LXXIV. THE DETERMINATION OF CHLORIDE IN ANIMAL TISSUES.

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DURING the course of an investigation of salt-cured meat, it became necessary to find a highly accurate method for the estimation of chloride in both salt-cured and fresh meat. It was essential that the method should be applicable to samples as large as 50 g., and that it should be simple and reliable. In order to determine the accuracy of various methods, estimations were carried out of the chloride content of (a) fresh meat, and (b) an equal weight of the same sample of fresh meat to which had been added a known amount of sodium chloride. Lean pork was minced three times and well mixed. 50 g. portions were placed in well-stoppered glass bottles with wide necks. The contents of half the bottles were analysed in order to determine the chloride content of the fresh meat. Known amounts of sodium chloride were added to the remaining bottles, which were kept at 0° for several days to allow the salt to react with the tissue. The chloride content of the salted meat was then determined.

There are two stages in the chloride analysis of meat. First, the chloride must be extracted from the tissues with as little protein as possible, and secondly the chloride content of the extract must be determined. For the second stage, the Volhard method was used in this investigation. The first stage, namely extraction, is frequently carried out after the organic matter has been charred by heat. This method gives low values for the chloride content because a certain amount of chloride is invariably lost by volatilisation. The results obtained by this method were lower than those obtained by any of the other methods investigated (see Table I). The incineration method has been modified by Weitzel [1920]. He mixed the substance to be analysed with calcium oxide before incineration. There is no doubt that this is an improvement, but even the modified method in our hands gave low results for the chloride of meat (see Table II).

As incineration was found to be unsatisfactory, an attempt was made to find other ways of obtaining the chloride in solution with minimal amounts of protein. It seemed probable that the most promising method would be to use a boiling aqueous salt solution which would extract the chloride and at

the same time coagulate the proteins. Solutions of sodium sulphate and of iron alum were used but gave results which were either too low or inconsistent. Distilled water alone, however, proved to be an excellent reagent for extraction.

Table I. *Typical results obtained for chloride estimation of meat.*

Various methods of extraction were used and in each case the extract was analysed by Volhard's method. The same sample of pork was used throughout.

Method of extracting chloride from meat	Chloride (expressed as g. NaCl) found in 50 g. of lean pork		NaCl recovered g.	Percentage recovery
	Chloride (expressed as g. NaCl) found in 50 g. of lean pork	Chloride (expressed as g. NaCl) found in 50 g. of lean pork to which had been added 2 g. NaCl		
Boiled with distilled water	0.055	2.062	2.007	100.4
Boiled with 20 % sodium sulphate	0.053	2.046	1.993	99.7
Boiled with 20 % sodium sulphate plus iron alum	0.056	2.036	1.980	99.0
Boiled with distilled water plus iron alum	0.046	2.002	1.956	97.8
Incinerated at a dull red heat	0.041	1.982	1.941	97.1

Table II.

The same sample of pork was used throughout. In each case the results are the average of two determinations.

NaCl added to 50 g. of pork g.	Extracted with boiling distilled water			Incinerated with calcium oxide (Weitzel's method)		
	NaCl found g.	NaCl recovered g.	Percentage recovery	NaCl found g.	NaCl recovered g.	Percentage recovered
Nil	0.054	—	—	0.051	—	—
1	1.044	0.990	99.0	1.026	0.975	97.5
2	2.051	1.997	99.9	2.003	1.952	97.6

The following procedure was finally adopted. 50 g. of finely minced meat are placed in a 300 cc. beaker and covered with distilled water. The beaker is placed in a boiling water-bath, and the contents are stirred in order to prevent the formation of large lumps during the coagulation of the protein. The beaker is then heated over a Bunsen burner until the contents boil. The precipitate is allowed to settle and the clear fluid filtered into a flask. The residue is again boiled with distilled water, and the solution decanted and filtered into the collecting flask. In the case of fresh meat this process is repeated 5 or 6 times and the filtrate is transferred to a 1000 cc. standard flask. With salted meat it is necessary to boil the residue 10 or 12 times with distilled water. The filtered solution in this case is made up to 2000 cc. Measured volumes of this solution (with fresh meat and with very low concentrations of sodium chloride the solution must be concentrated by evaporating 250 cc. to about 10 cc.) are then placed in boiling-tubes with an excess of silver nitrate. A volume of concentrated nitric acid equal to the volume of the mixture is added, and the tubes are heated in a boiling water-bath for

an hour and a half to destroy the proteins. When cool, distilled water is added to dilute the strongly acid solution. The silver chloride is filtered off and washed, and the excess of silver nitrate is estimated by the Volhard method.

A typical series of results is given in Table III.

Table III.

The same sample of pork was used throughout.			
Weight of NaCl added g.	Total NaCl in 50 g. meat g.	NaCl recovered g.	Percentage recovery
Nil	0.053 0.051 0.048	average 0.051	
0.2	0.246		97.5
0.2	0.248		98.5
0.5	0.552	0.501	100.2
0.5	0.553	0.502	100.4
1.0	1.050	0.999	99.9
1.0	1.055	1.004	100.4
2.0	2.051	2.000	100.0
2.0	2.041	1.990	99.5

These results show that sodium chloride which has been added to meat can be estimated quantitatively by this method. Although there is complete recovery of added sodium chloride, it does not necessarily follow that this method accurately estimates the chloride originally present in the meat. In order to test this point, the following experiments were carried out. After extracting the chloride by the method described above, the residues were cautiously incinerated at dull red heat. The charcoal formed was extracted with water and then ashed. No trace of chloride was detected in the charcoal extract or ash. Even when the extracted residues from 300 g. of fresh meat were bulked together for this purpose, no chloride could be detected. This indicates that the chloride is completely extracted from meat by this method. The question then arises as to whether the method is equally reliable when applied to commercially salt-cured meats, which contain saltpetre and often sugar in addition to sodium chloride. Estimations carried out on similar samples of such material gave remarkably concordant results, considering the difficulties of sampling. For example, six estimations of a sample of minced ham gave the following results for the concentration of chloride expressed as sodium chloride: 8.04 %, 8.02 %, 7.96 %, 7.88 %, 8.04 % and 8.02 %. Four estimations of a sample of cooked ham gave the following results: 4.94 %, 4.84 %, 4.84 %, 4.84 %. After extraction and incineration, the coagulated residue showed no trace of chloride.

An alternative method of estimating small quantities of chloride in biological fluids has been put forward recently by Christy and Robson [1928]. They add $N/29.25$ silver nitrate to the solution in the presence of nitric acid; the silver chloride is filtered off, and the excess of silver nitrate in the filtrate is titrated against $N/29.25$ potassium iodide in the presence of potassium

bi-iodate and soluble starch. Silver iodide is precipitated and, when the reaction is complete, further addition of potassium iodide results in the formation of iodine. According to Christy and Robson, the slightest excess of potassium iodide results in the formation of the well-known blue colour of starch iodide. I have found, however, that the end-point is obscured by the appearance of a yellow colour which changes the clear blue to a muddy green. Moreover, I found that the end-point is not reached until a considerable excess of KI has been added.

In order to test the method, I carried out preliminary analyses on a solution containing a known amount of sodium chloride. I followed Christy and Robson's procedure except that I used *M*/100 potassium bi-iodate, as it is not clear what is meant by *N*/100 potassium bi-iodate. In every case the end-point of the titration was greenish and not well defined, and the results were 5–10 % too low. A large number of control titrations were then carried out in order to discover the cause of these grave discrepancies. Using 10 cc. of *N*/29.25 silver nitrate, no end-point was obtained until more than 11 cc. of *N*/29.25 potassium iodide had been added. In Christy and Robson's method 1 cc. of *N*/100 potassium bi-iodate is used, but I found that the

Table IV.

<i>N</i> /29.25 silver nitrate cc.	<i>M</i> /100 potassium bi-iodate cc.	2 <i>N</i> nitric acid cc.	1 % soluble starch cc.	Water added cc.	Potassium iodide cc.
10	1.00	10	2	90	11.8
10	0.50	10	2	90	10.8
10	0.20	10	2	90	10.45
10	0.10	10	2	90	10.31
10	0.05	10	2	90	10.20
10	1.00*	10	2	90	10.92
10	0.50*	10	2	90	10.63
10	0.20*	10	2	90	10.26
10	0.10*	10	2	90	10.20
10	0.05*	10	2	90	10.30
10	1.0	10	1	90	11.81
10	1.0	5	1	95	11.54
10	1.0	2	1	98	11.26
10	1.0	1	1	99	10.92
10	1.0	0.5	1	99.5	10.80
10	1.0	0.2	1	99.8	10.36
10	1.0	0.1	1	99.9	10.32
10	1.0	10	2	90	12.02
5	1.0	10	2	90	6.47
2	1.0	10	2	90	2.91
1	1.0	10	2	90	1.66
0.5	1.0	10	2	90	0.84
Nil	1.0	10	2	90	0.05
10	1.0	10	1	90	11.72
10	1.0	10	1	80	11.90
10	1.0	10	1	60	11.87
10	1.0	10	1	40	11.78
10	1.0	10	1	20	11.80
10	1.0	10	1	10	11.92
10	1.0	10	1	Nil	11.93
10	0.10	10	1	Nil	10.30

* KIO_3 .

results agreed more and more closely with the expected figure as the concentration of bi-iodate was decreased until, in the presence of 0.10 cc., only 10.2 cc. of potassium iodide were needed for 10 cc. of silver nitrate. The end-point became progressively sharper as the concentration of bi-iodate was decreased, the muddy green colour being gradually replaced by a clear blue colour. With less than 0.05 cc. of *M*/100 potassium bi-iodate the end-point was indefinite because the blue colour was too pale. When potassium iodate was used instead of the bi-iodate, similar results were obtained. Other control titrations involved variations in the amounts of silver nitrate, nitric acid and water (see Table IV). The titration values of potassium iodide necessary to give an end-point are given in the last column.

On titrating in the absence of starch and filtering off the silver iodide formed, the filtrates obtained were yellow to orange-brown in colour. It was naturally suspected that the yellow colour was due to the formation of iodine, but this hypothesis at first appeared untenable because of the non-appearance of a blue colour with starch. Further experiments, however, indicated that iodine was actually present. It could be extracted by means of chloroform or obtained as a sublimate in the condenser on vacuum distillation. Since the yellow colour was due to iodine, it may be asked why no blue colour was obtained with starch. The answer to this question is the usually neglected fact that a solution of iodine in water is incapable of giving a blue colour with starch, except in the presence of a soluble iodide [Treadwell, 1921].

Thus, when solid iodine is allowed to remain in contact with a solution containing soluble starch, nitric acid and potassium iodate (or bi-iodate), no blue colour appears. Under these conditions, even if HI is formed, it immediately reacts with the iodate and nitric acid to give free iodine:

The fluid gradually becomes yellow and then orange-brown, and iodine can be extracted by shaking with chloroform. In all respects this fluid is similar to the yellow or orange-brown fluid previously described as occurring in the control titrations after filtering off the silver iodide. In the control titrations potassium iodide is run into a solution containing silver nitrate, potassium bi-iodate (or iodate), nitric acid and soluble starch. The blue colour of the starch iodide does not appear at the point when all the silver has been precipitated (as silver iodide) because further additions of potassium iodide react with the iodate or bi-iodate and nitric acid to give free iodine. This iodine gives no blue colour with starch because there is no soluble iodide present. From theoretical considerations we should not expect that a blue colour would be formed until enough potassium iodide has been added to decompose all the potassium bi-iodate present in the solution. In actual practice, however, it has been found that when starch is present the blue colour is formed before the theoretical amount of potassium iodide has all been added (see Table IV). This appears to be due to the fact that starch

iodide is a relatively stable compound and when once it has been formed it is difficult to decompose. This point is illustrated by the following experiment. When a large excess of potassium iodate (or bi-iodate) is added to a trace of potassium iodide in a solution of nitric acid, iodine is formed and all the potassium iodide is decomposed. On adding starch, no blue colour is produced. If however starch is added to the potassium iodide and nitric acid before the iodate or bi-iodate, a deep blue colour is immediately produced.

Christy and Robson's method is therefore subject to two sources of error. In the first place a considerable amount of free iodine may be present before the blue end-point is reached, and in the second place the excess of potassium iodide required varies with the concentrations of the reacting substances. It is conceivable that it might be used as an empirical method, but only under rigidly standardised conditions. This fact was emphasised by Bang [1913], who devised the procedure which forms the basis of Christy and Robson's method.

SUMMARY.

- (1) A method is described for the complete extraction of chloride from animal tissues.
- (2) Chloride in such extracts was estimated with a high degree of accuracy by means of a modification of Volhard's method.
- (3) A criticism is given of Christy and Robson's method for the estimation of chloride in biological fluids.

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