations.

Theory.

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

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.



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:

$$\frac{c_+ c_{\text{H}_2\text{O}}}{c_+^*} \cdot \frac{f_+ f_{\text{H}_2\text{O}}}{f_+^*} = K. \quad (20)$$

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

$$c_+ k_+ = c_+^* k_+^* \quad (21)$$

where k_+^* represents the probability of the irreversible transformation of a molecule $\text{Pn}^*\text{H}^+\text{H}_2\text{O}$ 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 assumption. 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

$$f_+ = f_+^* \quad (22)$$

the buffer complex and the penicillin ion having the same charge and almost the same molecular weight, since the difference is 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

$$k_+ = k_{(+)} \frac{f_{\text{H}_2\text{O}} c_{\text{H}_2\text{O}}}{c_{(\text{H}_2\text{O})}} \quad (23)$$

where $k_{(+)}$ is k_+ for a salt concentration of 0 and $c_{(\text{H}_2\text{O})}$ 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

$$k_+ = k_{(+)} \quad (24)$$

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

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

We shall now consider the dependence on the salt concentration of the activity coefficients of the equations (9) and (10). $\frac{f_0 f_{\text{H}^+}}{f_+}$ 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 on the salt concentration of the activity factor $\frac{f_{\text{H}^+}}{f_0}$ for 24 different acids which, as regards charge, are of the same type as acetic acid. On the basis of these measurements in connection with the determinations performed for α -dinitro phenol by HALBAN & KORTÜM (1934), HARNED & OWEN (1943) propounded the

rule that the variation of the activity factor with the ionic strength is approximately independent of the acid used, consequently the activity factor for an acid at a certain salt concentration may be taken to be the same as that of another acid for which this quantity has been determined at the salt concentration in question. Moreover the activity factor is, according to HARNED & OWEN, equal to $f_{H^+}f_{Cl^-}$, a quantity for which they give numerical values in a number of excellent tables. However, the law only 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 to 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_{\pm}/f_0 .

KIRKWOOD (1934) has devised a formula for the calculation of the activity coefficient f_{\pm} of amphi-ions. The formula holds on the following conditions:

- 1) The amphi-ions must be spherical.
- 2) The concentration of the amphi-ions must be so small that the inter-ionic forces between these ions may be neglected.
- 3) The dipole moment of the amphi-ions must be very large compared to that of the solution.
- 4) No deviations, except of electric nature, from the laws applying to diluted solutions must occur.

5) Corrections of the type stated by GRONWALL-LA MER (GRONWALL-LA MER & SANDVED (1928)) originating from non-linear terms of the Poisson-Boltzmann equation must be small.

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

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

$$\frac{f_{-}f_{H^+}}{f_0} = \text{constant} \quad (26)$$

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

we consider f_{H^+} in this equation to be independent of the acid used, we have

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

i. 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 indicator of the type ammonia-ammonium ion, irrespectively of the 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 a hydrogen ion. If we assume the velocity to be proportional to the 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 analogous to equation (26) will apply, hence

$$f_+ = f_0 \cdot \text{constant.} \quad (28)$$

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

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

The constant of this equation represents the effect of the charges upon the activity coefficient, and it is seen to be equal to f_{\pm} if we assume f_0 to be unity, i. e. if we neglect non-electrical contributions towards the activity coefficient. With a certain approximation we should be able to assume that it is this electrical contribution which we obtain from Kirkwood's formula, for which reason we shall replace f_{\pm} by f_{\pm}/f_0 in this formula, the more so as the latter quantity forms part of equation (10).

These considerations cannot, of course, lay claim to any exact 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:

$$-\frac{D}{D_0} \ln f = -\frac{Q_0}{2DkT} \cdot \frac{z}{1+za} - \frac{z^2}{2DkT} \sum_{n=1}^{\infty} \frac{(2n-1)Q_n}{(2n-1)(n+1)^2 a^{2n-1}} \cdot \frac{K_{n-1}(za)}{K_{n+1}(za) + \frac{nb^{2n+1}z^2 K_{n-1}(za)}{(n+1)(2n-1)(2n+1)a^{2n-1}}} \quad (30)$$

Q_n is found from the equation:

$$Q_n = \sum_{k=1}^M \sum_{l=1}^M e_k e_l r_k^n r_l^n P_n(\cos v_{kl}) \quad (31)$$

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

$$\begin{aligned} P_0(x) &= 1, \\ P_1(x) &= x, \\ P_2(x) &= \frac{3x^2-1}{2}, \\ P_3(x) &= \frac{5x^3-3x}{2}, \\ P_4(x) &= \frac{1}{8}(35x^4-30x^2+3), \\ P_5(x) &= \frac{1}{8}(63x^5-70x^3+15x) \end{aligned} \quad (32)$$

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

In (30) we further have

$$\kappa^2 = \frac{8\pi N \varepsilon^2 J}{1000 DkT} \quad \text{where}$$

N is Avogadro's number, $6.06 \cdot 10^{23}$

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

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

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 .

a 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. The value chosen for b must consequently to a certain extent be based on an estimate. If a spatial model of the molecule of G-penicillin 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 the diffusion constant of penicillin determined by KLENOW (1947), b is calculated from Riecke's equation to be 4.7 Å and from Stoke's equation to be 5.2 Å (BRODERSEN & KLENOW, 1947). The specific gravity of the molecule is in this case taken to be 1.3. From the formula for G-penicillin advanced by British and American scientists a molecular weight of 334 is obtained. On this basis b is calculated to be 4.7 Å for this molecular specific gravity.

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

From roentgeno-crystallographic data the mean radius of the sodium and the chloride ion is calculated to be 1.0 Å. HARNED & OWEN are of the opinion that at the moment of collision the distance between the centre of the sodium or chloride ion and

the surface of the other ion is greater than 1 Å. According to measurements performed by GRONWALL, LA MER & SANDVED (1928) this distance is about 2 Å. According to HARNED & OWEN 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 calculation 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 coefficient by means of Kirkwood's formula, it appears natural to use a value which has proved satisfactory in connection with this formula. Here we shall therefore consider the "effective mean radius" of the sodium and chloride ion to be 1.0 Å. 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 of the molecule to which the charges are attached is the same in both cases. WYMAN (1934) has determined the dipole moment of α -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_{\pm}/f_0 . From the quantities in formulae (9) and (10) we have as yet no knowledge of the hydrogen ion concentration. Like some of the experiments on

the effect of small salt concentrations dealt with in the preceding section, these experiments were performed in a 0.0095 m acetic acid, the hydrogen ion concentration of which is dependent on 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 analogous 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 of sodium chloride determined by means of formulae (9) and (10) are to be found in table 1. These two relations are represented by curves in fig. 4 in which the experimental results are plotted.

With regard to experimental method reference should be made to the section on weak salt solutions. The measurements dealt with here are a direct continuation of the experimental series performed for low concentrations.

Table 1.
(Referring to fig. 4).

J	c_{H^+} calculated	$\frac{f_{\pm}}{f_0}$ from Kirkwood's formula (30)	$-\log k$ from eq. (9)	$-\log k$ from eq. (10)
0.00	0.0003204	1.00	2.18	2.18
0.01	0.0003608	0.98	2.21	2.20
0.02	0.0003755	0.97	2.22	2.20
0.03	0.0003857	0.96	2.22	2.20
0.06	0.0004060	0.93	2.23	2.20
0.11	0.0004170	0.89	2.25	2.20
0.21	0.0004489	0.82	2.25	2.17
0.51	0.0004626	0.67	2.26	2.10
1.01	0.0004466	0.52	2.26	1.99
2.01	0.0003766	0.38	2.22	1.82
3.01	0.0003003	0.29	2.17	1.65

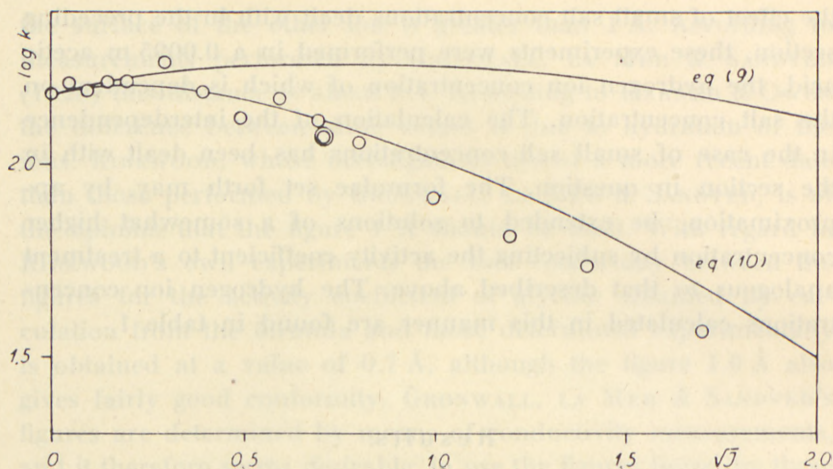


Fig. 4. The salt effect on the inactivation process in 0.0095 m acetic acid at greater salt concentrations. The curves are plotted from the equations (9) and (10), the first of which is based on the assumption that the molecule HPn is labile, and the second on the assumption that PN^-H^+ is labile. The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

It will be seen that there are considerable discrepancies between the experimentally determined figures and those calculated from formula (9), while the conformity with the course calculated according to (10) is quite good. As appears from the manner in which these two equations were deduced formula (9) is based on the condition that a penicillin molecule without charge is unstable, due to the presence of a hydrogen atom in the carboxyl group. Formula (10) is on the other hand based on the assumption that it is only possible to inactivate the uncharged penicillin molecule by converting it into an amphi-ion, the instability being caused by the combination of a hydrogen ion and the nitrogen of the amido group. The question is now whether it is warrantable, on the basis of these calculations and experiments, to accept as correct the theory on which (10) is based.

The inaccuracy of the calculation performed with equation (9) as a basis is essentially due to the inaccuracy attached to 1) the assumption that k_0 is constant, 2) the calculation of f_0/f_{H^+} , and finally, 3) the calculation of c_{H^+} , hence the second term of the parenthesis, at the hydrogen ion concentrations dealt with here, is rather small compared to k_0 . It is possible to obtain

an idea of the variation of k_0 with the salt concentration by considering the dependence of the rate of transformation of another uncharged molecule on the salt concentration. HARNED & PFANSTIEL (1922) have examined the dependence of the hydrolysis of ethyl acetate by 0.1 m hydrochloric acid on the concentration of potassium chloride, which was added in amounts varying from 0 to 3 m. They found a variation in $\log k$ of 0.02. Apart from this investigation the literature seems to present no examples of determinations of this type of dependence which appear reliable enough to warrant an application in this connection.

According to the statements in the theoretical section on the calculation of f_0/f_{H^+} the error to which this calculation is subject cannot be supposed to cause an error greater than 0.1 in $\log k$. As the calculation of the hydrogen ion concentration is based on the same theoretical suppositions as the calculation of the activity coefficient, the error originating from this calculation may be supposed to be of the same order of magnitude. As, however, the conformity between calculated and experimentally determined hydrogen ion concentrations is even better than might have been expected on this basis, the error is probably even smaller.

It is thus on the whole not very probable that the error to which the calculation of $\log k$ from equation (9) is subject is more than 0.4, if the hypothesis with regard to the mechanism of reaction on which (9) is based is correct. As the deviation determined experimentally in some cases is as great as 0.7, it may be said that most probably the assumptions on which formula (9) is based are incorrect.

If we now consider the calculations based on equation (10), it will at once be seen that the difference between the experimentally determined and the calculated values is smaller than the expected maximum error. This good conformity must, however, to some extent be ascribed to incidental circumstances, as the conditions on which the application of Kirkwood's equation is based are not completely fulfilled, and some of the assumptions we have made in order to be able to calculate the individual basic constants are rather uncertain. The conformity found shows, however, that the deviation from the course calculated according to equation (9) as regards direction and order of magnitude

corresponds to the deviation which should be expected to occur if the instability of penicillin is assumed to be due to the taking up of a hydrogen ion at the nitrogen atom in the four-membered ring.

It is thus highly probable that the factor which decides whether a penicillin molecule is stable or unstable in a slightly acid solution is the existence of a hydrogen ion linked to the above mentioned nitrogen atom, while the degree of dissociation of the carboxyl group only influences to a certain extent the velocity at which the inactivation takes place (BRODERSEN, 1947). This theory becomes even more probable when one considers that the inactivation must be assumed to consist in an opening of the four-membered ring and that inactivation in strongly acid solution, as shown in the above quoted work, is due to the taking up of a hydrogen ion at this point of the molecule.

Summary.

I. The result of the calculation of the salt effect on the inactivation of G penicillin in acid solution is dependent on the electric charge of the penicillin molecule and on whether we 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 calculated at small ionic strengths is deduced. This equation contains the electric charge of the acid penicillin molecule.

From this formula the salt effect on the inactivation in 0.029 and 0.0024 m hydrochloric acid and in 0.0095 m acetic acid to which sodium chloride is added is calculated for different values 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 effect it appears that the acid penicillin molecule is uncharged, while

the penicillin ion present in a neutral solution has a single negative charge.

III. An attempt is made to calculate the salt effect at high ionic strengths on the basis of the two theories on the reaction mechanism indicated above. To be able to carry out these calculations it is necessary to make a fairly large number of simplifying assumptions, which, of course, renders the results less reliable. A comparison with the experimentally determined figures shows, however, good conformity with the calculation method which is based on the assumption that the instability is due to the fact that a hydrogen ion has been taken up at the nitrogen atom in the four-membered ring.

An estimate of the errors to which the calculations are subject is shown to confirm this theory about the reaction mechanism, a theory which is also supported by previous investigations.

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