THE INFLUENCE OF SALTS ON THE ISO-ELECTRIC BEHAVIOR OF THE PROTEIN.

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Introduction. The point of maximum flocculation of such a difficult soluble protein as rice-glutelin or casein does not show the iso-electric point of the protein. We call this point an apparent iso-electric point.(3) This apparent iso-electric point is movable according to the concentration and kind of salts in the buffer solution. This is caused by the fact that the protein in the salt solution is not able to behave as an iso-electric protein, because the addition of salt causes a change in the hydrogen ion activity of the solution, and the cations and anions, which are derived by the dissociation of the salt, force the protein molecule to ionize in the opposite charge and in a different degree. Hence, the protein molecule will be ionized

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(3) K. Kondo and T. Hayashi, *Memoirs Coll. Agri. Kyoto Imp. Univ.*, No. 2, Art. 2 (1926).

according to the differences among the protein ionizing powers of the ions derived from the salt. Therefore, such a protein as casein or rice-glutelin under the presence of salts, cannot take an iso-electric form, even in the iso-electric solution. E. J. Cohn(1) has already stated that the presence of the foreign protein (with different solubility and different iso-electric point), of multivalent ions, or of neutral salts may shift the point of minimum solubility of the protein; hence the presence of these impurities renders impossible the determination of the true iso-electric point. This opinion may be reasonable from one point of view.

Moreover, if the concentration of the salt increase, the protein molecules may combine with one component of salt and form a complex compound. This complex-compound may be soluble or insoluble according to the concentration of the salt. Hence, the protein may not flocculate in a simple iso-electric form at the point of maximum flocculation in such a salt solution. These influences of salts on the iso-electric behavior of the protein are variable according to the kinds of salts and their concentration. The present work has for its purpose a more distinct elucidation of the above phenomena according to experimental results. The details are described in the Memoirs of the College of Agriculture, Kyoto Imperial University, No. 5.

Experimental Results and Conclusions. After the experiments described in the preceeding report(2) we know that the rice-glutelin, studied in this work, can flocculate at the optimum at paH 5.38–5.08 in a 0.10 n. sodium acetate solution, which is 0.02–0.04 n. in respect to acetic acid. Therefore, we have now to study the influence of salts on the rice-glutelin in such an apparent iso-electric solution as described above. The experiments were undertaken with chlorides, for comparing the influences of the various cations. The results are shown in the following figures. The figures, marked with "a", show the change of the iso-electric reaction, which forced after the addition of the various chlorides. And the figures, marked with "b", show the change of the precipitability of the iso-electric rice-glutelin under the addition of the chlorides.

As shown in the next figures, untill the salt-concentration increases to a certain degree, the higher the concentration becomes, the less the precipitability of the rice-glutelin. This phenomena may be explained by our theory, that is, the higher the salt-concentration increases to a certain limit, the greater the difference in protein-ionizing power between the cations and anions derived from the salts. Hence, the protein molecule may ionize as much as correspondent with the difference of powers, and in

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(1) E. J. Cohn, *Gen. Physiol.*, 4., No. 6 (1922), 697.

(2) K. Kondo, T. Hayashi and T. Matsushita, *Memoirs Coll. Agri. Kyoto Imp. Univ.*, No. 2, Art 3 (1926).

result the protein may dissolve a part. This is one of the principles on which the so-called solvent action of the salt on the protein is based. However, if the salt-concentration increase over the limit, the salt or one of its components may combine with protein and result in a further precipitation. This precipitation may be due to the formation of a complex compound

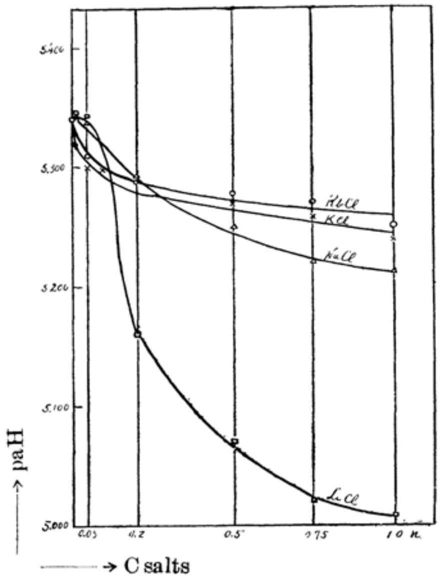

Fig. 1 a.

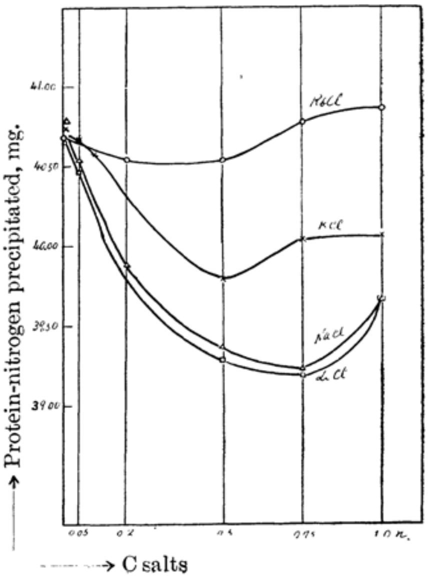

Fig. 1 b.

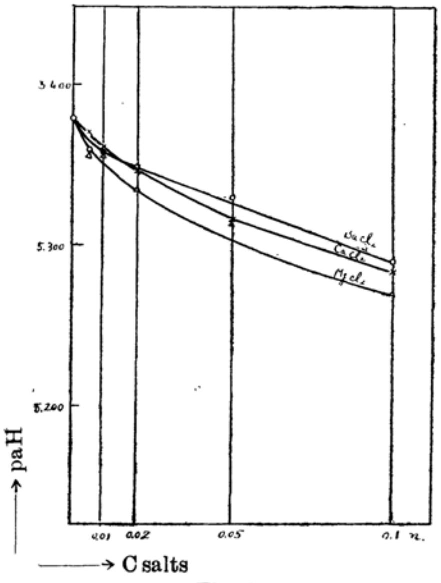

Fig. 2 a.

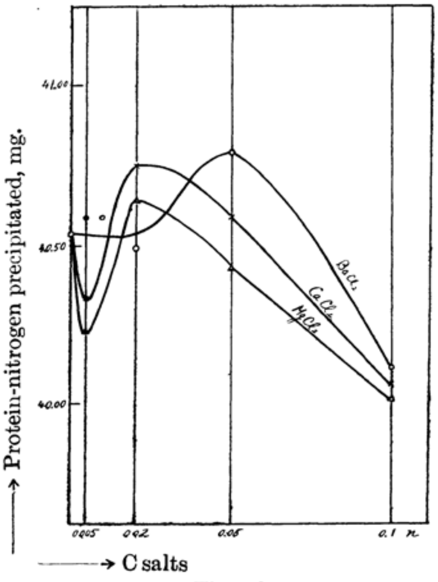

Fig. 2 b.

combining the protein and one component of the salt. This may be an original principle of the so-called precipitating action of the salt on the protein. Then, the protein-precipitate here obtained may consist of the mixture of the non-iogenic rice-glutelin and the complex-salt-formed rice-glutelin. Therefore, we can not assume the reaction of the solution, in

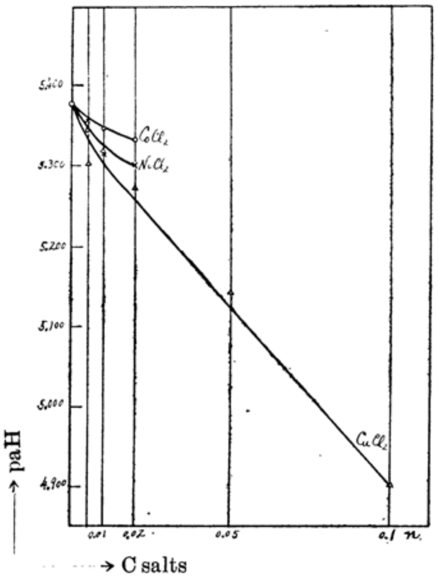

Fig. 3 a.

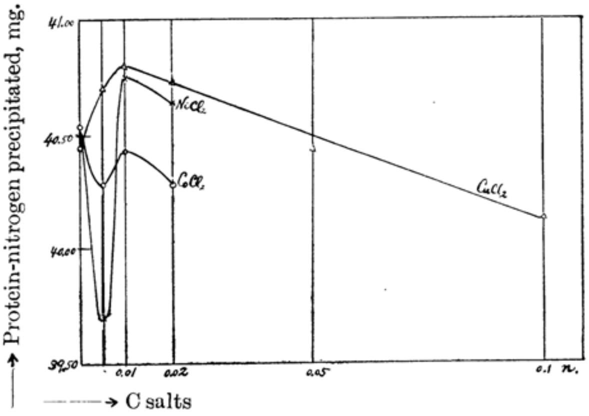

Fig. 3 b.

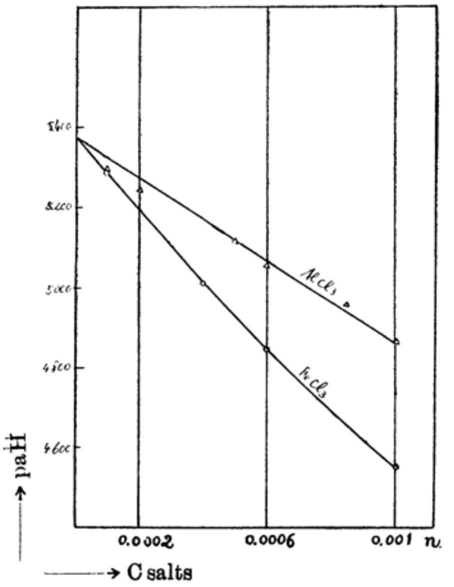

Fig. 4 a.

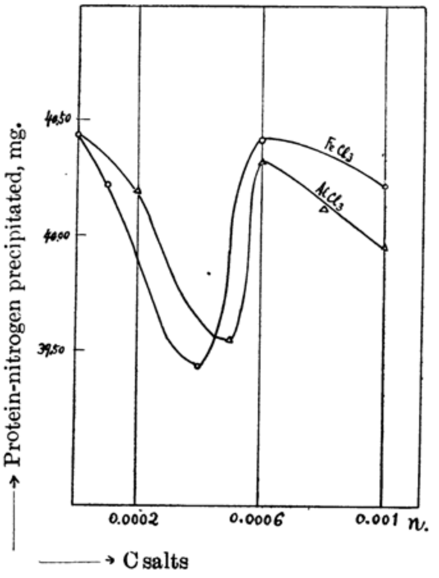

Fig. 4 b.

which, though the precipitability of the protein increased to a maximum point, to be an iso-electric reaction of the protein, when the salt-concentration is high. Because, the protein may not precipitate in all phases of the simple non-iogenic form in such a solution.

However, if the salt-concentration increase more than the above limit, the amount of the precipitated protein will decrease gradually in accordance with the salt-concentration. This may be due to the formation of a soluble or dissociable complex-compound formed of the protein and the cations derived from the salt. From other data⁽¹⁾ it may be inferred that a protein forms a complex-salt with one component of an inorganic salt. Moreover, the higher the concentration of the salt becomes, in a parallel manner, the greater the activity of the hydrogen ion, but the precipitability-curves turn at a certain salt-concentration. Herewith we are able to confirm the proposition that the salt itself, as well as the hydrogen ion activity, may play a great part in the rôle of the dissolution and precipitation of the protein. This action of the salt is due to the cations derived from the salt, and the action of the cations is peculiar to the kind of salt and its concentration. With chlorides we may arrange these cations with same valence in the order of their mobilities.

J. Loeb states that the influence of an acid, base or neutral salt on the physico-chemical properties of the protein solution is explained only by the valency rule. However, in the field, according to the phenomena alluded to, we must bear in mind the specific actions of ions as well as their valence. Therefore, we may agree with W. D. Bancroft, but not with J. Loeb, for the former sustains Hofmeister's rule which the latter does not.

Repeatedly going over the results shows us clearly that the influence of the chlorides on the behavior of rice-glutelin and the reaction of the solution becomes stronger with the increase of the valence of cations.⁽²⁾ In other words, the higher the valence of cations, the greater the difference, between chloride ion and cations in protein-ionizing power. That is, the precipitability curve of the protein has already been turned at the point of lower concentration of salt with the cations of higher valence. Therefore, in the presence of salt especially with multivalent ions, even in low concentrations, such a protein as rice-glutelin or casein may exhibit maximum flocculation at a hydrogen ion activity other than the iso-electric point of the protein.

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(1) E. J. Cohn, *J. Biol. Chem.*, **59**, iv and vii (1925); J. H. Northrop and M. Kunitz, *J. Gen. Physiol.*, **7** (1924–25), 25; D. E. Greenberg and C. L. A. Schmidt, *J. Gen. Physiol.*, **8** (1926), 271.

(2) Hardy (*J. Physiol.*, **24** (1899), 288 & **33** (1905), 251), Mellanby (*J. Physiol.*, **33** (1905), 338), Osborne and Harris (*Am. J. Physiol.*, **14** (1905), 151) and Hopkins and Savory (*J. Physiol.*, **42** (1911), 189) have found that the solvent or precipitating action of salts upon globulins increases distinctly with the valence of their ions.

The same theory has been discussed by Cohn.⁽¹⁾ Hereupon, it is clear that the presence of the salt plays an important part in the determination of the iso-electric point of the protein.

According to the results in Figures 3 and 4, $Cu^{\cdot\cdot}$, $Fe^{\cdot\cdot\cdot}$ or $Al^{\cdot\cdot\cdot}$, even in low concentration, forms a complex compound with protein. We know that $Cu^{\cdot\cdot}$ is very poisonous for organisms, especially for algae and fungus. We know also that the presence of iron and alumina in the soil is considered as one of the sources in the formation of acid soil. The sterility of plants in such a soil may be due to inhibition of the life-process in the formation of a complex compound between $Fe^{\cdot\cdot\cdot}$ or $Al^{\cdot\cdot\cdot}$ in the soil and the protoplasm-protein of the plant cell as well as to the acidification of the soil-reaction. The mechanism of the poisoning action of $Cu^{\cdot\cdot}$ or other heavy metal ions on organisms may be explained by the conception described above.

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(1) E. J. Cohn, *J. Gen. Physiol.*, **4** (1922), 697.