Reaction of 2-Bromo-4,4-dimethyltetralone-1 with Morpholine and Piperidine.—The reaction of this saturated α-bromoketone[3] with morpholine was carried out under four different sets of conditions. (a) A 1.27-g. (0.005 mole) sample of bromide and 1.3 g. (0.015 mole) of morpholine were allowed to stand for 11 days in 13 ml. of methanol in the dark at room temperature. Only 27% of the bromide had reacted after this time. Based upon the amount of bromide reacting, a 55% yield of the α-amino-α,β-unsaturated ketone (I) resulted. The unreacted bromide was largely recovered. (b) Using the same amounts of reagents at 65° for 28 hours, 80% of the starting bromide reacted. An 18% yield of (I) and a 20% yield of 4,4-dimethyl 1-keto-1,4-dihydronaphthalene were isolated from the reaction mixture. (c) A 1.26-g. sample of the saturated α-bromoketone was allowed to stand in the ice-chest for 27 days in 4 ml. of morpholine. The calculated amount of morpholine hydrobromide was obtained along with a 22% yield of (I) and a 72% yield of 4,4-dimethyl-1-keto-1,4-dihydronaphthalene from the reaction mixture. (d) Experiment (c) was repeated except that the reaction mixture stood for three days at room temperature. The calculated amount of morpholine hydrobromide, as well as a 31% yield of (I) and a 20% yield 4,4-dimethyl-1-keto-1,4-dihydronaphthalene were isolated.

Using piperidine in place of morpholine, experiment (d) was repeated giving the theoretical yield of piperidine hydrobromide, a 35% yield of (II), and a 47% yield of 4,4-dimethyl-1-keto-1,4-dihydronaphthalene.

Reaction of 2-Bromo-4,4-dimethyl-1-keto-1,4-dihydronaphthalene with Cyclohexylamine.—A mixture of 1.26 g. (0.005 mole) of the unsaturated α-bromoketone, 1.15 ml. (0.01 mole) of cyclohexylamine and 0.5 ml. of absolute ethanol was allowed to stand at room temperature in the dark for three days. The colorless oily product which could not be induced to crystallize was treated with dry hydrogen chloride gas in dry ether to produce 1.62 g. (95%) of a colorless solid; recrystallized from methanol and ether, m.p. 176–177° (VI).

Anal. Calcd. for $C_{18}H_{25}NOCl_2$: C, 63.16; H, 7.36; N, 4.09. Found: C, 63.12; H, 7.15; N, 3.96.

Using the technique described previously[7] the aminochloroketone hydrochloride (VI) reacted with acidic potassium iodide solution at 65° to release 32.8% of one equivalent of iodine in 15 minutes, 74.4% in 30 minutes and 85% in 45 minutes. Under these conditions 2-bromo-4,4-dimethyltetralone-1 released 100% in five minutes while 2-bromo-4,4-dimethyl-1-keto-1,4-dihydronaphthalene released no iodine in 30 minutes.

LINCOLN, NEBRASKA RECEIVED AUGUST 25, 1950

[CONTRIBUTION FROM THE GIBBS CHEMICAL LABORATORY, HARVARD UNIVERSITY]

Inhibition of Urease by Silver Ions

BY JOHN F. AMBROSE,[1a] G. B. KISTIAKOWSKY AND ANDREW G. KRIDL[1b]

The inhibition of urease by silver ions has been studied in a citrate buffer at *p*H 5.6 and 20°. The results indicate that the inhibition is produced by a reversible reaction of one silver ion with an active site in the enzyme molecule. Silver ions react also with inactive protein which was present in the repeatedly recrystallized samples of urease. The affinity for silver and the specific binding capacity of active urease and of the inactive protein were found to be the same, which suggests that the inactive protein was urease deactivated in the process of purification. Extrapolation to pure active enzyme shows that one mole of it is totally inhibited by reaction with three to four moles of silver ions. The significance of these findings is discussed.

Marked inhibitory effects of metal ions on the activity of urease have been reported.[2a,b,3] The action of silver ions has been the subject of a detailed study by Sumner and Myrback.[4] They concluded that seven ions suffice to inactivate one molecule of urease, from their measurements of the total concentration of silver ions required for half inactivation of the enzyme. The reversible nature of the inhibition as well as the uncertainty in the extrapolation of the half inactivation concentration to total inactivation was realized and emphasized by these writers.

Sumner and Myrback have shown also that the quantity of silver which is bound by the enzyme at higher concentrations of silver ions exceeds by at least a factor of ten the minimal amount necessary for total inhibition.

The inhibition of urease by *p*-chloromercuribenzoate has been studied by Hellerman and co-workers.[5] Their conclusions were not presented on a molecular basis, but using the data of Sumner[6] it may be calculated that twenty-two equivalents of the sulfhydryl reagent must be added per mole of enzyme before any effect is noted upon the enzymatic activity and twenty-two more equivalents are required before inhibition is complete.

This disparity between the numbers of ions or molecules of the inhibitor required for full inactivation of urease indicated that a more thorough examination of the inhibition by silver ions is desirable. Both of the previous studies evaluated only the total concentration of the inhibiting substance in the enzyme solutions. This left open the possibility that the reaction with the enzyme was not complete. In the present experiments both the total concentration of silver and the concentration of free silver ions were determined, making possible an application of the mass action law.

Experimental Details

Preparation of Pure Enzyme.—The crystalline enzyme was prepared from jackbean meal obtained from the Arlington Chemical Company, Yonkers, New York, batch numbers 490,428 and 490,208. The preparative procedure finally adopted involved repeated crystallizations from acetone–water mixture according to the general method developed by Sumner,[7] although in details some changes were made. In particular, no preservatives of any kind were introduced into the enzyme solutions.

(1) (a) Bell Telephone Laboratories, Murray Hill, N. J. (b) Monsanto Predoctoral Fellow, Shell Development Co., Emeryville, Calif.

(2) (a) J. B. Sumner, *Proc. Soc. Exptl. Biol. Med.*, **24**, 287 (1927); (b) J. B. Sumner and D. B. Hand, *J. Biol. Chem.*, **76**, 149 (1928).

(3) J. B. Sumner and G. F. Somers, "Chemistry and Methods of Enzymes," Academic Press, Inc., New York, N. Y., 1947, p. 157.

(4) J. B. Sumner and K. Myrback, *Z. physiol. Chem.*, **189**, 218 (1930).

(5) L. Hellerman, F. P. Chinard and V. R. Deitz, *J. Biol. Chem.*, **147**, 443 (1943).

(6) J. B. Sumner, N. Gralen and I. B. Eriksson-Quensel, *ibid.*, **125**, 37 (1943).

(7) See also A. L. Dounce, *ibid.*, **140**, 307 (1941).

The determination of the activity of enzyme solutions was performed under the conditions defined by Sumner and Hand.[8] The technique of the determination has been outlined in the previous paper.[9]

The protein content of the solutions was determined by micro Dumas procedure. The protein factor of 6.5 was used.

The Study of Inhibition.—The inhibition was studied in 0.1 *M* citrate buffer of *p*H 5.6 containing citric acid and 2.5 equivalents of sodium hydroxide. This buffer was chosen in preference to phosphate since it does not interact strongly with silver. This was shown by Sumner and Myrback[4] by using potentiometric titrations utilizing, in effect, concentration cells. No potential difference was noted by them when 10^{-4} *M* or lower silver nitrate solutions were used in two half-cells, one containing also sodium citrate and the other no foreign ions. We have studied the interaction of silver with the citrate buffer in roughly the same range of silver ion concentrations. The appropriate silver solutions were obtained by equilibration of buffer solutions and of water, containing known concentrations of sodium chloride, with the silver chloride crystals, as well as by dilution of standard silver nitrate solutions. The method used was to test the solutions thus obtained with the dithizone reagent. In the samples obtained by dilution the light transmission was the same, within the experimental error, in the solutions containing 0.1 *M* citrate buffer and those not containing any buffer. In the equilibration experiments the readings were also identical, after they were corrected for the effect of the ionic strength of the buffer solution on the solubility product of AgCl, using a mean ionic activity coefficient of 0.70. This shows that the solubility of silver chloride, aside from the ionic strength effect, is the same whether citrate ions are present or not; in other words, that the concentration of possible citrate–silver complexes is small compared to the concentration of "free" silver ions.

Solutions of enzyme, all in the range of 10^{-9} *M*, were made up in a 2 l. volumetric flask and transferred to a 3 l. Florence flask which was then thermostated at 20° and allowed to come to temperature equilibrium over a period of 1 to 2 hours. The solution was stirred mechanically all the time. After the temperature equilibrium had been established, the activity of two 20-cc. portions was measured, this being taken as the uninhibited activity. Small aliquots of a standard silver nitrate solution (usually 10^{-5} *M*) were then added, 20 cc. of the mixture being withdrawn after each addition for activity measurements. The activity was measured in the manner previously described,[9] the modification being to run the hydrolysis for only 1 or 2 minutes in order not to exceed the capacity of the buffer, and to run it in 3% urea solution to be as nearly as possible on the Michaelis plateau.

In general the additions of silver ions were of such magnitude that nearly twenty of them were necessary for an almost complete inhibition of the enzyme. Then a small amount of crystalline silver bromide and varying amounts of a standard sodium bromide solution were added until the activity (corrected for dilution) had reverted to its original value. In all runs the activity returned to within 10% of the original. In general the concentration of bromide ion necessary for this reversal of the activity was of the order of 0.05 *M*. Separate experiments showed that in absence of silver the activity of the enzyme in citrate buffer was not altered by making the solution 0.05 *M* in sodium bromide. The additions of bromide ion were made in such a sequence as to give roughly equal increments on the abscissas of a plot determining the order of the reaction with respect to silver ion (see below). All the runs were made at 20° and required from 3 to 5 hours for completion.

Sample 9 was investigated by a different procedure which, briefly, was as follows. In order to add known total quantities of silver ion to the enzyme, the buffer solutions containing known concentrations of bromide ion were equilibrated with a bed of large silver bromide crystals by passing the solution repeatedly through it. This gave the total concentration of the silver ion in the solution from the known solubility product constant of silver bromide, after allowing for the appropriate activity coefficients. To this solution was then added urease and its activity measured. To determine the inhibition in the presence of known concentrations of free silver ions, the buffered enzyme solutions containing a known amount of bromide ion were passed repeatedly through the same silver bromide bed. In these experiments, of course, the enzyme came into contact with large amounts of solid and this appeared to cause irreversible deactivation or elimination of the enzyme from the solution. In any case it was usually not possible to restore fully the original activity of the enzyme by adding bromide ions. The procedure, furthermore, was quite cumbersome and was therefore abandoned, but the experiment with sample 9 was deemed good enough for inclusion here, since enzymatic activity was restored to within 90% of the original, by adding bromide ions. Other experiments of this series gave qualitatively agreeing results, although their scatter was bad. This procedure was originally developed to minimize systematic errors that might arise from adsorption of silver ions on the walls of the glassware used, etc. The difficulties mentioned above made it not wholly satisfactory but the general agreement of data obtained by the two methods suggests, at least, that the adsorption was not of major significance. However it remains a possible source of systematic errors, the magnitude of which is impossible to estimate.

(8) J. B. Sumner and D. B. Hand, *J. Biol. Chem.*, **76**, 149 (1928).

(9) J. F. Ambrose, G. B. Kistiakowsky and A. G. Kridl, THIS JOURNAL, **72**, 317 (1950).

Results

The experiments showed a rapid and almost quantitative reaction of urease with silver ions, manifested by the inhibition of the enzyme. Figure 1 presents two plots which relate the activity of a typical enzyme preparation, expressed as the fraction of its activity in absence of silver, to the concentration of silver. Curve II shows the fractional activity as the function of the concentration of free silver ions, calculated from the known concentrations of bromide ions and a solubility product of silver bromide taken equal to 1.76×10^{-13} at 20°.

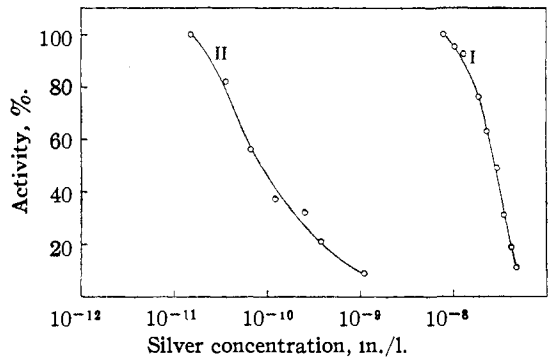

Fig. 1.—Activity of urease in presence of silver ions: curve I, total silver added; curve II, concentration of free silver.

Curve 1 shows fractional activity as a function of the total amount of silver added. The difference between the total and the free silver concentrations at any given fractional activity of the enzyme is the silver combined with the protein. If the fractional activity (A) is identified with the fraction of catalytically active sites in the enzyme molecules which have not combined with silver, then the data of Fig. 1 can be replotted as in Fig. 2, using the equation $(A_0 - A)/A = K_i(Ag^+)^n$, which is readily derived from the assumption that the equilibrium reaction is: enzyme + nAg$^+$ = (enzyme, Ag$_n^+$)$_{inactive}$. Such plots were made for all enzyme samples experimented with and gave sensibly straight lines having a slope near unity. Hence the equilibrium between silver and enzyme is representable by a single Mass Action constant and the stoichiometric equation is

enzyme + Ag^+ = (enzyme, Ag^+)$_{inactive}$. The straight line relationship observed in Fig. 2 could not be expected *a priori* because the enzyme might contain groups with different affinities for silver.

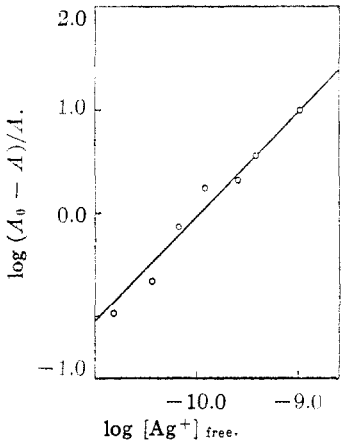

Fig. 2.—Order of the reaction.

Figure 2 demonstrates that in the investigated range of silver ion concentrations silver combines only with identical and mutually independent groups in the proteins. The plot does not prove that the reaction is limited to active urease. In fact, a simple extrapolation of the combined silver to total inhibition shows that this quantity depends on the degree of purity of the enzyme sample. This dependence can be shown more quantitatively with the aid of plots like the one shown in Fig. 3.

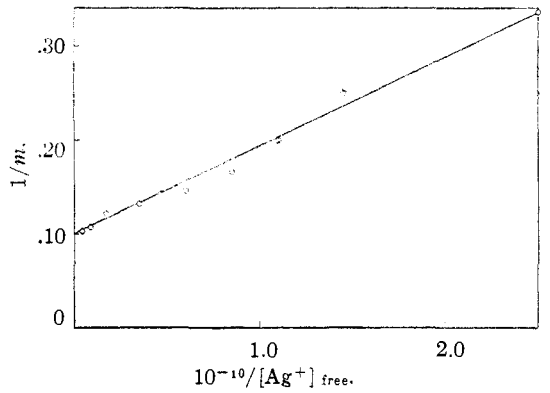

Fig. 3.—Binding of silver by a urease preparation.

They relate the amount of silver bound by the protein to the concentration of free silver. Assuming m_0 equivalent and independent groups, each reacting reversibly with one silver ion, the equation

$$1/m = K/(m_0(Ag^+)) + 1/m_0 \qquad (1)$$

is readily derived.[10] Figure 3 is a graphical representation of this equation, m, the number of silver ions bound per molecule of urease at a given ionic concentration of silver free in the solution (Ag^+) being expressed as the number of moles of silver ions per mole (483,000 g.) of urease. The molar concentration of urease was computed by dividing the activity per liter of urease samples by 133,000 × 483,000. Such plots were made for all enzyme samples and gave sensibly straight lines, the intercepts being $1/m_0$, the reciprocal of the number of silver ions causing total inhibition. Table I lists the results and shows that the number of silver ions bound at total inhibition decreases with increasing purity of the enzyme sample. It is therefore necessary to assume that the inactive protein possesses the same affinity for silver as does active urease. This is not an unreasonable conclusion because the method of preparation used might have resulted in materials which contained little else than the mixtures of active and deactivated urease. In the past the pure enzyme was obtained by the same crystallization procedure but with the addition of stabilizing agents, such as hydrogen sulfide or gum arabic. In the present work this protection was not practical and the technique of activity determinations precluded the use of concentrated enzyme solutions which are generally more stable. It was therefore expected that the solutions would contain variable amounts of inactivated enzyme, depending on the success of the efforts to avoid inactivation in the course of a preparation. As Table I shows, the results, in this respect, were far from uniform, although virtually identical purification procedure was applied to all enzyme samples, except Nos. 1 and 2 which were subjected to only one crystallization.

To determine the number of silver ions bound to a molecule of pure active enzyme the data of Table I require an extrapolation, which is shown in Fig. 4.

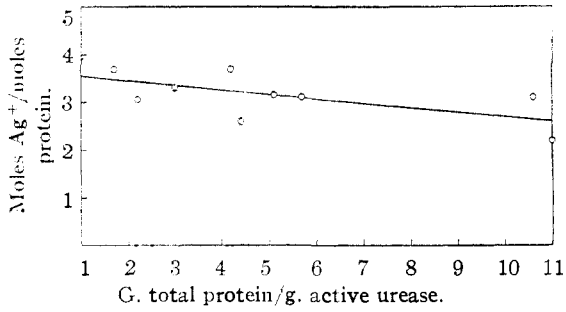

Fig. 4.—Binding capacity of urease preparations.

Assuming an activity of 133,000 units per gram for pure active urease,[6] the ratio of this quantity to the specific activity of a sample in Table I gives the number of grams total protein containing one gram of active urease for that particular sample. The ratio is at once a measure of purity, and a conversion factor from grams pure urease to grams total protein.

TABLE I

BINDING OF SILVER IONS AT COMPLETE INHIBITION

Urease sample no.	Purity, S.u./g.	Molarity × 10^9	No. of silver ions bound
1	12,600	3.95	32.8
2	11,900	3.33	24.2
3	23,500	8.04	17.4
4	26,000	4.38	16.0
5	32,000	3.80	15.6
6	30,000	4.73	11.4
7	45,000	4.38	10.0
8	60,000	5.22	6.7
9	78,000	7.90	6.3

(10) I. M. Klotz, *Arch. Biochem.*, **9**, 109 (1946).

If the m_0 values in Table I are divided by the value of this ratio for the particular sample considered, a value is obtained which represents the number of moles of silver ions bound at complete inhibition by 483,000 g. of total protein, rather than by 483,000 g., or one mole, of urease, as represented by m_0. The values so obtained may be plotted against the values of the ratio, which is likewise a measure of purity, as has been done in Fig. 4. It will be noted that an abscissa unity represents pure active enzyme, as one gram of total protein here contains one gram of urease. The plot clearly shows that the ordinate is nearly independent of enzyme purity, remaining between two and four. An extrapolation is hardly necessary to conclude that in the pure enzyme between three and four silver ions are bound at total inhibition. The constancy of the binding capacity of a constant mass of protein, coupled with the wide variation of the amount of active urease contained in it, argues strongly that the preparations contained only one species, urease, in active and inactive forms. However, the slight falling off of the binding capacity in the most impure samples may perhaps be attributed to the presence of another constituent.

One of three types of inhibition reactions may be involved here; silver may be reacting with the enzyme regardless of whether the latter combined with the substrate, urea; it may be reacting only with the free protein or it may be reacting only with the enzyme-substrate compound. If the first is the case, the addition of urea to the enzyme–silver solutions for the activity determinations does not affect the equilibrium; the second alternative means that upon the addition of urea some silver is displaced from the urease because the activity determinations were carried out at a urea concentration about hundred times higher than the Michaelis constant; the third means that silver combines with urease only after the addition of urea. Sumner and Myrback's experiments indicated competitive inhibition of the second type, but the authors emphasized the preliminary nature of the experiments. No explicit study of the character of inhibition was undertaken presently, but the non-competitive inhibition of the first type is made plausible by the following considerations. Enzyme solutions 1 to 8 incl. contained floating crystals of silver bromide as well as bromide ions, when studies of the free silver ion concentrations were made. These crystals should act as a source of additional silver ions if such were needed after the addition of urea; or they would serve as crystallization nuclei if excess silver ions were liberated upon the addition of urea. Sample nine (and others of that series) was a clear solution; the only silver contained in it was that already combined with the protein and that present to the limit of the solubility product of silver bromide. Hence the third of the above mentioned possibilities can be ruled out fairly securely: in sample 9 not enough silver was present to explain the inhibition if the reaction with urease took place only after the addition of urea. An elimination of the second alternative is less positive. The rate of hydrolysis was observed to be the same whether the experiment lasted one or two minutes; this agrees with the findings of Sumner and Myrback and shows that whatever shifts of equilibrium accompany the introduction of urea into the system, they must be exceedingly fast. This is perhaps possible in the presence of solid silver bromide, which provides the nuclei for the crystallization of excess silver ions, but is quite unlikely in clear solutions of such extreme dilution as characterized these experiments. Thus sample 9 should have shown different behavior from that of the other samples.

If the inhibition by silver is not of the competitive type, the data of Figs. 1 and 2 can be directly used to calculate the value of the inhibition equilibrium constant. The value obtained is about 10^{10} liters/mole, with some uncertainty because it rises slightly with the increasing purity of the enzyme. If competitive inhibition is occurring a combination of the reaction scheme: enzyme $+ n\mathrm{Ag}^+ =$ (enzyme, $\mathrm{Ag}_n^+)_{\text{inactive}}$ with the Michaelis–Menten mechanism of catalysis readily leads to the equation

$$\frac{A_0 - A}{A} = \frac{K_i K_m (\mathrm{Ag}^+)^n}{K_m + (\mathrm{S})} \qquad (2)$$

where A_0 and A are the original and the inhibited activities of an enzyme solution, K_i the inhibition constant, K_m the Michaelis constant, and (S) the concentration of the substrate. A plot of the type used in Fig. 2, therefore, still determines the order of the reaction and the finding that n is unity stands therefore. However, if the concentration of the substrate is much higher than the Michaelis constant, as was the case in the present experiments, the observed inhibition constant must be corrected by the factor $K_m/(\mathrm{S})$ to obtain the true equilibrium constant. This factor was of the order of 10^{-2} in the present experiments[11] and hence the true equilibrium constant is of the order of 10^{12} liters/mole.

Apart from the question of the competitive nature of the inhibition, the results here reported are not in real disagreement with the findings of Sumner and Myrback, when the combined uncertainties of both researches are taken into consideration. This is not true, however, of the findings of Hellerman that more than forty equivalents of *p*-chloromercuribenzoate are required for a total inhibition of urease, if this be interpreted as a measure of the number of active sites in a urease molecule. But Hellerman's work clearly indicates that *p*-chloromercuribenzoate reacts with some sulfhydryl groups which are not related to the enzymatic activity of urease; furthermore, the number of sulfhydryl groups found by him is in conflict with the observation of Smythe[12] that only twelve such groups are detectable with iodoacetamide. Finally, neither of these investigators allowed for the possibility of incomplete reaction of their reagents with the enzyme; their findings throw little light on the question of how many catalytically active sites are present in a urease molecule.

(11) K. M. Harmon and C. Niemann, *J. Biol. Chem.*, **177**, 601 (1949).
(12) C. V. Smythe, *ibid.*, **114**, 601 (1936).

The present experiments show that each catalytically active site, to which the Michaelis–Menten mechanism applies, is inhibited by combining with one silver ion. These sites must have identical affinity for silver and be mutually independent, to within the imperfect accuracy of the present measurements. The number of such sites cannot be larger than the number of silver ions required for total inhibition, *i.e.*, not larger than three or four; it may be smaller because some of the silver ions may react with some functional groups which are not a part of the catalytically active sites, but have identical affinity for silver. In fact the work of Sumner and Myrback has definitely shown that urease after total inhibition can react with considerable additional quantities of silver. However the affinity of this secondary reaction is relatively low; it should not have manifested itself in the present experiments. In all probability one deals here with electrostatic binding of silver ions to the protein, whereas the specific inhibition reaction is accompanied by such a large chemical potential change ($\Delta\mu^\circ \cong -15{,}000$ cal.) that a formation of covalent linkages is indicated. Cysteine has been observed[14] to form complexes with silver ions in acid *p*H which have an even higher stability. Also in basic solutions a rather stable cysteine–silver complex of the formula RSAg has been observed[15] and silver has been shown to combine with other proteins through the sulfhydryl groups.[16] Although all this evidence points to the inhibition of urease by silver through the reaction with sulfhydryl groups, it does not prove the occurrence of this reaction and the question of the exact nature of the chemical binding of silver must remain unanswered for the time being.

The observation that inactive protein present in recrystallized urease preparations has the same affinity and the same specific binding capacity for silver is of considerable interest. It explains, at least partly, the enhanced stability of impure enzyme preparations to heavy metal poisoning. It also suggests that within a catalytically active site there is present a functional group with a high affinity for silver ions; its reaction with silver causes the loss of catalytic activity by the site, but the inactivation of the site, in the course of crystallization operations, does not block the group from reacting with silver. Such behavior is readily understood if the catalytic activity of a site is due to a particular spatial configuration of several functional groups that may comprise it and the deactivation is due to the loss of this configuration rather than to direct chemical changes in the functional groups. Until the competitive or non-competitive character of the inhibition by silver has been established it is impossible to decide whether the group reacting with silver is involved in the primary reaction of the enzyme with the substrate or whether this group is essential to the second step of the Michaelis–Menten mechanism, the hydrolysis of urea.

(14) S. Valladas-Dubois, *Compt. rend.*, **231**, 53 (1950).
(15) I. M. Kolthoff and W. Stricks, THIS JOURNAL, **72**, 1952 (1950).
(16) R. Benesch and R. E. Benesch, *Arch. Biochem.*, **19**, 35 (1948).

CAMBRIDGE, MASS. RECEIVED SEPTEMBER 18, 1950

[CONTRIBUTION FROM THE NATIONAL BUREAU OF STANDARDS]

Specific Heat of Beryllium between 0° and 900°[1]

BY D. C. GINNINGS, T. B. DOUGLAS AND ANNE F. BALL

Using an ice calorimeter and furnace of improved design, the specific heat of two samples of beryllium was determined by measuring the changes of enthalpy between 0° and 900°. Both samples were more than 99% pure, and additive corrections were applied for the impurities present. Although one sample contained 0.4% magnesium and the other almost none, the specific heat values found for the two samples differed by less than 1% at every temperature investigated, a result in sharp contrast to the large variations found by other workers for various samples of beryllium of purities comparable to those of this investigation. Sources of error in the values obtained are examined. The values of C_v calculated from the data are found to fit the Debye function up to 500°, but near this temperature an abrupt change in slope occurs.

Introduction

The specific heat of beryllium has been determined by a number of observers, with reported values differing by as much as 30%. One observer claimed that the specific heat of beryllium depended on its heat treatment. Another claimed differences of 30% in heat capacity, due to only 1% impurity. The present investigation was undertaken in an effort to clear up some of these discrepancies and, if possible, to obtain accurate values for the specific heat of beryllium.

Samples.—Two samples of beryllium were used. Most of the experiments were made with Sample I, which was of the purest beryllium metal available (1947) from the Chemistry Division of the National Bureau of Standards. This sample, designated by them as BL, was in the form of pieces about 0.1 cubic millimeter in size. The analysis reported from this stock sample showed 99.5% total beryllium, 0.3% oxygen, 0.1% water and 0.3% total of numerous other metallic elements. The sample was used without further treatment except that some of its moisture may have been removed in the process of pumping preliminary to enclosing it. Further check experiments were made with a second sample of beryllium (Sample II, "Brush QT powder," in pieces about 100 cubic millimeters in size) having a somewhat similar analysis except that it contained more magnesium (0.4%), the other metals totaling 0.1%. There was essentially no magnesium in Sample I.

Method and Apparatus.—The method of meas-

(1) Presented before the American Chemical Society, Division of Physical and Inorganic Chemistry, April 20, 1950, in Detroit, Michigan.