CLX. THE ABSORPTION OF WATER BY GELATIN.

IV. THE INFLUENCE OF TEMPERATURE.

By DOROTHY JORDAN-LLOYD.

From the Laboratories of the British Leather Manufacturers' Research Association.

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INTRODUCTION.

ALTHOUGH the bulk of the work recorded in the present series of papers has been carried out at 18°, there are two drawbacks to this particular temperature. The first is that the gelatin must be considered as a mixture of the two heat-reversible forms, *A* and *B*, *A* being the sol-producing form, which begins to appear only at temperatures of 15° and upwards and has an optical rotatory power of -141° , *B* the gel-producing form, which exists only at temperatures below 35° and has an optical rotatory power of -313° [Smith, 1919]. The second objection is that considerable hydrolysis of the gelatin occurs in the alkaline solutions, thus adding an additional factor to a system already sufficiently complex in itself [Jordan-Lloyd, 1929]. In order, therefore, to study the problem in a form as simplified as possible, a number of readings were made at 0°. The pure gelatin used was prepared in the manner already described [Jordan-Lloyd and Pleass, 1927].

The solutions were placed in small glass jars fitted with ground glass stoppers. The jars intended for use with alkaline solutions were coated on the inside with hard paraffin wax to prevent any contact of the alkali and the glass. The solutions and the weighed gelatin pieces were left overnight at 0°, before the actual experiments were started. All alkaline solutions were made up with carbon dioxide-free water and experimental jars containing alkaline solutions were kept throughout the whole course of the experiments in closed containers over soda-lime. This precaution was found absolutely essential in any case where it was desired to keep an unbuffered alkaline solution at a constant p_H value for any length of time. The rapidity with which the carbon dioxide of the air is absorbed by these solutions creates a serious experimental difficulty. A lowering of the p_H value will occur even while an experimental jar has the stopper lifted only for the few minutes that are required to remove the piece of gelatin. For this reason it is impossible, with any degree of accuracy, to follow the swelling of the same piece of gelatin over a long period

of time if the experimental solution is alkaline (see Fig. 2). Every experimental jar containing an alkaline system can be used to obtain one experimental point and one only. The following method, therefore (based on a paper by Cameron [1929]), was adopted for determining the final p_H value of the experimental solutions. Comparator test-tubes, each containing 0.05 cc. of standard indicator solution, were taken to the cold chamber and 5 cc. of the experimental solution were placed in each tube which was then tightly corked. The series was matched for colour by titrating 10 cc. of a standard buffer solution with a standard $N/10$ solution of hydrochloric acid or sodium hydroxide. The relative volume of indicator present was maintained approxi-

Fig. 1. Rate of swelling of pure gelatin at 0° in various solutions.

• Water. × 0.2 M NaCl. ○ 0.2 M NaNO₃. + 0.001 N HCl, $p_H=3.1$.

mately constant throughout the titration by continual additions of appropriate small quantities. From the volume of standard acid or alkali required to bring the colour of the buffer solution to that of the experimental tubes the p_H value was read from a graph which had previously been prepared and which gave the titration curve for the standard buffer under the same working conditions. This colorimetric method, which gives p_H readings for the experimental tubes accurate only to 0.1 on the p_H scale, appears to be the most suitable one available for the determination of the p_H values of unbuffered dilute alkaline solutions. The colours do not appear to be noticeably influenced by a temperature change from 0° to 18°. In every case, with the dilute alkaline solutions

there was a noticeable change of colour at the surface in contact with the air. As long, however, as the tubes were kept stationary, this colour change did not travel further down the tube than a distance of about 5 mm., and this portion could easily be covered over in using the tube for a colour match. The method is only available over a range of p_H extending from about 1.5 to 9.5. The following indicators were used:

	p_H range
Thymol blue	1.2-2.8
Methyl orange	2.9-4.0
Bromophenol blue	2.8-4.6
Bromocresol green	3.6-5.2
"Universal indicator"	5.0-9.5 (approximately)

Fig. 2. Rate of swelling of pure gelatin in 0.001 *N* solutions of NaOH at 0°. The curves show the loss of alkalinity on each successive opening of the experimental jars after 42 days.

x = first opening, $p_H=9.9$. . = second opening, $p_H=9.5$. ⊙ = third opening, $p_H=9.2$.
 Δ = fourth opening, $p_H=9.9$. ▲ = fifth opening, $p_H=7.7$.

Attempts to extend the range on the alkaline side were not successful. The colour change with "Universal indicator" becomes too sluggish for accuracy beyond p_H 9.5; thymol blue and alizarin yellow were tested, but determinations made with these indicators were not found satisfactory. With the more alkaline solutions, however, the error due to manipulation in the electrometric method becomes relatively small and, therefore, in these cases, p_H determinations were made by means of the closed hydrogen electrode. On the acid side, the determinations not made colorimetrically were generally made with the glass electrode.

In all experiments, except where otherwise mentioned, 0.05 g. of gelatin was used with 50 cc. of solution. It has, however, previously been shown that the relative amounts of gelatin to solution do not influence swelling [Jordan-Lloyd, 1929, and in the press] except in alkaline solutions at 18°. Mixtures were given 10 days at 0° to reach equilibrium (see Fig. 1). In a few cases, experiments were made at 25° and at this temperature equilibrium is reached in 5 hours.

All experiments, except in strongly acid or alkaline solutions were carried out in the presence of toluene.

SWELLING CURVE FOR HYDROCHLORIC ACID AND SODIUM HYDROXIDE
AT 0°, 18° AND 25°.

In Fig. 3 is shown the swelling curve at 0° for gelatin in hydrochloric acid and in sodium hydroxide solutions for a range of p_H values from 0.1 to 14.

Fig. 3. Swelling of pure gelatin at various temperatures.

—•—•— 0°; p_H of neutrality, 7.35. - - - - - 18°; p_H of neutrality, 7.06.
× ······ × 25°; p_H of neutrality, 6.95.

On the same figure is drawn the curve for 18° as determined by earlier work [Jordan-Lloyd and Pleass, 1927]. In comparing the two curves it will be seen that, although generally similar, they differ in certain striking respects. Firstly, the curve at 0° is uniformly lower. (This is not in full agreement with earlier published work, but in this earlier work it had not been realised that for accurate figures both gelatin and solution must be cooled separately to 0° before the beginning of the experiment.) The p_H at maximum swelling in acid is not very different in the two curves, but where at 18° the acid curve shows a secondary increase of swelling, leading to solution at p_H values less than 1.1, at 0° the curve runs out to a level value. The solution of gelatin in concentrated strong acid at 18° is not due to hydrolysis [Jordan-Lloyd, 1920]. If it be due to any change in the molecule, the change is certainly reversible, since the gelatin can be recovered and will dissolve in hot water and set to a gel. Whatever the cause, solution does not occur at the low temperature. The solution that occurs in concentrated solutions of sodium hydroxide at 18° takes place also at 0°. Pure gelatin dissolved in water by warming is completely precipitated by half-saturation with ammonium sulphate, giving a clear filtrate that does not give a biuret reaction. Pure gelatin dissolved in strong alkalis

by standing at 0° gives (after neutralisation of the solution) a very heavy precipitate on half-saturation with ammonium sulphate. The clear filtrate from this gave in two experiments a very faint biuret reaction and a further very faint turbidity on full saturation with ammonium sulphate. The solution of the gelatin is, therefore, not due to hydrolysis, though it is accompanied by a slight hydrolysis. Solution, however, is accompanied by a change in character of the gelatin molecule, possibly due to racemisation [Jordan-Lloyd, 1920].

In the less concentrated alkaline solutions (p_H 5–13), the swelling curve at 0° presents two points of contrast with the curve at 18° ; the degree of swelling is considerably less and the p_H value of the external solution at the point of maximum swelling is considerably higher in the colder system. At 18° maximum swelling occurs at p_H 9.9; at 0° it is at about p_H 11.5. There is some evidence that at 25° maximum swelling is at p_H 7.2.

Another feature of very great interest in the curve for 0° is the existence of a flat plateau in the curve of alkaline swelling. At 18° , this flat portion of the curve lies between p_H 7 and 8. Between these two values, swelling is only very slightly affected by change of p_H value. At 0° this flat portion extends from p_H 7.5 to 10.5. At 25° the flat portion of the curve is no longer in evidence.

A few points have been obtained which indicate approximately the swelling curve at 25° . Under these conditions and with the gelatin used, complete solution occurred at p_H values less than 3 or greater than 10. In the acid zone, therefore, swelling increases with decreased value of p_H , but no maximum is reached, increased swelling leading to complete solution of the gelatin. On the alkaline side, there appears to be a peak of alkaline swelling at about p_H 7, followed by a second rise at p_H values greater than 9. At 25° all points in the alkaline zone ($p_H > 7$) are, however, liable to large errors on account of hydrolysis of the gelatin.

The shape of the curve at 25° indicates that swelling increases with temperature at all values of p_H except over a narrow range from p_H 5–6.5 (roughly). At 25° the characteristic form of the swelling curve, which is clearly shown at 18° as well as at 0° , has disappeared. The typical features of swelling curves may be attributed, therefore, to the presence of gelatin B.

The most striking feature of acid and alkaline swelling over the range of temperature examined is that with acid swelling the p_H value at maximum swelling is unaffected by temperature, whereas with alkaline swelling the p_H at maximum swelling decreases as the temperature is raised [see also Pleass, 1930].

SWELLING IN SALT SOLUTIONS AT 0° , 18° AND 25° .

The swelling in salt solutions was examined for the three sodium salts, chloride, nitrate and sulphate. The salts were used in concentrations of 0.2 *M*, 0.5 *M* and 1.0 *M* and were only examined at p_H 5.5–6.5 approximately. The temperatures of the experiments were 0° , 18° and 25° . The experimental results are recorded graphically in Figs. 4, 5 and 6.

Fig. 4. Swelling of pure gelatin in solutions of sodium chloride at p_H 5.5.

Fig. 5. Swelling of pure gelatin in solutions of sodium nitrate at p_H 5.5.

Fig. 6. Swelling of pure gelatin in solutions of sodium sulphate at p_H 5.5.

The slope of the curve of swelling plotted against concentration of salt shows a characteristic value for each salt as long as solution of the swollen jelly has not actually set in. This shows well in the chloride curves (Fig. 4) where the slope of the curves for swelling plotted against salt concentration is the same at 18° and 25° as at 0°. The influence of the chloride on the swelling of gelatin is, therefore, equally affected by concentration at all temperatures. With nitrates, when the gelatin is on the verge of solution at 18°, the curve is different in form at 18° and 0° (Fig. 5). At 25° solution occurred in all three solutions of nitrates.

It is interesting to note that, in the sulphate system at 0°, the coagulation of the gelatin that is characteristic of concentrated solutions of sulphates at 18° cannot occur since these concentrations are in excess of saturation at the lower temperature. At 25° the extent of coagulation is the same as at 18°, but there is very little swelling due to the action of the sulphate in the solutions with a concentration of 0.5 *M* or less, the level for these points being the same as in distilled water at the same temperature.

In all the experiments with salt solutions, the influence of temperature on salt action appears to be the same at all concentrations of salt, as long as the jelly is not on the verge of solution or as long as the salt remains in solution. The broken line curves in Figs. 4, 5 and 6 are reproduced from the previous papers in this series. They were made for experiments at 18°.

DISCUSSION.

An examination of the slope of the curves of swelling of gelatin, in the presence of various electrolytes and over a range of temperature from 0° to 25°, shows that in any investigation of gelatin as a colloidal jelly, *i.e.* as a colloidal system characterised by the existence of the colloids in the form of coherent fibrils, only experimental data gathered at the lower temperatures present a true picture of the behaviour of the jelly. The forces of cohesion between the gelatin aggregates that lead to the orientation of these and the formation of the ultra-microscopic fibrils which are characteristic of the jelly are strongly affected by temperature and diminish rapidly with rising temperature. They are obviously also affected by the condition of the gelatin either as uncharged aggregates or as charged micellae, since the critical temperatures at which the forces of cohesion are overcome by the forces of solution are influenced by the p_H value of the system; isoelectric gelatin in water remaining coherent at temperatures below 35°; gelatin chloride at p_H 2.6 or less and sodium gelatinate at p_H 9.5 or greater, becoming completely dispersed at 25°. The swelling curve at 0°, a temperature at which the forces of cohesion remain considerable in all but strongly alkaline solutions ($p_H > 13$), presents, therefore, features of special interest. The striking feature of this curve is the lack of symmetry about the point of minimum swelling, which may be assumed, in the absence of dissolved salts, to approximate very closely to the isoelectric point. On theoretical grounds, if due regard is paid to the fact that the jelly is a system consisting

not only of gelatin but also of water, besides either an acid or an alkali, this lack of symmetry is not surprising, for although for the gelatin the dividing line between the properties of gelatin functioning as a base and gelatin functioning as an acid might be expected to occur at the isoelectric point of the gelatin, namely p_H 5, as regards that extremely important component of the system, water, the expectation undoubtedly would be that an axis of symmetry would occur at absolute neutrality, namely p_H 7.35 at 0°, 7.06 at 18° or 6.95 at 25° [Michaelis, 1914]. This important point appears previously to have been overlooked, but it is certainly merely an assumption that the internal pressure and cohesion of water will be equally affected by an excess of hydrogen ions and by an excess of hydroxyl ions. On the acid side of the isoelectric point of gelatin, therefore, there will be no reversal of properties either of gelatin or of water with increasing concentration of acid. In concordance with this, the curves of swelling of gelatin in acid solutions are comparatively simple. The general form of the curve is not affected by temperature, the p_H of maximum swelling measured in the external solution is at p_H 2.6 for all temperatures and the swelling can be interpreted equally well on Procter's theory at 0° as at 18° or 25°. This theory is based on the assumption that there is an excess of diffusible ions in the jelly compared with the external solution, which leads to an osmotic pressure that causes the jelly to swell until the swelling forces are balanced against the cohesive forces of the jelly. This theory has been established by a considerable body of experimental data obtained by Procter himself [1914], Procter and Wilson [1916] and by Loeb [1922]. None of these writers discusses the mechanism of the forces which lead to the appearance of the osmotic pressure, but the kinetic theory of osmotic pressure is generally implied in their work. An understanding of what osmotic pressure actually is is essential to a full understanding of the problem of gelatin swelling. An excellent review of recent work on the subject is given by Lewis [1925]. This review includes an account of Tinker's theory of osmotic pressure, which attributes the force to a solvent pressure, which is greatest in the pure solvent and falls in solutions owing to the constraint due to the presence of the solute molecules. Even if it be assumed, in accordance with Tinker's theory of osmotic pressure, that the force actually causing swelling in a jelly is a pressure due to the solvent, in this case water, there is no need to modify the Procter theory of swelling in acid solutions. As long as the mechanism of osmotic force can be regarded as hydrostatic in nature, the interpretation of the results adapts itself easily to modern views as to the nature of osmotic action. In fact, certain phenomena become easier of interpretation, particularly the observation that acid-swollen jellies extrude water in an atmosphere of saturated vapour, for, assuming that osmotic equilibrium is established in the jelly when the solvent pressure on one side of the jelly-water interface is equal to the solvent pressure on the other, then it is easy to see why the removal of the external fluid phase, and hence of the external solvent pressure, should be followed by a flowing out of water from within the jelly. The implications of

Tinker's interpretation of the mechanism of osmotic pressure cannot, however, be followed here.

To return to the consideration of the swelling curve at 0°, there remains for consideration the effect of temperature on the swelling of gelatin in solutions alkaline to the isoelectric point (not necessarily alkaline themselves). In these systems, as the p_H value increases from 5, there is a reversal in the properties of the water which changes in character from acid to alkaline, *i.e.* it changes from a solution having an excess of hydrogen over hydroxyl ions, to one in which the reverse condition is true. How far this change is of primary importance in determining the shape of the alkaline swelling curve, it is, at the moment, difficult to say. The fact remains that the curve of alkaline swelling cannot be interpreted on the same simple osmotic basis as applies on the acid side. The form of the alkaline curve changes considerably with temperature. The p_H value at which solution occurs and also the p_H value of maximum swelling decrease with rising temperature, and conversely the range of p_H over which the p_H value is without influence on swelling becomes more and more extended as the temperature falls. This interesting characteristic of the alkaline system is also shown in the independent observations of Pleass [1930] on the swelling of gelatin in solutions of calcium hydroxide. The zone of constant degree of swelling at variable p_H is well marked at 0°, considerably abbreviated at 18° and non-existent at 25°.

The interpretation of alkaline swelling, therefore, is not easy, for, in addition, the alkaline solutions tend to produce irreversible chemical changes in the gelatin molecule [Dakin, 1912-13]. There is no doubt, however, that the characteristic peak of alkaline swelling is due mainly to forces of an osmotic nature. Over this range of p_H , the alkali-swollen gelatin resembles acid-swollen gelatin in that the addition of small quantities of normal salts leads to a suppression of the swelling, or transference of the swollen jelly from a liquid to a vapour phase is followed by the extrusion of water by the jelly. Over the zone of alkalinity lying towards the isoelectric point, this osmotic interpretation will not hold. Gelatin at the isoelectric point and in pure water does not swell by osmosis (*i.e.* by solvent pressure) but by imbibition. The latter is a process characterised by evolution of heat and contraction of volume. The forces of imbibition are not hydrostatic since very large pressures fail to inhibit imbibitional swelling. There is no doubt that the water of imbibition combines in some manner with the gelatin and that there is a loss of free energy to the system. Imbibition occurs in all aqueous solutions and, under all conditions, a part of the swelling of dry gelatin is to be attributed to it. Imbibition does not seem to be greatly affected by acid conditions, but a certain amount of circumstantial evidence suggests that it may be influenced by dilute alkalis and by salt solutions. The latter stimulate gelatin swelling. Their action, however, is not osmotic, *i.e.* it is not to be attributed to the creation of a Donnan equilibrium on either side of the gelatin-water interface. Salt action is primarily due to direct influence on the cohesive forces

of the gelatin fibril. (It has already been pointed out elsewhere that gelatin, swelling in salt solutions, loses all coherence and is completely dispersed if the swollen weight reaches the figure of 4000 % of the dry weight, while gelatin swelling in acid or alkaline solutions can maintain coherence up to 7000 or even 10,000 %.)

Solutions of sodium hydroxide have a similar influence on the swelling of many different kinds of fibres. Hairs, for instance, which are physically very different from jellies, swell in dilute solutions of the alkali or alkaline earth hydroxides [Kaye and Marriott, 1925]. Wool fibres show the same behaviour. This property, however, is not due to the protein nature of these fibres, since cellulose fibres swell in these alkaline solutions in exactly the same manner. Speakman [1930] attributes the swelling of the wool in water to hydration at the peptide links of the elongated protein molecule. The swelling of the viscose fibre in alkaline solutions has also been attributed to hydration, though the locality in the molecule at which this may occur has been left vague. An important point about fibre swelling is that in the wool fibre, at any rate, it brings about very little change in the X-ray diagram of the fibre [Eroles and Speakman, 1930]. This suggests that imbibition is not accompanied by any displacement in the molecular components of the long keratin molecule and hence is more probably to be attributed to the separation of these elongated molecules allowing water to pass between. In other words, the volume of the free space between the fibrils (or molecules) is increased and therefore the total volume of the fibre is increased. How far the increased hydration due either to dilute alkalis or to salts can be explained on the same basis is still uncertain.

The application of this theory to the case of the gelatin jelly follows readily. A fibre is to be regarded as a body composed of long molecules or fibrils, all arranged parallel to the axis of the fibre. A jelly can be regarded as composed of exactly the same long molecules or fibrils, only arranged haphazardly throughout the jelly, *i.e.* without geometrical order. The same forces, however, which cause a spreading of the fibrils of the fibre with increase of volume, will also cause spreading of the fibrils of the jelly, again with increase of volume. The swelling of gelatin in very dilute alkaline solution can, therefore, be attributed to the spreading of the fibrils brought about by hydration of the fibrils. This type of swelling is most definitely marked at p_H 5.5 to 8.0. It is not to be confused with the osmotic swelling due to the formation of gelatin salts and their influence on the solvent pressure on either side of the gelatin-water interface. Some osmotic swelling will undoubtedly be present at all reactions alkaline to the isoelectric point. At 0° the osmotic swelling is small compared with hydration until the alkalinity becomes greater than p_H 10. At p_H 11.5 osmotic swelling (due to solvent pressure) is at a maximum. At 18° the maximum of osmotic swelling is at p_H 10.

The hydration swelling of alkalis resembles in many respects the swelling induced at the isoelectric point by salts. It is important to notice in this respect that salt solutions at high temperatures and neutral reactions have

the same swelling and solvent action on viscose fibres as solutions of the alkali hydroxides [von Weimarn, 1927]. With the gelatin jelly, the swelling at 0°, 18° and 25° is independent of the volume of salt solution and is directly proportional to the logarithm of the concentration of the salt. If swelling be called S , then

$$S = a \log C,$$

where a is a constant depending on the nature of the salt, but, as long as swelling has not proceeded so far that the coherent framework of the jelly starts to rupture, a is independent of the temperature. This property should give a clue to the nature of the forces causing salt swelling. It is an interesting observation that with salt swelling which leads to solution, the degree of swelling plotted against time is a sigmoid curve, a fact which suggests that solution occurs at a critical point at the moment of rupture of the jelly framework.

It appears probable to the writer that no interpretation of the whole range of water absorption of gelatin is possible until further information is obtained on the influence of very dilute alkalis and of solutions of salts on the properties of water. It is, however, abundantly clear that with gelatin and water, three mechanisms of swelling are in action:

- (1) imbibition, or restoration of water to a preformed structure;
- (2) osmotic swelling, due to an unequal solvent pressure in the solution and in the jelly: this is characteristic of acid solutions and alkaline solutions with a p_H value greater than p_H 7;
- (3) swelling of hydration, characteristic of salt solutions and of solutions of p_H greater than 5.5 but less than 7.

SUMMARY.

1. In hydrochloric acid, the swelling of gelatin at 0° rises to a maximum at p_H 2.6 and falls with increasing hydrogen ion concentration to a value which becomes stationary over a long range of p_H . Gelatin does not dissolve in concentrated solutions of the acid at 0°.

2. At 0° the swelling of gelatin in sodium hydroxide solutions rises with increasing value of p_H from 5 (the point of minimum swelling) to 7.5. There is an extended range of p_H from 7.5 to 10, over which variation of the alkalinity has little effect on the swelling. In solutions more alkaline than p_H 10, there is a well-defined peak of maximum swelling at p_H 11.5. Gelatin dissolves at 0° in alkaline solutions at p_H 13 and upwards.

3. The p_H of maximum swelling in acid solutions is not affected by temperature.

4. The p_H of maximum swelling in alkaline solutions is strongly affected by temperature; the lower the temperature, the greater the p_H value at the point of maximum swelling.

5. In solutions of sodium chloride or nitrate at or near the isoelectric point, the absorption of water by gelatin increases with rising temperature.

Unless the system is on the verge of solution, the influence of temperature is the same over a wide range of concentration.

6. Coagulation of gelatin gels by concentrated solutions of sodium sulphate cannot occur at 0° , owing to the decreased solubility of the sulphate.

7. At 25° gelatin dissolves in acid solutions of $p_H < 3$; in alkaline solutions of $p_H > 9.5$. There is no maximum of acid swelling, but a maximum of alkaline swelling occurs at $p_H 7.2$.

8. In acid solutions, the swelling of gelatin jellies can be attributed to osmotic pressure (solvent pressure). In alkaline solutions, the swelling is only partly due to osmotic pressure (solvent pressure); it is also partly due to hydration.

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REFERENCES.

- Cameron (1929). *J. Amer. Leather Chem. Assoc.* **24**, 76.
Dakin (1912-13). *J. Biol. Chem.* **13**, 357.
Eroles and Speakman (1930). *Proc. Roy. Soc. Lond.* B **105**, 600.
Jordan-Lloyd (1920). *Biochem. J.* **14**, 147.
— (1929). *Koll. Z.* **48**, 342.
— and Pleass (1927). *Biochem. J.* **21**, 1352.
Kaye and Marriott (1925). *J. Inter. Soc. Leather Trades Chem.* **9**, 591.
Lewis (1925). A system of physical chemistry, 4th ed. (London).
Loeb (1922). Proteins and the theory of colloidal behaviour (New York).
Michaelis (1914). Die Wasserstoffionenkonzentration (Berlin).
Pleass (1930). *Biochem. J.* **24**, 1472.
Procter (1914). *J. Chem. Soc.* **105**, 313.
— and Wilson (1916). *J. Chem. Soc.* **109**, 307.
Smith (1919). *J. Amer. Chem. Soc.* **41**, 135.
Speakman (1930). *Chem. Ind.* **49**, 209 T.
v. Weimarn (1927). *Koll. Z.* **43**, 43.