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STUDIES ON BOUND WATER IN THE FISH MEAT MUSCLE (I)

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I. INTRODUCTION

1. The definition of Bound Water.(1)

The problems of Bound Water have past time been studied from the standpoint of Biology and Physiology(2), and also will have many interesting points in future from the stand point of the applied field.

At first, Bound Water (or Living Water) was defined as that a part of the water in the living bodies bound with the muscle tissues. The other water which exists freely in muscle is called Free Water. This Bound Water has been considered as a factor for psychrotoleranty of living bodies, desictoleranty or maintaining lives of living bodies.

Lately it has been considered in colloidal chemistry and molecular theory that hydrophilic colloids such as protein or carbohydrates which are compositions of the body bind with Bound Water to form stable colloidal system, that is to form a protoplasm. In this case, the first reason for the binding of water with the tissue is as follows: the nitrogen-, oxygen- and hydrogen-atoms in the protein containing carboxyl-, hydroxylic-, imino- and amino-radicals combine with hydrogen- or oxygen-atoms of water and form hydrogen-binding. Therefore all proteins are considered to have Bound Water(3). According to the studies of Weidinger and Pelser(1a) on gelatine, Bound Water is water which exists within the mycel of gelatine and Free Water is water which exists between the mycels.

Thus there are many definitions of Bound Water. In a word, the idea of Bound Water is different from the samples, the purposes of studies which investigators employ. But since bodily juice has always a close relation with protein(3c,4), therefore it is necessary that we have abundant studies in the problems of Bound Water, when we discuss the protein in fish muscles.

2. The methods of estimating Bound Water.

Methods of estimating the amount of Bound Water which have been used up to the date are classified into three groups. But they agree that the amount of Bound Water is the difference between the total water content and the Free Water in the sample. The total content of water in the sample is calculated from the difference of the initial weight of the sample and the ultimate weight which is obtained by drying the sample for some time at 100°~110°C.

There are some different methods of estimating the amount of Free Water according to various ideas concerning Bound Water. One of the methods is the estimation of the frozen water contents from the assumption that Free Water will be frozen and Bound Water will not be frozen even though the muscle is kept at some constant low temperature such as -20°C. And another is based on the assumption that Free Water behaves as a solvent, but Bound Water does not do so. And the rest is the chemical estimating method.

Those methods contained few other methods can be summarized as follows:

(1) Freezing methods: (a) Calorimetric method(5); A sample which was frozen at -20°C is put into the Calorimeter and Free Water is estimated from the latent heat of the melting of the frozen sample. (b) Dilatometric method(6); The quantity of the ice (this is Free Water frozen) in the sample which was frozen at -20°C is estimated from the change of the volume of the ice by the Dilatometer. (c) Direct estimating method of the amount of water which is equivalent state to ice(7).

(2) Solvent methods: (a) Method concerning the depression of the freezing point(8); This method is suitable when the sample is liquid. Free Water is estimated from the depression of the freezing point in Centigrade degrees; when 0.01 M of saccharose is dissolved in 10 gm of test solution, if the test solution is Free Water only, a normal depression of the freezing point will be noted, but if there is some Bound Water in the sample, the depression of freezing point will be correspondingly lower for the concentrated solution. (b) Method concerning the depression of the vapour tension(9); This method is the same in the principle as the method concerning the depression of freezing point.

(3) Vapour tension method(10): This method is determined from the equilibrium curve which show the relation between the amount of water contained in the sample and its vapour tension.

(4) Swelling tension method(7b,11): This method is determined from the equilibrium curve which show the relation between the amount of water contained in the sample and its swelling tension.

(5) Chemical methods: (a) Cobaltous chloride method(12); A sample, which was added with cobaltous chloride powder or was steeped in the cobaltous chloride

solution and dyed to pink color is dried at the temperature of 25°～30°C, then the sample will turn from pinkish blue to pure blue. At the time of changing color, the amount of water in the sample is considered as that of Bound Water. (b) Copper sulphate method(13); This method is determined by estimating the change of concentration of dilute copper sulphate solution into which a sample is steeped. (c) Alcohol method(1a): This method is determined by estimating the change of the specific gravity of alcohol in which a sample is steeped.

(6) Method by studying of physical properties(14); This method is determined by studying the coefficient of friction of the surface, adsorptions-ability of the surface and other properties concerning the state of the surface of a gel membrane which hydrates with water at various temperatures, and the amount of Bound Water is discussed.

Recently Higashi and his coinvestigators(1) have studied the degree of binding strength of water by the electric method. In studying the various aspects of Bound Water, the electric method will have some greater significance for the studies in Bound Water.

I. HISTORY OF STUDIES ON BOUND WATER

1. Studies on Hydrophilic colloids and Meat muscle.

Studies on Bound Water were at first done about the state of water frozen in various substance. But recently the water in hydrophilic colloids and meat muscle has rather been studied from the standpoint of physical chemistry or physiology than from pure quantitative analysis.

Thoenes(5b) has studied the meat muscle tissues of dogs and other animals and said that 20～30% of water in their tissues is not frozen and this unfrozen water is Bound Water. He has also observed that in childhood of animals there are larger amount of Bound Water in their tissues, but in old age Bound Water decreases. Hardy(15) has demonstrated the existence of unfrozen water in gelatine which was frozen.

Moran(7b,16) has observed that when myogen and egg-albumin are frozen at −20°C, so 0.48 gm and 0.31～0.38 gm of water in these samples respectively per gm of dried matter remained unfrozen. He also has observed that when a disk of gelatine gel is frozen very slowly, such as at −20°C, water which exists in the neibourhood of the surface of the disk of gelatine gel is frozen and there remains 0.53 gm of water unfrozen per gm of dried gelatine in the disk. He has also observed that when the muscle of cattle and of frogs are cooled gradually from 0°C, the amount of the unfrozen water becomes constant at about −40°C, that is to say, 0.43 gm of water per gm of cattle meat muscle and 0.45 gm of water per gm of frog meat muscle remains unfrozen, and he considered that these unfrozen water are Bound Water. Briggs(10c) has observed from vapour tension measurement of the gelatine gel that each gm of gelatine remains 0.33 gm of unfrozen water at −20°C. Kinoshita(17) has also observed the existence of unfrozen water in gelatine which was frozen. Kistler(18) examined qualitatively the existence of unfrozen water from the degree of condensation of frost that was formed from the fog in the tube which was cooled by dry ice and he has obtained the following result that a certain quantity of water remained unfrozen at even low temperature of −72°C. From the physiological and biological standpoints, Plank(19) has advocated so-called freezing phase. He has devided into four phases the freezing process of living things from −1° to −60°C, and he has pointed out the existence of biochemical Bound Water and colloidal Bound Water. But after that there is no reexamination about this problem.

There are many studies on the hydrophilic colloid of vegetable juice from old time, but the literatures on these studies are omitted.(8a,20)

Heiss[21] has said that the change of animal protoplasm during the refrigeration of animal kingdom foods are due to the variation of the volume of Free Water and the dehydration of the colloidal Bound Water in them, and he observed that two factors influencing these changes are the velocity of freezing and the pressure on cells, those factors are concerned with the temperature for freezing, various sizes of ice crystals in muscle, density of substance and thermal conductivity in muscles. Recently Joslyn(22) has discussed the relation between Bound Water and irreversibility in the change of colloidal properties during the freezing of foods.

According to the results of studies of each above mentioned investigators, in the case of refrigeration of colloidal matters or meat muscle, the possibility of the existence of Bound Water which is independently isolated from Free Water has been practically shown. And we know that in the case of refrigeration of foods Bound Water has important properties.

2. Bouud Water contained in the meat muscle of marine animals.

There are a few studies on Bound Water in meat muscle of marine animals. Higuchi[23] has studied the variation of the amount of Bound Water of the squid and flatfish meat in the course of drying at room temperature using the method of the depression of the freezing point and the calorimetric method. According to his results, the amount of Bound Water is 3 to 5% of the initial weight of the fish meat muscle, but it gradually increases during the drying, and when the ratio of the drying, ($^{W}/_{W_0}$), reaches 0.30, the amount of Bount Water of the fish muscle becomes from 6 to 10% of the weight of the dried fish muscle and it has a tendency to increase in proportion to the degree of drying. Here W_o is the initial weight of the sample, and W is the weight after drying. Kawakami(24) has considered that if the same amount of water in fish meat muscle as the amount of water in fish meat muscle that is frozen to ice is evaporated by drying, the depression of freezing points of both remaining solution should be the same, and he studied the relation between the depression of the freezing point and the variation of the weight of fish meat muscles in the course of drying by using carp meat muscle and observed that his results was exactly equal to Finn's result 25) which shows the relation between the freezing point and the amount of frozen water. From this point of view it will be assumed the existence of Bound Water in the carp meat muscle.

As stated above the probelms on Bound Water in the meat muscle are discussed from various ideas of estimating it from the standpoints of biological, physiological and colloidal chemistry and molecular theory. And in applied field there had been many studies on this Bound Water, but we have not yet remarkable results contrary to our expection(26).

The writer has wished to study various properties of Bound Water in the fish meat and to know the relation between Bound Water and protein of fish meat, taste of fish meat, and the growth of micro-organisms, etc. and wished to apply these results to the manufacture or processing of marine products.

Acknowledgement

Before going further, the author wishes to express his hearty thanks to his respected teacher who first suggested this problem, Prof. Dr. Eiichi TANIKAWA, of Faculty of Fisheries (Hakodate), Hokkaido University, for his sympathetic guidance and encouragement throughout these experiments and his kindness for reading and correcting of this manuscript.

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II. STUDIES ON ESTIMATING METHODS OF BOUND WATER

1. Examination of the comparison between the methods of cobaltous chloride with vapour tension method and with the method by electric resistance.

As stated above, there are many methods for estimating the amount of Bound Water. But some of them have different ideas as to the principle of the estimating method. Owing to the upper mentioned difference of the ideas, the difference of freezing temperature of the water in the sample or the kind of added matters in the solvent method, from even the same sample we can not always obtain the same result. The reason for the disagreement of the results was discussed from various standpoints. Among these discussions, according to Briggs' theory(10b,c), the amount of water contained in the sample controls the activity coefficient of water (the water-activity) "a" at the existing amount of the water.

The water-activity of the sample, "a", is calculated by dividing the vapour tension of the water in the sample p_x by the vapour tension of the pure water at the same temperature p_o, and these results are illustrated by a curve of the relative vapour tension p_x/p_o ($a = p_x/p_o$) and the weight of the amount of the water in the sample (the amount of water per gm of dried matter of the sample). This water-activity may be taken as a measuring rod defining the physical status of the water present in a system. When the water-activity is varied, it is an indication of a corresponding change in the free energy content of that water. That is to say, the less the water content of the sample becomes, the less the value of the water-activity, "a", becomes.

The water-content—water-activity curve which indicates the relation between the amount of water contained in the sample and the water-activity at the existing amount of the water, forms an identical continuous curve from the same sample even by various estimating methods. Because the various thermodynamic methods, for example even if vapour tension method, the method of the depression of the freezing point, Calorimetric method, Dilatometric method, Swelling tension methods, are only a means to obtain the water-activity in the sample.

As the estimating method of the amount of Bound Water, beside the above stated methods of thermodynamic methods, there are some methods by pure chemical operation, for example the cobaltous chloride methods by Hatschek or Oyagi. In these methods the sample can be handled at room temperature, and the operation is simple and convenient, so that these methods have been employed among investigators of Bound Water. But these methods have still some problems to be reexamined. Because in these methods also, they are suitable or unsuitable for some kinds of the samples, and there is an individual error, that is to say, the turning point at which a pure blue color from pink color of dyed sample in the course of the dehydration at temperature from 25°—30°C, is different according to the individual investigators.

The author has always employed this cobaltous chloride methods for estimating the amount of Bound Water in fish meat muscle. And he has found some suspicious point in these methods, then he has wished to try to reexamine various conditions. By the comparison of the results by the cobaltous chloride method with various other thermodynamic methods stated above, the author wished to make the significance of quantitative estimation of the amount of Bound Water in the sample clear.

The author will report here the results of the comparative examination of the states of water about the same sample by the cobaltous chloride methods, vapour tension method and the method by electric resistance.

(1) Experimental Methods.

(I) Sample

As sample the author has at first used the gelatine which has been employed for the study of Bound Water, and has compared the results from the gelatine with results of fresh fish meat muscle: Hatahata (Sandfish; *Arctoscopus japonicus STEINDACHNER*), Hokke (Atka Mackerel; *Pleurogrammus azous JORDAN et METZ*) and Yariika (A species of squid; *Loligo bleekeri KEFERSTEIN*). The samples of gelatine are one which is on the market (water content 13.04 %, ash 0.74 %) (The author calls it Sample No. 1) and one (water content 17.95 %, ash 0.69 %) (Sample No. 2) and the other which was purified by the Second Faculty of Engineering of Tokyo University (water content 18.66 %, ash 0.43 %) (Sample No. 3). These gelatines were supposed to be manufactured by acid treatment. The water solutions of these gelatines were pH 7.4.

(II) Cobaltous chloride methods.

The author has employed both the methods by Hatschek and Oyagi. Both these methods are the same in the principle of the estimating method. That is to say, the sample dyed with cobaltous chloride turns from a pink color to a pure blue color at one point in the course of the dehydration of the sample, and the amount of Bound Water will be known from the difference of the total content of water and the amount of Free Water which was estimated at the point of the change of color.

(A) Hatschek's method(12a)

Mix 10 gm of the sample of gelatine (as dried matter) and 5 gm of cobaltous chloride ($CoCl_2 \cdot 6H_2O$) and add an adequate amount of water to the mixture and dissolve it by heating. Pour the dissolved sample into a wooden cylindrical vessel (dia. about 3 cm, height about 0.7 cm), which is laid on a metal plate having a smooth surface.

After the cooling and solidifying of the gelatine, remove the cylindrical vessel from the metal plate, and dry gradually by standing it in the drier at 25°~30°C. Then the color of the cylindrically shaped gelatine turns gradually from pinkish red to violetish red and then to bluish violet from the outside of the cylinder of gelatine. After the color has turned homogeneously to pure blue, cut off cylindrically (dia. about 1 cm) the center part of dried blue colored gelatine. Put this cylindrical gelatine into a weighing bottle, and weigh the weight of bottle with the sample, W_1, and then dry it further in the drier at 100°~110°C until obtaining the constant weight, W_2.

In the case of estimation of the amount of Bound Water of fish meat muscle by using the Atka Mackerel meat, the author has employed the same method as above stated: Grind the fish meat, mix this fish meat with the definite quantity of cobaltous chloride. The following procedures are the same as the gelatine. In the case of fish meat, the using of cylindrical vessel was experienced to be unsuitable, because the meat shrunken by drying falls off from the vessel.

The calculation by Hatschek's method shows the following :

(a) The amount of water in the blue colored sample (gm): $W_1 - W_2 = W$

(b) The weight of dried matter of the sample in the blue colored sample (gm): $G = W_2 \times \frac{g}{C+g}$ Here "g" is the weight of dried matter in the sample taken after the preparation of the sample. C is the weight of added cobaltous chloride without crystal water.

(c) The percentage of the amount of Bound Water in the dried matter of the sample : $\frac{100W}{W+G}$

(d) The amount of Bound Water per gm of the dried sample (gm):

$$\frac{W}{G} = \frac{W_1 - W_2}{W_2 \times \frac{g}{C+g}}$$

(e) The amount of Bound Water per gm of the dried sample which was mixed with cobaltous chloride (gm) : $\frac{W_1 - W_2}{W_2}$

(B) Oyagi's method(12c)

Cut the air dried gelatine or fresh fish meat in slices about 1 cm square, and dye to a pink color by steeping the slices in a 10% cobaltous chloride solution. After 24 hours, take out this sample and absorb the solution attached to the surface of the sample with a filter paper. Put this sample into a weighing bottle or on a watch glass which has been previously dried and then weighed, and the initial weight of the meat is obtained as W_0. Then let this sample alone in the oven from 25° to 30°C, and dry until it turns to pinkish blue from pink color and then to pure blue. At the time of changing to pure blue color the sample must be weighed rapidly. The weight at this changing point of the color is obtained as W_1. Then dry further this sample for several hours in the oven of 100° to 110°C and obtain the constant value of the weight of the sample, W_2, according to the usual method. The calculation is shown as follows :

(a) Percentage of the total amount of water in the dyed sample : $\frac{W_0 - W_2}{W_0} \times 100$

(b) Percentage of the amount of Free Water in the dyed sample : $\frac{W_0 - W_1}{W_0} \times 100$

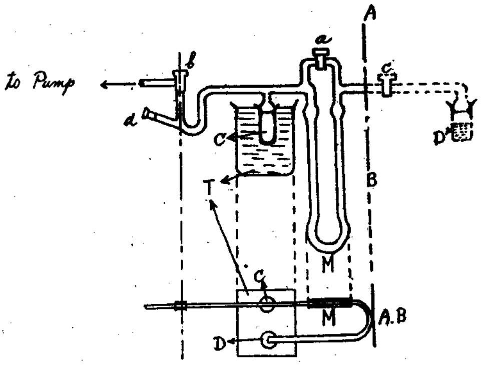
(c) Percentage of the amount of Bound Water in the dyed sample : $\frac{W_1 - W_2}{W_0} \times 100$

(d) The amount of Bound Water per gm of the dried matter containing cobaltous chloride without crystal water: $\frac{W_1 - W_2}{W_2}$

In Oyagi's method, the weight of the dried matter (without cobaltous chloride) of the dyed sample is not calculated. So the author shows the amount (gm) of Bound Water per gm of the dried matter containing cobaltous chloride as the weight of the amount of Bound Water per gm of the sample. The author has calculated the weight of the anhydride of cobaltous chloride which penetrated into the sample when the sample was steeped in 10% cobaltous chloride solution from the increasing difference between the weight of the dried matter of the original sample and the weight of the dried matter of the sample which was dyed in cobaltous chloride solution. The author has thus obtained the weight (gm) of Bound Water per gm of the dried sample, and compared the results by Hatschek's method with Oyagi's method.

(III) Vapour tension method.

Fig 1. A Estimating apparatus of Vapour Tensi



The main part of the apparatus used for the vapour tension method consists of oil-manometer (M), sample bottle (C), distilled water bottle (D) which runs parallel with (C) by bending the glass tube attached to (D) from the A-B line and exhaust cocks (a), (b), (c), (d) (See Fig. 1). Bottles (C) and (D) are submerged in the same thermostatic vessel (T). Put 5～10 gm of the sample (in the case of the sample of gelatine, gelatine gel containing 80% of water is ground and added into (C)-bottle.) After (C)-bottle is attached to the apparatus, open (a)-cock and (c)-cock, close (b)-cock and (d)-cock, drive the air pump (guaranteed vacuum 0.1 mm Hg), then open (b)-cock slowly. After air in the apparatus is exhausted for about 5 minutes, close (b)-cock and (a)-cock at the same time. Read the equilibrium pressure between the vapour tension p_0, of pure water in the (D)-bottle on the scale of the manometer (M). After the estimation of the equilibrium pressure, open (a)-cock slowly, then open (b)-cock and let air fill the apparatus. Take out the (C)-bottle from the apparatus and wipe grease attached to the mouth of the bottle with absorbent cotton moistened with toluene, and then put another cap on the mouth of the bottle and weigh the weight of the bottle with the sample,

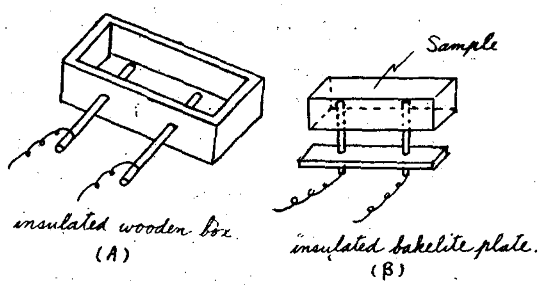
W_1. By repeating this treatment, after some time of exhausting of air from the apparatus, estimate the equilibrium pressures of p_2, p_3, p_4---p_n and weight of samples, W_2, W_3. W_4----W_n, at the pressures of p_2, p_3; p_4---p_n respectively. At last, after drying of (C)-bottle containing the sample, weigh the dried matter of the sample, W_a. From these results the weight of the total amount of water in the sample at each estimating time is calculated.

For the correction from the pressure by oil manometer to the pressure by Hg manometer, the formula $p_x = p_o - 0.0714p_y$ is ascertained within the range of the temperatures between 5° and 16°C. Here p_x is the vapour tension of the water in the sample (Hg. cm) at the estimating temperature t°C; p_o is the vapour tension of pure water (Hg. cm) at the same temperature; p_y is the pressure read (cm) on the scale of the oil manometer.

(IV) The method by electric resistance (50 cycles resistance).

For the estimating of electric resistance of gelatine, the sample of gelatine containing various amounts of water, is dissolved and then these sol are poured into an insulated wooden box (A) (4.4×1.5×1.0 cm). (See Fig. 2, A).

Fig 2. Insulated wooden box and bakelite plate for the estimation of electric resistance.



Two copper poles are thrust through one outside wall of the box, and then cooled and solidified. (The length of the pole thrust into the sample is 0.7 cm, the diameter of the poles is 2.3 cm). Then this apparatus containing the sample is placed in a desiccator of 15°~16°C in which electric wire is arranged, and the electric resistance was made by Fuji Radio Co. and its cycle was 50, and its type was Wheatstone's bridge.

In the case of the estimation of the electric resistance of fresh fish meat, two copper poles which are fixed to the insulated bakelite plate are thrust through the fish meat filet (4×2×2 cm). (See Fig. 2, B). The distance between the two poles is 1.5~3.0 cm. The length of the pole thrust the sample is 0.7 cm and the diameter of the pole is 0.75 mm. In the case of the dried fish meat, the temperature of drying of fish meat is 20°~30°C, and then the sample which was thrust through by the pole is placed in the desiccator at 15°~16°C for some time before estimation of the electric resistance.

(2) Experimental results on gelatine.

(I) Results from the estimation of the amount of Bound Water in the gelatine by cobaltous chloride method were compared with the results from the vapour tension method and method by electric resistance.

Table 1 (Fig. 3) shows the variation of the amount of Bound Water in the sample No. 2 of gelatine estimated by the cobaltous chloride methods (Hatschek's and Oyagi's methods) at intervals of 30 minutes.

Table 1. Experimental course which show the variation of the amount of Bound Water at intervals of 30 minutes in the drying of the sample No. 2 of gelatine by the Cobaltous chloride Methods. (Hatschek′s and Oyagi′s Methods)

	Hatechek′s Method				Oyagi′s Method			
Drying time in hours.	Weight of sample (gm)	Gm of water per gm of dried matter which mixed with $CoCl_2$.	Percentage of the amount of water (gelatin containing $CoCl_2$)	Drying temperature (°C)	Weight of sample (gm)	Gm of water per gm of dried matter which mixed with $CoCl_2$.	Percentage of the amount of water (gelatin containing $CoCl_2$)	Drying temperature (°C)
0	0.9310	1.10	52.1	25° ~ 30°C	0.6027	8.35	89.3	25° ~ 30°C
0.5	——	——	——		0.3211	3.99	80	
1.0	0.7714	0.743	42.6		0.2032	2.15	68.3	
1.5	——	——	——		0.1702	1.64	62.1	
2.0	0.7408	($H.B.P_2$)0.695	41.0		0.1256	0.948	48.7	
2.5	——	——	——		0.1201	0.863	46.3	
3.0	0.6998	0.580	36.7		——	——	——	
3.5	——	——	——		0.1151	0.785	44	
4.0	0.6752	0.525	34.5		0.1096	($O.B.P_2$)0.699	41.1	
4.5	——	——	——		0.1079	0.672	40.1	
5.0	0.6556	0.480	32.4		0.1036	0.606	37.8	
5.5	——	——	——		0.1009	0.564	36.06	
6.0	0.6498	0.468	31.9		0.1008	($O.C.P_2$)0.564	36.06	
6.5	——	——	——		0.0780	0.219	17.96	100° ~ 110°C
7.0	0.6332	0.430	30.07		0.0645	0.00	0.00	
7.5	——	——	——		0.0645	0.00	0.00	
8.0	0.6332	0.430	30.07					
8.5	0.6330	($H.C.P_2$)0.429	30.02					
9.0	0.5501	0.242	19.46	100° ~ 110°C				
10.0	0.4884	0.103	9.33					
11.0	0.4429	0.00	0.00					
11.5	0.4428	0.00	0.00					

The signs of $H. B. P_2$ or $O. B. P_2$ shown in Table 1 (Fig. 3) are points at which the color of pieces of dyed gelatine that were treated by Hatschek or Oyagi's method respectively, turns to pure blue from pink color in the course of drying at 30°C. At these points of $H. B. P_2$ or $O. B. P_2$ the amount of water estimated in the sample is recognized as the amount of Bound Water. But as shown in this experiment there are other points of $H. C. P_2$ or $O. C. P_2$ which are recognized as the apparent constant weight of water in the sample in the course of drying at 30°C from 1 to 3 hours after the appearance of the point of $H. B. P_2$ or $O. B. P_2$ (Each sign such as $H. B. P_2$ or $H. C. P_2$ has small type figure 2 under the letter P on the right; these figures show the No. 2 of the sample employed. Those

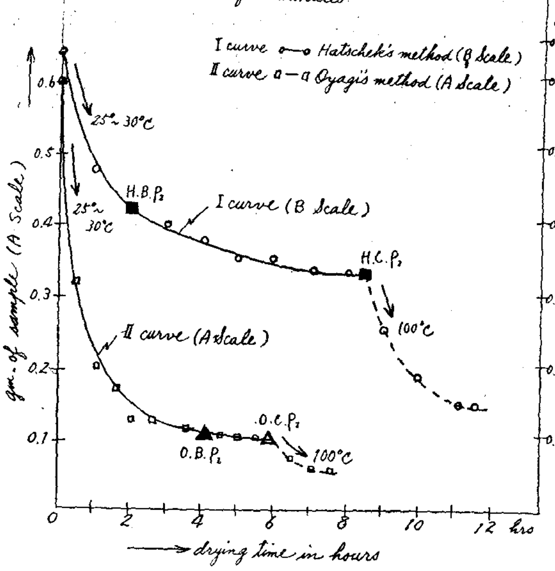


Fig 3. Experimental course which show the estimation of the amount Bound Water in the sample No. 2 gelatine by the cobaltous chloride method at intervals of 30 minutes.

figures that follow are the same).

When the amount of Bound Water is disputed from the point of view of binding strength of water to the colloidal matter, the amount of water in the sample at the point of B. P. (H. B. P or O. B. P) or C. P. (H. C. P. or O. C. P.) which was determined by Hatschek's or Oyagi's method respectively will be recognized as the amount of Bound Water. The author has called the amount of the water estimated at B. P. (H. B. P. or O. B. P.) the maximum value of the amount of Bound Water and the one at C. P. (H. C. P. or O. C. P.) the minimum value of the amount of Bound Water.

Here the author has compared the amount of Bound Water at the points of B. P. (H. B. P. or O. B. P.) and C. P. (H. C. P. or O. C. P.) with the results from the vapour tension method and the method by the electric resistance.

Table 2 shows the amount of Bound Water in the sample of No. 1, No. 2, No. 3 of gelatine by the cobaltous chloride methods.

In considering the difference between the values of the amount of Bound Water in the sample, the difference is owing to the kind of the sample and the initial amount of water in the sample. The estimation of the amount of Bound Water was made by Oyagi's method in Experiment I, II, III, IV, and V about the sample of No. 1 gelatine, air dried sample of No. 2 gelatine, bone dried sample of No. 2 gelatine, air dried sample of No. 3 gelatine, and bone dried sample of No. 3 gelatine respectively. In experiment VI, the author has tried the following treatment according to Hatschek's method: After the addition of cobaltous chloride to gelatine sol having various amounts of water, the mixtures were poured into each cylindrical wooden mold and solidified and then the solidified gelatine was dried at 20°~30°C, the center of the cylindrical dried gelatine was cut off cylindrically at the time of turning blue of the sample. As

Table 2. Estimating results of the amounts of Bound Water in the Sample No.1, No.2 and No.3 gelatine by the cobaltous-chloride methods. (Hatschek's and Oyagi's Methods.)

Methods	Experiments	Samples		Percentage of the amount of Total Water in the original sample before used.	Percentage of the amount of Total Water after treating with $CoCl_2$.	Calculated from the values at the appearent constant point (C. P.) in the course of drying at 25°~30°C. (Minimum Values of the amount of Bound Water)						Calculated from the values at blue point (B. P.) in the course of drying at 25°~30°C. (Maximum values of the amount of Bound Water.)			Remarks.
						Percentage of the amount of Free Water after treating with $CoCl_2$	Percentage of the amount of Bound Water after treating with $CoCl_2$.	Gm of Bound Water per gm of dried matter which was mixed with $CoCl_2$. (g'_1)	Gm of Bound Water per gm of dried matter of gelatine (g_1)	Percentage of the amount of Bound Water in the sample of gelatine-gel.	Gm of $CoCl_2$ per gm of the mixture of gelatine and $CoCl_2$.	Gm of Bound Water per gm of dried matter which was mixed with $CoCl_2$ (g'_2)	Gm of Bound Water per gm of dried matter of gelatine (g_2)	Percentage of the amount of Bound Water in the sample of gelatine.	
Oyagi's Method	Experiment I.	Sample No.1	(1)	13.04	83.87	78.77	5.10	0.316	(0.500)	(33.3)	Calculated from the assumpt on as 0.368	—	—	—	Volume and Weight after steep in 10% $CoCl_2$ solution. (1) 1×1×0.3 cm (0.5gm), (2) 1×1×0.1 (0.1gm)
			(2)	13.04	85.03	80.04	4.99	0.335	(0.530)	(34.6)					
			average	13.04	84.45	79.40	5.05	0.325	(0.515)	(34.0)					
	Exp. II	Sample No.2 (bone dried sample)	(1)	0.00	82.74	74.79	7.95	0.458	(0.612)	(38)	(0.254)	—	—	—	—
			(2)	0.00	82.93	74.63	8.30	0.486	(0.648)	(39.4)	(0.250)				
			(3)	0.00	83.00	74.29	8.71	0.512	(0.680)	(39.8)	(0.247)				
			(4)	0.00	83.07	75.71	7.36	0.435	(0.581)	(36.7)	(0.252)				
			av.	0.00	82.94	74.86	8.08	0.[illegible]73	(0.630)	(38.5)	(0.251)				
	Exp. III	Sample No.2 (air dried sample)	(1)	17.95	89.51	84.03	5.48	0.[illegible]22	(0.849)	(45.9)	(0.385)	0.710	(1.151)	(57.2)	
			(2)	17.95	88.92	83.12	5.80	0.[illegible]23	(0.811)	(44.3)	(0.355)	0.665	(1.030)	(51.4)	
			(3)	17.95	89.31	82.90	6.41	0.[illegible]00	(0.949)	(48.7)	(0.367)	0.766	(1.210)	(54.8)	
			(4)	17.95	89.30	82.75	6.55	0.[illegible]64	(0.969)	(49.1)	(0.364)	0.699	(1.097)	(54.6)	
			av.	17.95	89.26	83.20	6.06	0.564 (O.C.P'_2)	0.894 (O.C.P_2)	(47.2)	(0.368)	0.710 (O.B.P'_2)	1.097 (O.B.P_2)	(54.6)	
	Exp. IV	Sample No.3 (bone dried sample)	(1)	0.00	85.5	80.00	5.50	0.377	(0.590)	(37.1)	(0.36)	0.453	(0.710)	(41.5)	
			(2)	0.00	84.5	79.35	5.15	0.333	(0.497)	(33.2)	(0.33)	0.385	(0.575)	(36.5)	
			av.	0.00	85.0	79.67	5.33	0.355	(0.542)	(35.1)	(0.345)	0.419	(0.643)	(39.0)	
	Exp. V	Sample No.3 (air dried sample)	(1)	18.66	86.1	81.42	4.68	0.337	(0.552)	(35.5)	(0.39)	0.645	(1.055)	(52.6)	
			(2)	18.66	87.1	80.71	6.39	0.495	(0.677)	(40.4)	(0.27)	0.419	(0.575)	(36.5)	
			av.	18.66	86.6	81.06	5.54	0.413	(0.616)	(38.1)	(0.33)	0.532	(0.815)	(44.6)	
tschek's Method	Exp. VI	Sample No.2 pour into the drying mold)	(1)	85.63	82.35	75.49	6.86	0.389	0.516	34.05	0.247	0.484	0.643	39.2	Hatschek had estimated of the amoun oft Bound Water of the several kinds of gelatine by his method. and he determined that these gelatine contained 0.44~0.51 gm of Bound Water per gm of dried sample of gelatine (30.4~34% to the dried gelatine) at the blue turning point.
			(2)	73.43	69.74	56.44	13.30	0.439	0.567	36.20	0.225	0.484	0.626	38.5	
			(3)	62.22	56.20	39.00	17.20	0.393	0.500	33.30	0.214	0.493	0.627	38.5	
			(4)	52.44	48.20	25.94	22.26	0.430	0.544	35.20	0.210	0.531	0.673	40.2	
			av.	—	—	—	—	0.413 (H.C.P'_2)	0.533 (H.C.P_2)	34.70	0.224	0.491 (H.B.P'_2)	0.642 (H.B.P_2)	39.1	
	Exp. VII	Sample No.2 (dried on the watch glass)	(1)	85.63	82.35	74.83	7.52	0.426	0.565	36.1	0.247	—	—	—	
			(2)	73.43	69.74	57.16	12.53	0.416	0.538	35.0	0.226				
			(3)	62.22	56.20	38.51	17.73	0.405	0.519	34.2	0.220				
			(4)	52.44	48.20	27.20	21.00	0.405	0.513	33.9	0.210				
			av.	—	—	—	—	0.413	0.534	34.8	0.224	—	—	—	

the Experiment Ⅶ, the author has employed the Hatschek's method of preparation of sample and Oyagi's method of estimation as follows: Small piece of gelatine gel (the weight of about 0.5 gm) which was added with cobaltous chloride powder was laid on the watch-glass, and was dried at 25°～30°C, and the amount of Bound Water in the sample was estimated. In each experiment, each estimation was carried out in duplicate or triplicate.

In Table 2, the percentage of the total amount of water in the sample after treating with cobaltous chloride (which is written in 5th column of Table 2) is shown as the total amount of water in dyed sample after steeping in cobaltous chloride solution in the case of Oyagi's method, and is shown as the total amount of water in the sample after adding with cobaltous chloride to the sample of gelatine having various amount of water in the case of Hatschek's method respectively.

Table 3 and Fig. 4-1 shows the relation between the vapour tension of the water in the sample, p, (estimated temperature was 13° ± 0.5°C) and the water content of the samples; Fig. 4-2 shows the relation between the water-activity of the samples, "a", and the weight of the amount of the water per gm of the bone dried gelatine of the samples, "g". This relation was obtained from the results obtained by the vapour tension method on No. 2 sample of gelatine, and No. 2 sample of gelatine to which was added cobaltous chloride (the weight of cobaltous chloride was 0.213 gm per gm of dried matter of the mixture). Table 4 and Fig. 5 show the relation between the electric resistance at 50 cycles of frequency and the total amount of the water in the above stated No. 2 samples.

In Hatschek's method the quantity of cobaltous chloride containing 6 molecules of crystal water to be added, is about 50% of the weight of the dried gelatine.

(Ⅱ) Discussion of results on gelatine.

(A) Discussion of results obtained by cobaltous chloride methods.

(a) The comparison of the amounts of Bound Water between sizes and kinds of samples of gelatine by Oyagi's method.

In Experiment Ⅰ of Table 2 which dealt with the sample of No. 1 gelatine, various shapes of gelatine having varied volume and weight were employed, and the experimental results by Oyagi's method shown much the same amount of Bound Water per gm of the dried matter of these samples and shown the mean value of 0.325 gm per gm of dried matter of samples. As shown in Experiments Ⅰ,Ⅲ,Ⅳ, the amount of Bound Water in different kinds of samples of gelatine were recognized to be different.

In conclusion, the amount of Bound Water in various sizes of the sample of

Table 3. Estimating results of the vapour-tension of No. 2 gelatine having various amounts of water at 13°C. ($p_0 = 1.1231$ Hg.cm)

Sample No. 2 of gelatine				Sample No.2 of gelatine containing cobaltous chloride. (The weight of $CoCl_2$ was 0.213gm per gm of bone dried matter of the mixture.)				
Water content in %	gm of water per gm of bone dried gelatine (g_1)	vapour-tension in Hg. cm. (p) 13°C	water-activity of the samples $a=(p/p_0)$ 13°C	water content in %	gm of water per gm of bone dried mixture (g'_2)	gm of water per gm of bone dried gelatine (g_2)	vapour-tension in Hg. cm. (p) 13°C	water-activity of the samples $a=(p/p_0)$ °C
75.0 %	3.01 gm	1.112 cm	0.990	97.8 %	44.5 gm	56.5 gm	1.097 cm	0.977
66.4	1.97	1.109	0.987	88.0	7.33	9.31	1.098	0.977
60.75	1.54	1.102	0.981	80.6	4.15	5.26	1.076	0.958
55.80	1.26	1.087	0.968	65.8	1.92	2.44	1.029	0.916
39.70	0.66	0.989	0.881	59.6	1.48	1.88	1.001	0.891
30.50	0.44	0.886	0.789	56.6	1.30	1.65	1.029	0.915
21.20	0.269	0.707	0.639	48.55	0.945	1.20	0.938	0.836
19.80	0.246	0.518	0.461	45.00	0.820	1.04	0.927	0.825
15.30	0.180	0.196	0.174	36.85 (B.P)	0.584	0.741	0.816	0.726
15.20	0.179	0.163	0.145	33.98	0.515	0.655	0.727	0.647
13.20	0.152	0.041	0.037	31.04	0.450	0.572	0.674	0.600
				30.40	0.436	0.554	0.600	0.534
				29.50	0.418	0.531	0.606	0.537
				29.40	0.416	0.528	0.509	0.453
				23.65	0.310	0.394	0.317	0.282

Fig. 4-1. Relation between the vapour tension of water in samples No.2 of gelatine and their water content (Temp.=13°C. p_0=1.1231 Hg cm)

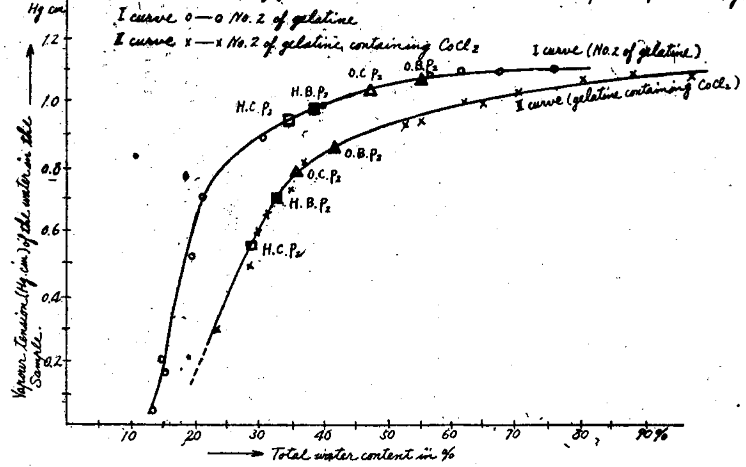


Table 4. Relation between the electric resistance of 50 cycles and the total amounts of water in gelatine.

Sample No.2 of gelatine		No.2 gelatine containing cobaltous-chloride (The weight of $CoCl_2$ was 0.216 gm per gm of bone dried matter of mixture.)	
water content in %	Electric-resistance in KΩ	water content in %	Electric-resistance in KΩ
98.0	2.5	98	0.32
90.0	1.0	90	0.13
85.6	1.1	80	0.08
73.4	1.4	70.8	0.075
70.0	1.5	51.2	0.18
62.2	1.75	40.2	1.28
58.1	2.1	35.6	3.6
56.7	2.3	34.0	4.6
56.0	3.6	32.8	9.5
44.3	20.0	31.4	11.0
38.2	80	30.6	40
35.5	200	29.6	45
30.4	470	28.8	50
29.4	650	28.0	60
27.4	2,400	27.3	90
25.5	10,000	—	—

Note: Order of the specific resistance of distilled water which was used in this experiment was about 10^{-4}Ω at 18°C.
Apparent electric resistance of distilled water was 25kΩ at which poles distance is 2.3cm.

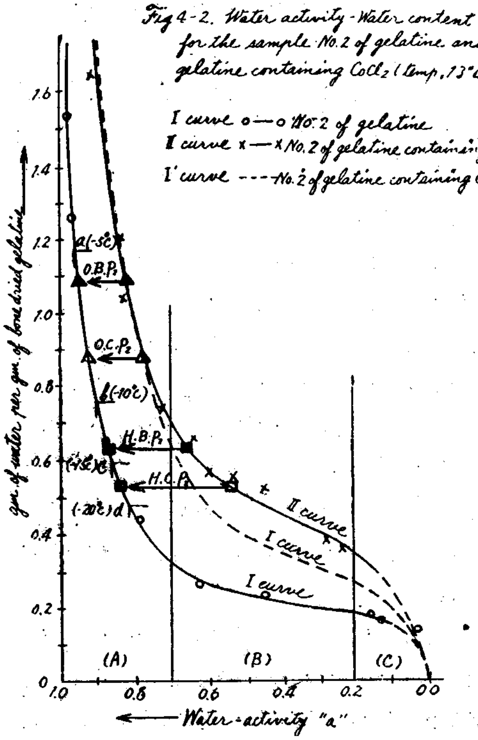


Fig 4-2. Water activity - Water content Curve for the sample No. 2 of gelatine and of gelatine containing $CoCl_2$ (temp. 13°C)

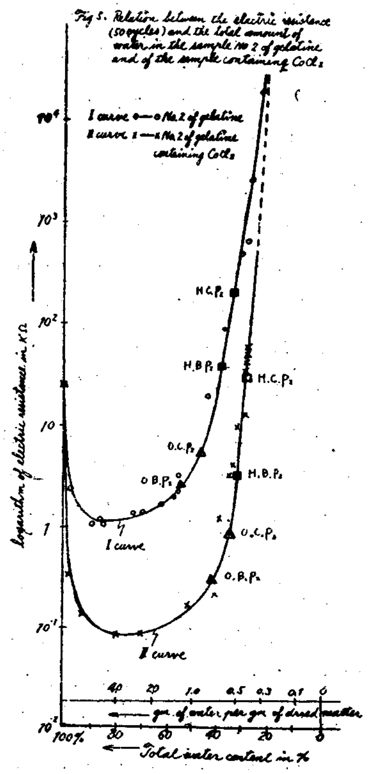


Fig 5. Relation between the electric resistance (50 cycles) and the total amount of water in the sample No 2 of gelatine and of the sample containing $CoCl_2$

gelatine were almost the same value within the range of 0.1—0.5 gm of the sample, but the amount of Bound Water was different according to the kinds of samples of gelatine.

(b) The comparison of the amount of Bound Water between the air dried sample and the bone dried sample at 110°C. (by Oyagi's method)

As shown in Experiment II, III, and Experiment IV, V of Table 2, the amount of Bound Water determined by Oyagi's method in the air dried sample was larger than in the bone dried same sample in a drier at 110°C. In this case, the total amount of water in the air dried sample after steeping it in cobaltous chloride solution was greater than the bone dried same sample in drier at 110°C and the degree of the swelling of the former was also greater than the latter. These results have already been recognized by Lloyd and coinvestigators$^{(4b,c)}$. And these facts perhaps due to that gelatine which has been dried at 110°C in drier lost water which must have been present as Bound Water in the gelatine during steepng in the cobaltous chloride solution. Therefore the fact that there is some difference according to the kinds of sample, is perhaps caused by the different conditions of drying in the process of manufacturing gelatine.

(c) The comparison of the amounts of Bound Water determined by Hatschek's and Oyagi's methods.

As shown in Experiment Ⅲ and Ⅳ of Table 2, the amount of Bound Water which was determined by Oyagi's method was recognized to be somewhat larger than that of the same sample by Hatschek's method. This is recognized to be due to the different drying mechanisms in both methods.

In Hatschek's method, the quantity of anhydrous cobaltous chloride was mean 0.224 gm per gm of the dried matter of gelatine containing cobaltous chloride powder in a definite proportion as shown in Table 2. That is to say, the quantity of the dried matter of the gelatine was 0.776 gm per gm of the same sample. And in Experiment Ⅲ in Oyagi's method, the quantity of anhydrous cobaltous chloride which penetrated into the sample of gelatine was mean 0.368 gm per gm of the dried matter of the sample of gelatine which was submerged in 10% cobaltous chloride solution. That is to say, the quantity of the dried matter of the gelatine was mean 0.632 gm per gm of the same sample.

Therefore if we show the estimated amount of Bound Water per gm of dried matter of gelatine containing cobaltous chloride by Oyagi's method as the amount of Bound Water per gm of the dried matter of gelatine by Hatschek's method, we must divide the estimated value from Oyagi's method by the quantity of dried matter of gelatine per gm of the dried sample containing cobaltous chloride, for example by the mean value 0.632 above stated, as a factor. The author has given the values thus obtained in parenthesis in the 11th column of Table 2.

From those results by the comparison of the methods of Hatschek and Oyagi, the author has obtained the following conclusion that the values of the amount of Bound Water at the points of B. P. and C. P. by Oyagi's method are larger than values of the amount of Bound Water at the points of B. P. and C. P. by Hatschek's method. That is to say, the amount of Bound Water determined by Oyagi's method is greater than the amount of Bound Water determined by Hatschek's method.

(d) The comparison of the values of the amount of Bound Water obtained by Hatschek's reformed method and the old Hatschek's method.

The values of the amount of Bound Water obtained at the respective point of C. P. in the two different treatments by Oyagi's and Hatschek's methods are almost the same, as shown in each mean value of Experiment Ⅵ and Ⅶ of Table 2. If the purpose of employing the drying mold in Hatschek's method is merely a means to obtain homogeneous drying of sample, the author thinks that it is better to employ a reformed Hatschek's method above stated, because the Hatschek's method (old) needs a comparatively larger amount of sample and longer time for the estimation of the amount of Bound Water. The reformed Hatschek's method is the most simple in treatment of samples. Moreover the results (Experiment VII of Table 2) obtained by the process of the reformed Hatschek's method agreed with the value by Hatschek's method (old), so that it is a useful method to determine the amount of Bound Water in samples.

(e) The comparison of the amount of Bound Water between the different initial concentrations of gel of gelatine in the Hatschek's method.

The author was unable to recognize any difference in the values of the amount of Bound Water in the sample of gelatine of which initail concentrations are different in the Experiment VI. But he was able to recognize the followiug difference from the results of Experiment VII that the less the water content of gel of gelatine becomes (i. e. the larger the concentration of gel of gelatine), the less the amount of Bound Water is estimated. The difference of the amount of Bound Water in samples of gelatine having different concentrations of gel has been recognized by Jones and Gortner(6a) according to the dilatometric method, and by Frick(37) according to the method by estimating the dielectric constant. They agreed that the larger the concentration of gelatine becomes, the less the amount of Bound Water in gelatine becomes. The fact that by the cobaltous chloride method which is completely different from the dilatometric method and method by estimating dielectric constant in their principle, much the same result was obtained as the results by the author's method, is very interesting. Therefore in Hatschek's method the same sample having different water content at the time of sampling shows not necessarily the same result.

(B) The comparison of results of the estimated amount of Bound Water by vapour tension method and cobaltous chloride methods (Hatscheh's and Oyag's methods).

In Fig. 4-1, the curves I and II show the curves of the depression of vapour tension in proportion to the decreasing of the water content of the sample of No. 2 gelatine, and the same sample to which was added cobaltous chloride, respectively.

H.B.P$_2$ (H.B.P$_2'$), H.C.P$_2$ (H.C.P$_2'$) and O.B.P$_2$ (O.B.P$_2'$), O.C.P$_2$ (O.C.P$_2'$) which are shown on the curves I and II indicate the points which show the maximum value and minimum value of the amount of Bound Water calculated from the values estimated at B. P. and C. P. according to the Hatschek's and Oyagi's methods respectively. Mean values which were calculated from the amount of Bound Water per gm of dried matter of gelatine containing cobaltous chloride are written in the 8th and the 12th columns of Experiments Ⅲ and Ⅳ of the Table 2, and are plotted on the curve Ⅱ.

As shown on the curve Ⅱ, the vapour tension decreased at first gradually in proportion as the total amount of water in the sample decreased, and it decreased more rapidly at the points showing the Bound Water content of the sample estimated by cobaltous chloride methods.

In fact, when the water content of the sample of gelatine containing cobaltous chloride reached 36.85% (at neighbouring point of O. C. P_2') as shown in the 5th column of Table 3, the sample has been observed to turn to pure blue.

When the observed values of the amount of Bound Water in the sample shown on the curve Ⅱ are converted into the values of the amount of Bound Water per gm of dried gelatine without cobaltous chloride(as shown as mean values in Experiments Ⅲ and Ⅳ in 9th and 13th columns of Table 2), the points showing the observed values on the curve Ⅱ will be able to be transferred to the curve Ⅰ. Points which were transferred on to the curve Ⅰ from the curve Ⅱ are shown as H. B. P_2, H. C. P_2 and O. B. P_2, O. C. P_2 respectively.

It is clear from Fig. 4-1 that these points on the curve Ⅰ give the values which are considered as the amount of Bound Water in the sample by means of the cobaltous chloride methods even before the sudden depression of vapour tension happens.

The relation between the points on the curve Ⅰ and Ⅱ can be understood as follows: for example, when we added cobaltous chloride to the gelatine which has the water content as much as O. B. P_2 on the curve Ⅰ (the quantity of cobaltous chloride ($CoCl_2 \cdot 6H_2O$) added to the dried gelatine is 50% as stated above), the water content of the gelatine which was added with cobaltous chloride decreases to the water content at O. B. P_2' on the curve Ⅱ, and the vapour tension also depresses until the vapour tension at the point O. B. P_2' on the curve Ⅱ. In Fig. 4-2, the curve Ⅰ shows the water-content—water-activity curve for No. 2 of the sample, and the curve Ⅱ shows the water-content—water-activity curve for No. 2 of the sample to which was added cobaltous chloride powder. The reason to believe that the water-content—water-activity curve for the gelatine to which are added cobaltous chloride will be shown as the curve Ⅱ, is because the quantity of cobaltous chloride which penetrates into the sample in the case of Oyagi's method is almost equal to the quantity of cobaltous chloride which is added to the sample in the case of Hatschek's method. Thus points of B. P. and C. P. which were obtained by the cobaltous chloride methods were plotted on the curve Ⅱ in Fig. 4-2.

The water-content per gm of dried gelatine is shown on the ordinate of Fig. 4-2, therefore points which were plotted on the curve II can be transferred horizontally on to the curve I. In Fig. 4-2, the water activity "a" corresponding to the range of the curve I which is recognized as the amount of Bound Water in the gelatine without cobaltous chloride by means of the cobaltous chloride methods was between 0.95 and 0.85.

However, the water-activity "a" corresponding to the range of the curve II which is recognized as the amount of Bound Water in the gelatine to which is added cobaltous chloride decreased to between 0.85 and 0.5, because the water activity decreased owing to the existence of cobaltous chloride in the gelatine.

The water-content—water-activity curve was formerly discussed by Katz(10a), Moran,(7) Brooks(10d) or Briggs(10b,c) and recently by Higashi(1), but it is difficult to decide what range of the curve is the boundary of Free Water and Bound Water, because the ideas for Bound Water are different from the stand points of Biology, Colloidal chemistry and Molecular theory respectively.

However, as a characteristic of the curve of S shape, the water-content—water-activity curve has generally three parts, A, B, C. The part of B is a gentle grade and its range is 0.2~0.7 of the values of "a" that is to say, the variation of the water-content of the sample of gelatine is small. The part of A and C are steep grade and the range of the part A is 1.0~0.7 of the values of "a" and the range of the part C is 0.2~0.0 of the values of "a".

(a) Discussion of the amount of Bound Water from the standpoint of Molecular theory.

In the discussion of the amount of Bound Water from the standpoint of Molecular theory according to Herman, Gerngroβ and Abitz(23) and Katz and Derksen(29) who have examined independently the gelatine containing various amounts of water from the defraction by X-ray and Sponsler, Bath and Ellis(8b) who have examined the hydration of water molecules of amino acids which are components of gelatine, it is considered that 0.5 gm of the water-content is at least necessary to constitute molecules of gelatine. According to Adair and Callow(30), the amount of Bound Water is independent of the water-activity, and it takes a definite value of 0.5.

Briggs(10b,c) said that when the water-activity "a" is below 0.8~0.7, water corresponding to that water-activity does not act as a solvent. Recently Bull(31) has examined the relation of water-content—water-activity of gelatine and other various high molecular compounds, and analyzed his results by the equation of

B. E. T.(32) He has discussed water whose activity is below 0.7 of water-activity "a".

From analyzed result of the curve I and II in author's experiments using the equation of B. E. T., about 0.35 gm and about 0.7 gm of the water-content per gm of the dried gelatine respectively were considered to be necessary to saturate each hydrating point in the molecule of gelatine. But the author will in detail report elsewhere.

Here, however, the author wishes to have an opportunity to say the following: about 0.35 gm of the water content per gm of the dried gelatine in the case of the curve I, and about 0.7 gm in the case of the curve II correspond respectively to about 0.7 of the water-activity "a".

From thinking thus, the amount of Bound Water at the point of H. C. P_2 on the curve I by means of Hatschek's method will give the approximate value of the amount of Molecular theoretical Bound Water. The amounts of Bound Water at the point of O. B. P_2, O. C. P_2 and H. B. P_2 are Bound Water having some weak binding strength, that is to say, it has more or less properties of Free Water.

(b) Discussion from the standpoint of colloidal chemistry on Bound Water.

Next the author will discuss from the standpoint of colloidal chemistry. In the case of the determination of the relation of water-content—water-activity by the method by the depression of freezing point and the calorimetric method the water-activity "a" varies according as the variation of freezing point of the sample (T′°K), and the water-activity of the sample is calculated from the following equation (1) under the assumption that colloidal matter has generally no heat of dilution(33).

$$\log a = -0.004211\,(273.1 - T') + 0.0000022\,(273.1 - T')^2 \text{----} (1)$$

From equation (1), the relation between the water-activity and freezing point of pure water will be calculated as Table 5.

Table 5. The relation between the water-activity and freezing point of pure water.

Water-activity "a"	Freezing point °C
0.9526	− 5
0.9071	−10
0.8630	−15
0.8220	−20

That is to say, the less the water-activity of the sample becomes, the lower the freezing point of the sample is, and the value of the water-activity becomes the approximate value of 1, in proportion as the freezing point approaches to 0°C.

Assuming that water in the sample conforms to the law of true solution, the values of the water-activity corresponding to various freezing points (shown as Table 5) are plotted on the curve I of Fig. 4-2, where they are shown as points of a, b, c, d. From the position of these points, the author knows that each value of the amount of Bound Water corresponding to each value of the water-activity shows the range extending over the water-contents per gm of bone dried gelatine at the points from "a" (water-activity 0.9526) to "d" (water-activity 0.822). (That is to say, the points correspond to freezing points; −5°～−20°C).

In estimating the amount of Bound Water in the gelatine or other colloidal matters by solvent methods (e. g. method concerning the depression of the freezing point) or by Freezing methods (e. g. Calorimetric method, Dilatometric method), the water-content of the samples corresponding to −5°～−20°C in the former case and to −20°C in the latter case respectively, were obtained. The amount of Bound Water in the samples according to the cobaltous chloride method is the water-content corresponding to the same range of values of water-activity as the methods stated above. In a word, the values of the amount of Bound Water in the sample estimated by various methods are almost the same.

The water content at points of O. B. P_2, O. C. P_2 which were obtained by Oyagi's method and plotted on the curve I of Fig. 4-2 and the water content at point of H. B. P_2 which was obtained by Hatschek's method and plotted on the curve I, are too large as the amount of colloidal Bound Water. But in Oyagi's method the water content per gm of dried matter containing cobaltous chloride is considered as the amount of Bound Water in pure gelatine as above stated (I) (Ⅱ) (B), so that if the water-content of 0.564 at O. C. P_2 in the 8th column of Experiment Ⅱ in Table 2, and 0.710 at O. B. P_2 in the 12th column of Experiment Ⅱ in the same Table are plotted on the curve I, the point of O. B. P_2' will be situated near the point of H. B. P_2, and the point of O. C. P_2', will be situated near the point of H. C. P_2.

However, since the water content which was looked upon as the amount of Bound Water in gelatine according to the Hatschek's method agreed with the water content which was looked upon as the amount of Bound Water in gelatine according to various other methods, Hatschek has proposed that his method should be employed for the estimation of the amount of Bound Water.

The author has gone a step further and explains as follows: This agreement is due to the fact that the amount of Bound Water in the samples is water having the same water-activity.

The water content at the points of O. B. P_2' and O. C. P_2' are shown as propriately as the amount of colloidal Bound Water from the idea as stated above.

In a word, the values which are obtained from the cobaltous chloride methods are considered to agree rather with the values of the amount of colloidal Bound

Water than with the value of the amount of Molecular theoretical Bound Water.

However, whether the amount of colloidal Bound Water is always larger than the amount of Molecular theoretical Bound Water cannot be determined until the meanining of molecular theoretical Bound Water is settled.

(c) The influence of addition of salts to the sample in the estimation of the amount of Bound Water.

When the curve II of Fig. 4-2 is compared with the curve I of the same Fig., the water-activity of the former is smaller than the water-activity of the latter at the same water-content, because the curve II is influenced by the addition of cobaltous chloride.

If cobaltous chloride which was added to the sample dissociates 100% and if three ions - (viz. one ion of Co^{++} and two ions of Cl^-) are dissociated from one molecule of cobaltous chloride, the curve I′ will be theoretically formed from the curve I according to our calculation as well as to Briggs.(10b)

The theoretical curve of water-activity — water-content is shown as a dotted line (I′—curve) in Fig. 4-2.

The water-content "g" per gm of dried gelatine corresponding to various water-activities "a" is measured from the curve I of Fig. 4-2, and the freezing point (Δ°C) of water at each water-activity "a" is calculated by equation (1), these values are shown in the 1st, 2nd and 3rd columns of Table 6.

The values in 4th column of Table 6 are the water-content "g" per gm of the gelatine with cobaltous chloride added at the same water-activity as the curve II.

The quantity of cobaltous chloride in the sample is 0.213 gm per gm of the dried matter, so that 2.08×10^{-3} Mol of cobaltous chloride is equivalent to 1 gm of the dried gelatine.

Therefore the water-content which is necessary for showing freezing point corresponding to various water-activities "a" in the case of dissolving the quantity of cobaltous chloride as stated above, is calculated from the following equation (2).

$$W = 3 \times \frac{2.08 \times 10^{-3} \times 10^{3} \times 1.86}{\Delta} \qquad \text{----------(2)}$$

The results obtained from the equation (2) are shown in the 5th column of Table 6.

The 6th column of Table 6 shows the total amount of hydrated water in gelatine and hydrated water dissolved in cobaltous chloride. Each water-activity of hydrated water in both materials is the same. The water-activity of this

Table 6. Calculated results of the amounts of water (gm of water per gm of bone dried gelatine) at the each water-activities in the system of No. 2 gelatine, cobaltous-chloride and water. (Temp = 13°C, p_0 = vapour tension of pure water = 1.1231 Hg. cm.)

		A (obs.)	B (obs.)	W (calc.)	(A+W) calc.	(A+W)/B
Water-activity $a = p/p_0$	Freezing point Δ C	Gm of water per gm of bone dried gelatine for the system of No.2 gelatine and water. (enterpolated from I curve in Fig. 4—2)	Gm of water per gm of bone dried gelatine for the system of No.2 gelatine, $CoCl_2$ and water.	Gm of water per gm of dried $CoCl_2$ wihch is able to hydrate with $CoCl_2$.	Theoretical amount of water (gm of water per gm of bone dried gelatine) for the system of No. 2 gelatine, $CoCl_2$ and Water.	Ratio of the calc. and obs. (I′ curve in Fig. 4—2)
0.95	5.2	1.15	——	2.23	——	——
0.90	10.8	0.76	1.80	1.074	1.834	1.020
0.85	16.6	0.56	1.255	0.699	1.259	1.002
0.80	22.6	0.456	0.990	0.514	0.970	0.980
0.75	29.1	0.380	0.808	0.399	0.779	0.965
0.70	36.0	0.330	0.700	0.323	0.652	0.932
0.65	43.3	0.296	0.634	0.268	0.564	0.890
0.60	51.3	0.270	0.586	0.226	0.496	0.847
0.55	59.8	0.253	0.552	0.194	0.447	0.810
0.50	69.0	0.243	0.523	0.168	0.411	0.786
0.45	79.1	0.232	0.492	0.147	0.379	0.771
0.40	90.1	0.221	0.465	0.129	0.350	0.753
0.35	102.6	0.213	0.438	0.113	0.326	0.744
0.30	116.8	0.206	0.410	0.099	0.305	0.745
0.25	134.0	0.198	0.383	0.086	0.294	0.769
0.20	148.2	0.189	0.363	0.078	0.267	0.736
0.15	174	0.179	0.322	0.067	0.245	0.761
0.10	210	0.165	0.267	0.055	0.220	0.824
0.05	270	0.128	0.185	0.043	0.171	0.925
						average 0.848

total amount of hydrated water is considered as the water-activity of the system of gelatine, cobaltous chloride and water. The 7th column of Table 6 shows the comparison of theoretical values which are calculated as above stated and the observed values.

According to Table 6 and Fig 4-2, the theoretical curve and the observed curve are the same when the values of water-activity "a" are in the range of 1.0—0.8, but when the values of water-activity "a" are below 0.8, the ratio of the theoretical value to the observed value of water-content is below 0.95. Therefore it is seen that curve I′ slips gradually down from the curve I.

But it is a noteworthy fact that the depression of the freezing point of the concentrated electrolyte solution is not necessarily proportional with the increasing of its concentration, as often observed. That is to say, the high concentrated solution of electrolytes shows usually abnormal depression of freezing point (abnormal depression of the water-activity "a"). For example Rudorff(34) has estimated the freezing point of various kinds of high concentrated solutions of cobaltous chloride, and his results are shown in the Ist and 3rd columns of Table 7.

Table 7. Relation between the freezing point (or water-activity) and the amount of hydrated water in the various concentration of cobaltous-chloride ($CoCl_2 \cdot 6H_2O$) solution. (Rudorff)

Gm of $CoCl_2 \cdot 6H_2O$ per 100gm of water	W (obs.) Gm of water per 0.2706 gm of $CoCl_2$	A (obs) Freezing point Δ°C	Water-activity at A	W′ (calc.) Gm of water per 0.2706 gm of $CoCl_2$	A′ (calc.) Freezing point Δ°C	Water activity at A′	Ratio of calc. and obs. W′/W=A′/A	Ion number (degree of ionization)
10	5.18	2.05	0.980	5.66	2.24	0.978	1.092	2.74
20	2.71	4.50	0.956	2.58	4.28	0.960	0.952	3.15
30	1.88	7.35	0.930	1.58	6.17	0.941	0.840	3.57
40	1.47	10.35	0.905	1.12	7.90	0.926	0.762	3.94
50	1.215	13.80	0.874	0.84	9.55	0.911	0.692	4.32
60	1.05	16.80	0.848	0.69	11.04	0.899	0.657	4.56

The 2nd column of the Table 7 shows the amount of water "W" which is added to 2.08×10^{-3} Mol of cobaltous chloride to prepare the concentration of cobaltous chloride solution used by him,

The values in the 4th column are shown as the values of water-activity corresponding to the measured freezing points from the equation (1). W′ in the 5th column and A′ in the 6th column of the Table 7 are the water-content and freezing point (Δ°C) respectively which are calculated theoretically from the equation (2). The values in the 7th column of Table 7 are shown as the ratio of theoretical value to observed value. According to Table 7, when the water-activity "a" is below 0.956, the ratio of Calc./Obs. becomes gradually smaller, and when the water-activity is about 0.85, the ratio is about 0.66. On the contrary, in order to make the theoretical value agree with the observed value, the number of ions, 3, shown in the equation (2) should apparently be increased more than 3 in proportion to the increase of the concentration of the cobaltous chloride solution. But the more the concentration of the electrolyte increases, the less the electrolytic dissolubility becomes, so that the number of ions necessary is not considered more than 3.

After all, in high-concentrated cobaltous chloride solution the fact of abnormal depression of freezing point (or the fact of abnormal depression of vapour tension) is observed as well as the presence of Bound Water in colloidal solution. Therefore the theoretical value does not agree with the observed value, that is to say, the curve I′ in Fig. 4-2 does not fit in with the practical case, and when the water-activity "a" is below 0.9, the water-content—water-activity curve which will theoretically agree with the observed value slips from the curve I′ as shown in Table 6, so that, this curve is properly considered as slipping down to the right hand of the curve I′ and Ⅱ at the same amount of water. (slipping down at upper position at the same water-activity.)

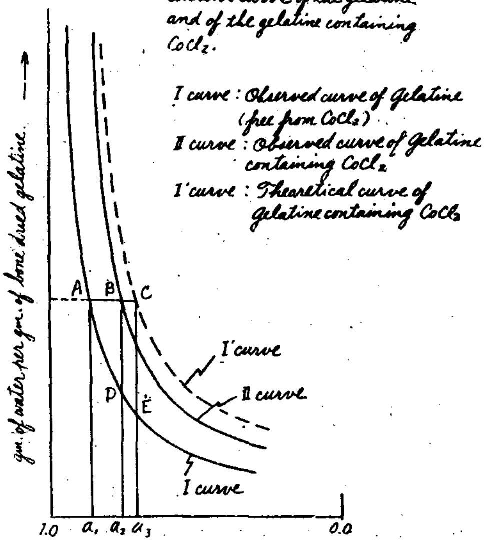


Fig 6. Water activity - water content curve of the gelatine and of the gelatine containing $CoCl_2$.

In Fig. 6 the curve I is the system of gelatine and water, the curve II is the system of gelatine, salts and water, and the curve I′ is the curve which will theoretically agree with the curve II of gelatine containing cobaltous chloride. The water-activity (or the freezing point Δ°C) of the hydrated water in gelatine and the hydrated water in salts are supposed to be the same even after the mixing of gelatine with cobaltous chloride as stated above.

Now, the author calls the point A which indicates the water-content per gm of dried gelatine corresponding to the water activity a_1 on the curve I; and also the points of juncture of the straight line which is drawn horizontally from A against a-axis (abscissa) with the curve II and I′, he calls B and C respectively. The water-activities corresponding to B and C are a_2 and a_3. The author calls the points of juncture of straight lines of a_2-B and a_3-C with the curve I, D and E respectively. As the ordinate of Fig. 6 indicates the water-content (gm) per gm of bone dried gelatine, the water contents at the points of A, B, C are of course the same. Therefore, when a definite quantity of cobaltous chloride is added in to the system of gelatine and water having water-content corresponding to the point of a_1 of the water-activity, the water-activity "a_1" drops theoretically to "a_3" by influence of the existence of the salt. If the curve II agrees with the curve I′, according to the ideas of Newton and Gortner[8a] or Hill[9] that Bound Water does not act as a solvent, the water-content between the points of C and E will be Free Water and the water-content between the points of a_3 and E will be Bound Water, but the water-activities of both waters will equally be a_3.

However, the curve II does not agree with the curve I′, the system of gelatine containing salts and water is shown as the curve II as above stated, so that the normal depression of water-activity ($a_1 \rightarrow a_3$) (the depression of freezing point according to Newton and Gortner; the depression of vapour tension according to Hill) does not take place, but the water-activity drops practically to a_2.

From considering these phenomenon, if there is a definite quantity of Bound Water in a system at the point of a_1 of the water-activity, (supposing that gelatine combines with water having a definite binding strength corresponding to the water-activity of gelatine), all the quantity of added cobaltous chloride will combine not only with Free Water in the system, but also with a part of water bound with gelatine. But it will not combine with all the water corresponding to the water-content between the points of C and E which will be considered as Free Water, in other words, it will combine practically with Free Water corresponding to the water-content between the points of B and D. The reason for this is that the stronger the binding strength of Bound Water for gelatine becomes in proportion to the depression of the water-activity, the less the strength of hydration of the added cobaltous chloride becomes. This fact is also owing to another reason, that not all of the added cobaltous chloride takes a part of the hydration, that is to say, a part of it precipitates as solid in the system, and the rest alone hydrates with hydratable water (e.g. water corresponding to the water-content between the points B and D) and indicates the water-activity "a_2". The rest of the water which is not bound with cobaltous chloride is water combining with gelatine, this water indicates also the same activity "a_2". So that whole system indicates the same water-activity "a_2". That is to say, the system of gelatine and salt solution consists of the following three systems at eutectic point of the mixture of the samples. (1) Gelatine and water which combines with gelatine, (2) Salt and water which hydrates with salt, (3) Precipitated salt.

There are probably some questions about the consideration of salt and gelatine that are unable to be explained from the author's experiments, but the author intends to explain these question by further experimental results later on.

(C) Results from the estimation of the amount of Bound Water in the gelatine by cobaltous chloride method and these results were compared with the results from the method by electric resistance.

The curves I and II of Fig. 5 show the relation between the water content of No. 2 sample of gelatine and the same sample containing cobaltous chloride (the same sample as employed in the estimation of vapour tension) with 50 cycle resistance respectively. The ordinate of Fig. 5 shows logarithms of values of electric resistance (kilo-ohm), the abscissa shows the water-content (%) of the samples.

As shown in these two curves, the less the water-content of the sample becomes, the less gradually the electric resistance decreases, but the electric resistance starts gradually increasing at 70~80% of the waterc-ontent. The electric resistance increases suddenly from the water-content at the point of O. B. P_2, but it is difficult to measure such low frequency of electric resistance as that below the water-content corresponding to the point of H. C. P_2. This fact will be understood by the following considerations: as stated at (B), the value of the amount of Bound Water estimated by cobaltous chloride methods gives the amount of colloidal Bound Water, and it takes a part of the affinity of intermolecules, and it does not act as a solvent of salts or other existing substances in the sample. On this subject, Robinson(1a) has stated that the greater the amount of Bound Water is in the sample, the less the electric conductivity becomes.

The less the water-content in the gelatine containing cobaltous chloride becomes, the higher the concentration of electrolyte becomes. As shown in the curve Ⅱ, the increasing of electric conductivity and the decreasing of electric resistance are seen in the first course of the decreasing of water-content of the sample. But when the concentration of gel increases further, and Free Water in the total amount of water of the sample decreases, that is to say, the amount of Bound Water proportionally increases (This fact is clear from experiment Ⅳ of Table 2), it is considered that there is a point which shows the maximum amount of Bound Water in the course of increasing of the concentration of gel. At that point, the electric resistance decreases to the minimum point

With the decreasing of water-content in the sample, if the state of water becomes to molecular Bound Water from the colloidal Bound Water, the electric resistance is considered to increase suddenly. The curve Ⅱ shows that this explanation is right.

As shown in the curve-I, the depression of electric resistance of the first step in accordance with the decreasing of water-content is considered to be owing to the increase of the concentration of electrolyte from the reason that the sample has 0.52% of electrolyte as NaCl in ash, and the sample was not iso-electric protein. Frick(27) has investigated the relation between the gel concentration and dielectric constant (ε) in the wide range of wave lengths, his result was that the dielectric constant (ε) is the maximum at 40% of the concentration of gel. Recently, Takeda(35) has discussed Frick's result and has concluded that the decreasing of dielectric constant (ε) is owing to the decreasing of the amount of Bound Water. As stated in (A), Newton and Gortner(8a) have investigated on Bound Water in gelatine by Dilatometric method and the present author has investigated the same subject by Cobaltous chloride methods and they have admitted that the greater the concentration of gel is, the less the amount of Bound Water becomes. From these results, the fact that when the total amount of water decreases below 60% in the sample, the electric resistance increases, is owing to the decreasing of the amount of Bound Water. Because water which has strong binding strength rests only in the sample.

Sheppard, Houck and Dittmar(36) have investigated the electric conductivity of gelatine. From their result, it was clear that the gelatine shows the minimum electric conductivity at its iso-electric point, and the less the amount of water in the sample becomes, the less the electric conductivity becomes, and the relation between the water content of below 20% and the logarithm of electric conductivity shows a straight line. The author has considered that when the total amount of water in the sample is below 20%, the electric resistance increases proportionally to the decreasing of the amount of water.

At last, the fact of the decreasing of electric resistance at the initial decreasing of water-content in the curves of Fig. 5, is explained from the physical consideration besides the upper mentioned consideration as follows: when the concentration of gel increases, the so called "water-channel" which is temporarily charged with electricity will be formed between mycels of gelatine, and therefore the electric conductivity will increase and the electric resistance will decrease

When water which existed in mycels (this is considered as Free Water) evaporates out in accordance with increasing of the concentration of gel, that is to say, when only Bound Water remains in the sample, the electric resistance is considered to increase.

From considering thus, even if the employed sample was isoelectric protein, the electric resistance will be considered to decrease temporarily at the initial decreasing of water-content in the curves of Fig. 5. But this consideration will not yet be conclusive by the author's results at this time.

It is of interest that the physical state of water in the sample can be determined from the estimation of such lower frequency of electric resistance.

(3) Experimental results on the fish meat muscle.

(I) Results from the estimation of the amount of Bound Water in the fish muscles by the cobaltous chloride method were compared with the results from the vapour tension method and the method by electric resistance.

Table 8 and Fig. 7 are the experimental course showing the amount of Bound Water in Hatahata-fish (Sandfish : *Arctoscopus japonicus STEINDACHNER*) by cobaltous chloride method (Oyagi's method) at intervals of 30 minutes.

In the case of the estimation of the amount of Bound Water in the fish meat muscle by Oyagi's method, it is observed that there is the maximum value of the amount of Bound Water which is estimated at the point of B. P. (O. B. P.) in drying (at 30°C) of dyed fish meat muscle by cobaltous chloride solution and the minimum value of the amount of Bound Water which is estimated at the point of C. P. (O. C. P.) as well as in the case of gelatine. The author has eatimated two values of the amount of Bound Water at both points, B. P. and C. P., and has compared them with other methods.

Table 9 shows the results of the estimated amount of Bound Water in fresh Sandfish, Atka Mackerel, and Squid by cobaltous chloride methods. The experimental results by Oyagi's method are shown as gm of water-content per gm of dried matter containing cobaltous chloride. Table 10 shows the relation among the vapour tension, the water-content and the water-activty of the fresh Atka Mackerel meat and the same meat containing cobaltous chloride powder (about 50% of $CoCl_2 \cdot 6H_2O$ powder was added to the dried matter of fish meat by Hatschek's method). Table 11 shows the experimental results of vapour tension in Sandfish meat and Squid meat muscle. Fig. 8 shows the curve of the depression of vapour tension in accordance with the decreasing of water-content (%) of samples, which were written in Table 10 and 11.

Fig. 9-1 shows the relation between the water-content "g" and the water-activity "a" in Atka Mackerel meat muscle (the amount of water-content "g" (gm) per gm of dried fish meat muscle).

Fig. 9-2 shows the relation between the water-content "g" and the water-activity "a" in Sandfish meat and Squid meat which were written in Table 11.

Table 12-1 and Table 12-2 show the relation between the electric resistance of 50 cycles and the water-content in Atka Mackerel and Squid meat muscle. These reults are shown in Fig. 10-1, Fig. 10-2.

(II) Discussion on the experimental results of the fish meat muscle.

(A) Experimental results of Bound Water by the cobaltous chloride method.

When we compare the experimental results by Oyagi's method with these Hatschek's method about Atka Mackerel meat in Table 9, the amount of Bound Water per gm of dried matter "g" at B. P., (Blue turning point) by Oyagi's

Table 8. Experimental course which show the variation of the amount of water at intervals of 30 minutes in the drying of Sandfish meat muscle by the cobaltous-chloride method. (Oyagi's method)

Drying time in hrs.	Weight of sample (gm)	Gm of water per gm of dried sample which was mixed with cobaltous-chloride (gm)	Water-content in %	Temperature (°C)
0	0.1561	5.99	85.7	
0.5	0.0651	1.91	65.6	
1.0	0.0514	1.31	56.8	25°
1.5	0.0413	0.86	46.3	
2.0	0.0372	0.67 (O. B. P)	40.1	
2.5	0.0360	0.62	38.2	~
3.0	0.0340	0.53	34.6	
3.5	0.0304	0.37	27.0	30°C
4.5	0.0302	0.356 (O. C. P)	26.3	
5.0	0.0251	0.130	11.5	100°
5.5	0.0223	0.00	0.00	
6.0	0.0223	0.00	0.00	~ 110°C

Table 9. Estimating results of the amount of Bound Water in the fresh Atka Mackerel, Sandfish and a Sqiud meat muscles by the cobaltous-chloride methods. (Hatshek's and Oyagi's Methods)

		Atka Mackerel (HOKKE; *Plourogrammus azous JORDAN et METZ*)		Sandfish (HATA-HATA; *Arctoscopus japonicus STEINDACHNER*)		A species of Squid (YARI-IKA; *Loligo bleekeri KEFERSTEIN*)	
		Gm of Bound Water per gm of dried matter which was mixed with $CoCl_2$.	Percentage of the amount of Bound Water.	Gm of Bound Water per gm of dried matter which was mixed with $CoCl_2$.	Percentage of the amount of Bound Water.	Gm of Bound Water per gm of dried matter which was mixed with $CoCl_2$.	Percentage of the amount of Bound Water.
Oyagi's Method	The maximum value of the amount of Bound Water at O.B.P.	0.961 gm (O.B Pa)	49%	0.670 (O.B.P$_b$)	40.1	—	—
	The minimum value of the amount of Bound Water at O.C.P.	0.372 (O.C.Pa)	27.1	0.356 (O C.P$_b$)	26.2	(1) 0.417 (2) 0.536 average 0.477 (O.C.Pc)	29.4 34.9 32.3
Hatschek's Method	The maximum value of the amount of Bound Water at H. B. P.	0.420 (H.B Pa) The amount of Bound Water (gm) per gm of dried sample is 0.539gm (35%)	29.6 The weight of $CoCl_2$ was 0.221 gm per gm of dried matter of mixture.	—	—	—	—

Table 10. Estimating results of vapour-tension of Atka mackerel meat muscles having various amounts of water at 15°C. (p_0 = 1.2788 Hg. cm)

Atka Mackerel meat (sample I.)				Atka Mackerel (II) (Cobaltous-chloride added to the sample I. The weight of $CoCl_2$ was 0.221gm per gm of dried matter of mixture)									
						B (obs)				W (calc.)	A (obs.)	(W+A) calc.	(W+A)/B
Water content in %	Gm of water per gm of bone dried meat muscle (g_1)	Vapour tension in Hg.cm (p) 15 C	Water activity of the samples (a) 15°C	Water content in %	Gm of water per gm of bone dried matter of mixture (g'_2)	Gm of water per gm of bone dried meat muscles (g_2)	Vapour tension in Hg.cm (p') 15°C	Water activity of the samples (a') 15°C	Freezing point (Δ°C)	Gm of water which will be able to hydrate with $CoCl_2$	Gm of water which was enterpolated from g_1-a curve	Theoretical amount of water (gm of water per gm of bone dried meat muscles) which containing $CoCl_2$ sample.	Ratio of calc. and Obs.
78.3	3.61	1.274	0.996	38.1	0.616	0.792	0.956	0.748	29.3	0.415	0.38	0.795	1.004
60.0	1.50	1.194	0.934	25.8	0.348	0.447	0.763	0.597	51.7	0.235	0.223	0.458	1.023
39.3	0.646	1.079	0.844	18.8	0.231	0.297	0.623	0.487	71.2	0.171	0.169	0.340	1.145
25.4	0.341	0.932	0.729	17.8	0.216	0.278	0.514	0.402	89.9	0.135	0.146	0.281	1.010
17.3	0.209	0.691	0.540	15.1	0.178	0.229	0.439	0.343	104.5	0.116	0.141	0.257	1.1210
12.9	0.148	0.317	0.405	14.4	0.168	0.216	0.374	0.293	119	0.102	0.138	0.240	1.1110
10.9	0.122	0.256	0.210	13.1	0.151	0.194	0.371	0.290	120	0.1013	0.137	0.238	1.228
10.0	0.111	0.130	0.102	—	—	—	—	—	—	—	—	—	average 1.092

Table 11. Estimating results of vapour-tension of the fresh Sandfish and Squid-meat muscles having various amount of water at 15°C (p_0 = 1.2788Hg . cm)

Sandfish meat muscle				A species of Squid meat muscle			
Water content in %	Gm of water per gm of bone dried matter (g'_3)	Vapour tension in Hg cm (p'') 15°C	Water activity of the sample (a'') 15 C	Water content in %	Gm of water per gm of bone dried sample (g'_4)	Vapour tension in Hg.cm (p''') 15°C	Water activity of the sample (a''') 15°C
72.6	2.66	1.110	0.867	80.13	4.03	1.272	0.995
44.4	0.79	1.090	0.853	70.06	2.33	1.208	0.945
31.1	0.45	1.042	0.816	55.88	1.26	1.102	0.862
17.9	0.22	0.870	0.680	42.31	0.734	0.978	0.765
15.2	0.180	0.745	0.582	34.50	0.527	0.894	0.699
13.1	0.150	0.574	0.449	28.17	0.392	0.815	0.638
11.8	0.133	0.388	0.303	22.30	0.287	0.710	0.555
9.84	0.109	0.261	0.204	16.40	0.196	0.503	0.394
9.11	0.100	0.175	0.137	13.26	0.152	0.327	0.256
8.16	0.088	0.155	0.123	11.27	0.127	0.144	0.113

Table 12—1. Relation between the electric resistance at 50 cycles and the total amount of water in the fresh Atka Mackerel meat muscles.

Percentage of total water (%)	Gm of water per gm of dried matter (gm)	Distance of poles and electric resistance in KΩ		
		1.5 cm	2.0 cm	3.0 cm
80	4.0	0.450	0.520	0.710
70.3	2.36	0.410	0.470	0.550
48.4	0.937	0.450	0.630	0.760
38.2	0.618	0.650	0.870	1.150
30.0	0.428	1.02	1.45	2.2
24.5	0.324	5.5	7.5	10.0
20.2	0.253	60	160	170
14.8	0.174	1000 over	—	—

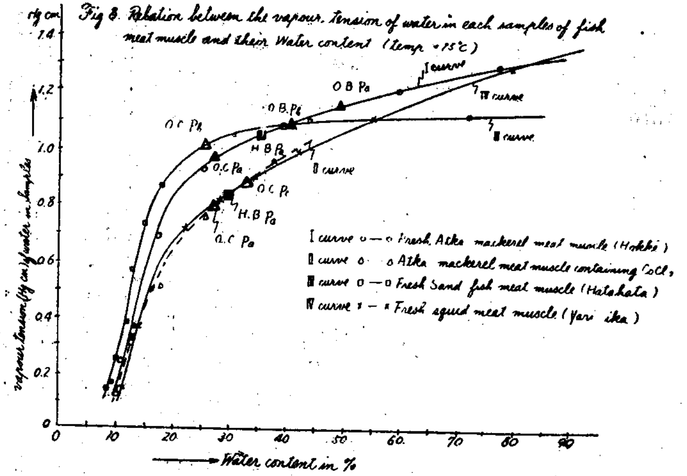


Fig 8. Relation between the vapour. tension of water in each samples of fish meat muscle and their Water content (Temp = 15°C)

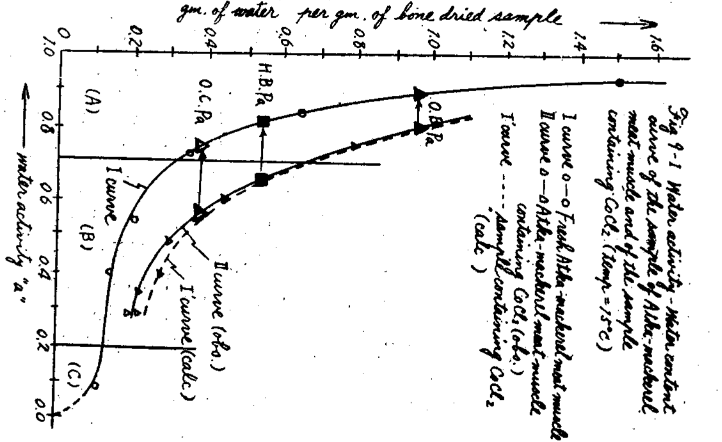


Fig 9-1 Water activity-Water content curve of the sample of Atka-mackerel meat muscle and of the sample containing CoCl₂ (Temp = 15°C)

I curve o—o Fresh Atka-mackerel meat muscle.
II curve ▵—▵ Atka-mackerel meat-muscle containing CoCl₂ (obs.)
I' curve ---- sample containing CoCl₂ (calc)

Table 12—2. Relation between the electric resistance at 50 cycles and the total amount of water in the fresh Squid meat muscles. (Pole-distance was 1.5cm)

Percentage of total water (%)	Electric resistance (KΩ)
79.86	0.77
58.6	1.07
33.5	2.5
26.0	15
22.98	25
22.35	35
20·26	46
18.65	90
15.8	360
13.0	700

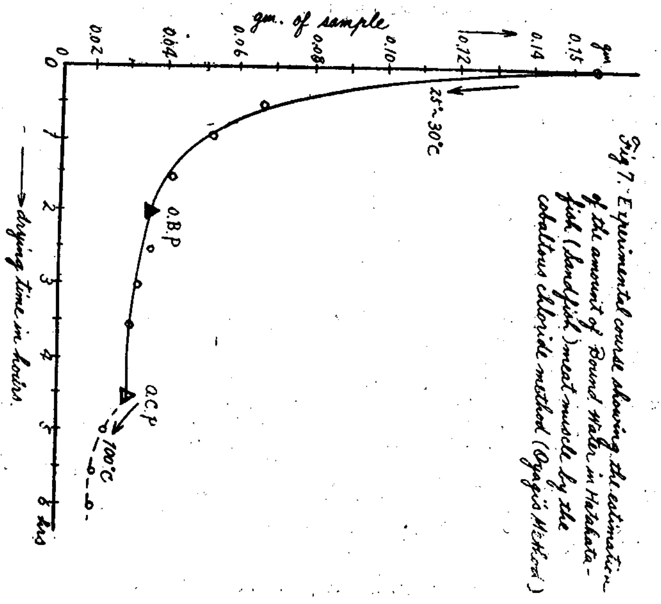


Fig 7. Experimental course showing the estimation of the amount of Bound Water in Hatakata-fish (Sandfish) meat muscle by the cobaltous chloride method (Oyagi's Method)

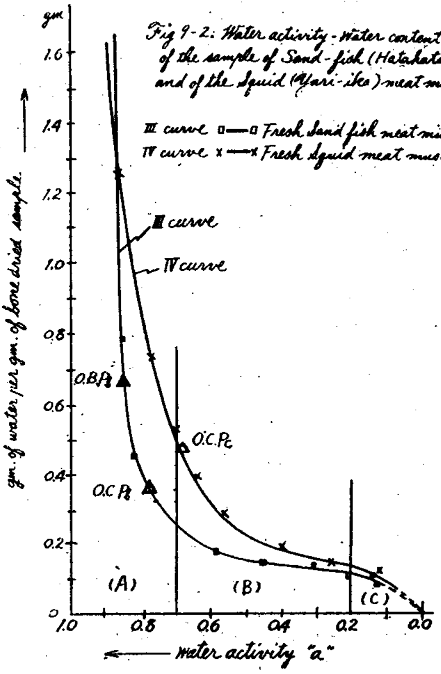


Fig 9-2: Water activity - water content curve of the sample of Sand-fish (Hatahata) meat and of the Squid (Yari-ika) meat muscle.

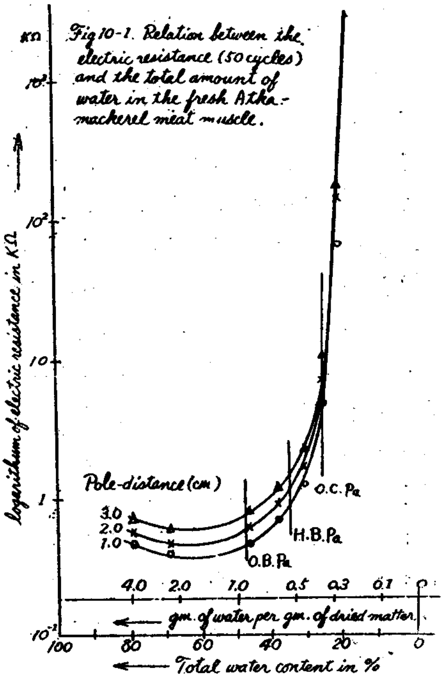


Fig 10-1. Relation between the electric resistance (50 cycles) and the total amount of water in the fresh Atka-mackerel meat muscle.

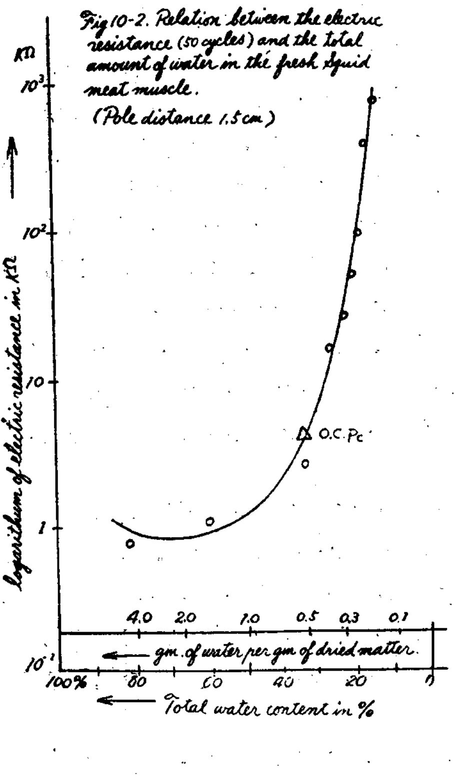


Fig 10-2. Relation between the electric resistance (50 cycles) and the total amount of water in the fresh Squid meat muscle.
(Pole distance 1.5 cm)

method is greater than by Hatschek's method. This result is the same as in gelatine. This difference is owing to the different condition of fish meat muscle tissue treated by Oyagi's and by Hatschek's methods.

Therefore the mechanism of evaporation of water from the fish meat is of course different. In general, the fish meat treated by Hatschek's method is ground meat, and its tissue is comparatively rough, therefore the evaporation of water from the fish meat is uniformly done. Therefore the time of turning of pink color to blue color from the environment of the sample to the center is short, and excess drying takes place. But it is more difficult to identify the blue turning point in the estimation of Bound Water by

Hatschek's method with fish meat muscle than with gelatine.

(B) The comparison of the results of vapour tension with the results of cobaltous chloride method.

The curves I and II of the relation between the water-content and vapour tension of fish meat muscle shown as in Fig. 8 are the curves for Atka Makerel meat and for the same meat containing cobaltous chloride (0.221 gm of cobaltous chloride per gm of dried fish meat) respectively.

H. B. P_a 'and O. C. P_a are situated before the sudden change of depression of vapour tension on the curve II. Each sign such as H. B. P_a or O. C. P_a have small type figure "a" under the letter P on the right, those figures show the kind of fish, e. g. Atka Mackerel. Those figures that follow are the same. H. B. P_a of the curve II which indicates the amount of Bound Water will be transferred on to H. B. P_a on the curve I, when the amount of Bound Water of Atka Mackerel meat will be calculated for the same meat containing no cobaltous chloride. This point of H. B. P_a of the curve I corresponds to the value of the amount of Bound Water of 0.539 gm (35%) per gm of dried meat which is written in the 3rd column in Table 9.

The amount of Bound Water which was estimated by Oyagi's method is shown by the amount of Bound Water per gm (g') of sample containing cobaltous chloride, therefore as shown in the sample of gelatine, O. C. P_a and O. B. P_a of the curve II will be transferred vertically on the curve I.

As shown on the curve I, according to Oyagi's method, the maximum amount of Bound Water will be seen as about 49% of the water-content (at the blue point) and the minimum amount of Bound Water will be seen as about 27% of the water-content (at constant point by drying at 30°C). According to Hatschek's method, the maximum amount of Bound Water will be seen as about 35% of the water-content. O. B. P_a, O. C. P_a and H. B. P_a of the curve I are situated on the curve of which the vapour tension begins to decrease gradually.

The curves III and IV in Fig. 8 are curves of the water-content—vapour tension for Sandfish and Squid respectively.

The amount of Bound Water of those samples by Oyagi's method which were written from the 5th to the 8th columns in Table 9 are shown as O. B. P_b, O. C. P_b and O. C. P_c on the curves III and IV respectively. As seen in the curves III and IV, the vapour tension decreases suddenly from the blue turning point (B. P.) and it decreases suddenly from the constant point (C. P.), as well as in the case of Atka Mackerel meat above.

In Fig. 9-1 the curve I is shown as the curve of the water-content—water-activity of fresh Atka Macherel meat containing no cobaltous chloride, and the curve II is shown as Atka Mackerel meat containing cobaltous chloride.

In the case of Atka Mackerel meat containing cobaltous chloride, the boundary of Bound Water and Free Water at the blue turning point (H. B. P_a) is seen as 0.65 of the activity of water in the fish muscle. But the value of the amount of Bound Water on the curve II will be determined as the water-content corresponding to 0.8—0.83 of the water-activity when the point of H. B. P_a of the curve II will be transferred horizontally on the curve I in order to show the amount of Bound Water in the fresh Atka Mackerel containing no cobaltous chloride.

O. B. P_a and O. C. P_a on the curve I show the amount of Bound Water (g′) per gm of the dried matter containing cobaltous chloride, therefore the points of O. B. P_a and O. C. P_a must be transferred to the upper positions on the same curve respectively.

In general, according to Oyagi's method the maximum amount of Bound Water will be determined as the water-content corresponding to 0.95—0.9 of the water-activity "a" at the point of B. P. and the minimum amount of Bound Water will be determined as the water-content corresponding to 0.85—0.8 of the water-activity at the point of C. P.

In the case of Oyagi's method and Hatschek's method the points of O. B. P_a, O. C. P_a and H. B. P_a are situated in the range of 1.0—0.8 of the water-activity on the curve I.

Here, the author knows that the values of the amount of Bound Water which were estimated by cobaltous chloride methods do not agree with the value of the amount of the molecular Bound Water, but rather agree with the approximate value of the amount of the colloidal Bound Water as in the case of gelatine. That is to say, according to the cobaltous chloride method, we can not estimate the amount of Bound Water when the water-activity is below 0.8—0.7, even if we employ the value at blue turning point or at apparent constant point in the course of drying.

The author has the same conclusion on the curve III of Sandfish meat and the curve IV of Squid as in the case of Atka Mackerel meat.

The curve I′ (dotted line) of Fig. 9-1 shows the curve of the theoretically calculated values for the Atka Mackerel meat containing a known quantity of cobaltous chloride from the curve I of the same fresh meat as well as in the case of gelatine. These calculated values are shown from the 11th to the 14th columns of Table 10.

In this case, the quantity of cobaltous chloride per gm of the dried matter of Atka Mackerel meat containing cobaltous chloride is 0.221 gm, therefore 2.18×10^{-5} Mol of cobaltous chloride is equivalent to 1 gm of dried fish meat. The amount of hydrated water to cobaltous chloride at each water-activity is calculated by the equation (2) as well as in the case of the sample of gelatine above.

As shown in Fig. 9-1, the curve I′ which was theoretically formed as above stated, agrees perfectly with the curve II which was observed by experiments. The ratio of the theoretical value to the observed value was 1.092 on the average. But as described in the case of the sample of gelatine, the theoretical curve I′ does not fit with the actual system of cobaltous chloride and water, because when we compare the observed value with the theoretical value in the system of water and cobaltous chloride, the observed values for the water-content of the system are determined as lower water-activities in the same water-content. Here supposing that there is a curve which fits the practical case, the author calls it as the curve I″. Therefore the curve of the observed value for the fish meat containing cobaltous chloride (the curve II) is situated under the supposed curve, I″. For this reason, not all of the molecules of the added cobaltous chloride hydrate with the water in the fish meat muscle by the presence of Bound Water, but a part of the added cobaltous chloride precipitates as solid, and the remainder of it hydrates with water in fish meat muscle in the predetermined quantity. The hydrated water with cobaltous chloride indicates the same water-activity of the water which is hydrated in fish meat muscle.

(C) The comparison of the results by the electric resistance with the results by cobaltous chloride method.

As seen in the relation between the water-content in Atka Mackerel and Squid meat and electric resistance of 50 cycles which is shown in Table 12-1, 12-2 and Fig. 10-1, 10-2, as well as in the case of gelatine, the electric resistance decreases more or less in the initial decreasing of the water-content of the curve, and begins to increase at 55~60% of the water-content, and it increases more and more with the decreasing of the water-content afterwards. Below 20% of the water-content, the relation between the water-content and the logarithm of electric resistance shows a straight line, and the electric resistance increases suddenly at that water-content.

That is to say, the electric resistance shows gradual increasing at the point of B. P. by Oyagi's and Hatschek's methods, and the point of C. P. by Oysgi's method is situated before the sudden increasing of electric resistance.

These facts will be understood from the reason that Bound Water does not act as a solvent for electrolytes, and the value given at C. P. by cobaltous chloride methods is probably colloidal Bound water, as well as in the case of gelatine.

The decreasing of electric resistance at initial decreasing of water-content is admitted clearly in the case of Atka Mackerel meat in Fig. 9-1, when the pole-distances are 1.5, 2.0 and 3.0 cm.

These facts will also be understood as well as in the case of gelatine from the reason that the concentraion of salt solution in fish meat muscle increases in accordance with the decreasing of Free Water and the so called "water-channel" will be formed in the tissue of fish meat by the shrinking of fish meat in the initial course of drying.

2. Conclusions on the comparison of estimation of Bound Water by the cobaltous chloride methods with the vapour tension and the method by electric resistance.

As the examinations of the estimation of the amount of Bound Water, the author has estimated the amount of Bound Water in gelatine and fish meat muscle by the cobaltous chloride methods, the vapour tension method, and the method by electric resistance of 50 cycles, and these results obtained were compared with each other, and the following conclusions were obtained.

(1) In the case of the estimation of Bound Water in the samples by the cobaltous chloride methods (Oyagi's and Hatschek's methods), the author was able to estimate the amount of Bound Water at the point of B. P. (the blue turning point) and C. P. (the point of apparent constant weight in the drying at 30°C) on the basis of ideas for the Bound Water. The author has called the water-content at B. P. the maximum amount of Bound Water and the water-content at C. P. the minimum amount of Bound Water.

(2) When the amount of Bound Water in gelatine and fish meat was estimated by the cobaltous chloride methods, and these results were compared with the values on the curve of water-content—water-activity obtained by the vapour tension method, the amounts of Bound Water per gm of the dried matter of the sample at the points of H. B. P. and H. C. P. by Hatschek's method and O. C. P. by Oyagi's method were ascertained to be the water-content in the sample corresponding to above 0.8~0.7 of the water-activity, and these values will give the probable value which has been considered before as the amount of colloidal Bound Water,

and the amount of water (gm) per gm of the dried matter of samples (containing cobaltous chloride) at the points of O. B. P′. and O. C. P′. will give also the value of the amount of colloidal Bound Water.

(3) In the cobaltous chloride methods, cobaltous chloride which was added to the samples or which penetrated into the sample decreases the activity of water in the sample.

The degree of decreasing of the water-activity is different from the activity of water hydrated with the sample which is added with cobaltous chloride. (That is to say, it is different from binding strength of sample with water.) In this case, when we suppose that water which is hydrated with cobaltous chloride shows the same water-activity as water which is hydrated with the sample, the hydration of cobaltous chloride which is added to the sample is restricted in accordance with the increasing of binding strength of the sample with water, but all the quantity of the added cobaltous chloride does not take a part of the hydration of water in the sample.

(4) In the results of the estimation of the amount of Bound Water, the electric resistance decreases in the range of from 80% to 50～60%, and it begins increase at about 50% of water-content. The electric resistance gradually increases at the point of B. P. which was considered to be shown as the maximum amount of Bound Water (the water-content 50～30%) by the cobaltous chloride method, and it increases rapidly at the point of C. P. which was considered to be the minimum amount of Bound Water (30～20%) by the same method. At below 20% of the water-content, the relation between the increasing of electric resistance and the water-content showed a logarithmic straight line.

(5) From the results of the estimation of the amount of Bound Water in gelatine and fish meat by the cobaltous chloride methods, the following facts are shown.

(a) The amount of Bound Water per gm of dried matter is almost the same in the sample (gelatine) of 0.5～0.1 gm in size in the author's experiment.

(b) The amount of Bound Water in the air dried gelatine is greater than the bone dried gelatine.

(c) The amount of Bound Water is different in the kinds of the same sample..

(d) Oyagi's method gives a greater amount of Bound water than the Hatschek's method.

(e) The greater the concentration of gel of gelatine increases, the less the amount of Bound Water becomes.

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