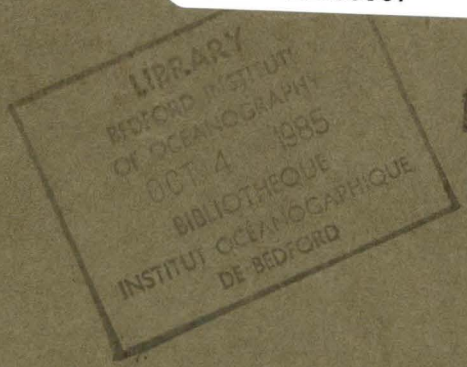

THE DISCOLORATION OF CANNED LOBSTER

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No. 28.

The Discoloration of Canned Lobster.

By A. Stanley Cook

Preliminary Experiments.

The blue black discoloration which frequently occurs in canned lobster has been the subject of several investigations. As this previous work has been discussed by Reed and MacLeod (1) in connection with their own study of the problem, there is no necessity for discussing it any further here. Reed and MacLeod demonstrated clearly that the principle if not the only cause of lobster discoloration, is bacterial action prior to sterilization of the cans. They recommend that no more than four hours be allowed to elapse between boiling the live lobsters and sterilizing the cans and that none but live lobsters should be canned.

The following experiments were conducted at the suggestion of Dr. Leim in the endeavour to throw some light on the following questions.

(1) Does any change affecting the discoloration take place in the composition of lobster muscle, during the gradual loss of vitality by the lobster but before it is actually dead as judged by the practical method of prodding?

(2) Since in canneries it is often impossible to deal with all the lobsters on hand within four hours, is it advisable to boil them and then hold them on ice or is it better to attempt to keep them alive until they can be processed?

It should be mentioned at the start that the few experiments reported here can only be taken as pointers to further research, it being impossible in the space of two months to work out definite answers to both these questions. Also an experimental answer to question (2) to be valid should be on a factory scale.

I

The first experiments were performed on perfectly fresh lobster muscle with a view to finding if any free volatile sulphur exists in the lobster muscle and to give a leader for choosing an analytical method.

Experiment I

A. The tail was broken off of the live lobster, meat dissected out and passed through a mincer. 10 gm. samples of this were aerated in the presence of a buffer of pH5 (about) for 30 minutes. Caprylic alcohol was used as antifoam and saturated lead acetate solution to catch the H_2S. The apparatus was the ordinary Van Slyke-Cullen urea apparatus. At the end of 30 minutes the lead acetate solution was filtered through a small filter paper and the paper spread out and examined for blackening.

16.7 - ♂Al condition Sodium acetate-acetic acid buffer- No blackening.
18.71 - ♀Al " 2 gms. potassium acid phosphate "- " "
18.72 - ♂Al " " " " " "- " "
25.7 - ♀Dead " " " " " " - " "

B. The same procedure was used except that the 10 gms. of minced tail meat were steam distilled with 2 gms. KH_2PO_4 for 10 minutes. The distillate was collected as before in lead acetate solution, filtered and the filter paper examined.

17.7 - ♀ condition Al Distilled 15 mins.- Distinct blackening X
18.71 - ♀ " Al " 10 " - " " X
18.72 - ♂ " Al " 10 " - " " XX

C. In these experiments the lobsters were first boiled for 10 minutes and when cool treated the same as A or B. In two cases the distillation was continued for a longer time and successive portions of the distillate collected in fresh lead acetate solution.

21.71 ♀ condition Al Steam distilled and distillate collected for two successive 10 min. periods.
1st Period - Distinct blackening XX
2nd " - " " X

21.71 ♀ " " Aerated 30 minutes - No blackening.

21.72 ♂ condition A1 Steam distilled 20 min., 10 min., and 10 min.
1st period - Distinct blackening XX
2nd " - Trace
3rd " - Distinct blackening X

21.72 ♂ " " Aerated 30 min. - No blackening.

Discussion: The inferences drawn from these experiments were:

1. There is no free volatile sulphur present in fresh raw lobster tail meat.

2. There is present a labile sulphur group which is removed by steam distillation. Removal is not complete in 40 minutes.

3. Previous boiling of the lobster by dropping it into boiling water and leaving it there 10 minutes does not appreciably increase the amount of sulphur removed.

4. There is no pronounced difference between male and female lobsters in the amount of volatile sulphur given off.

The preceding experiments were only qualitative and very rough. It was desired to find a suitable micro analytical method which would detect slight changes in volatile sulphur evolved. The method first tried was that of I. St. Lorant (2) which is designed for the micro estimation of sulphur in blood. This method is extremely sensitive and depends on the formation of methylene blue when hydrogen sulphide reacts with p-phenelene diamine. In the original method all sulphur compounds are reduced to H_2S by hydriodic acid in a special apparatus and the H_2S is passed into a zinc solution where it is trapped, reagents for colour development being added later. In adapting this method to this problem, the distillate from the lobster muscle was trapped in the zinc solution in the special cylinder prescribed and after cooling, the reagents for colour development were added exactly as in the original method. The detailed procedure is as follows:

Sulphur compounds are reduced to hydrogen sulphide by means of an acid mixture (HI + Formic + Phosphoric). The liberated H_2S is distilled into 20 cc. zinc acetate solution in a glass stoppered 50 cc. graduate. The cylinder is filled

to the 35 cc. mark with distilled water. 7.5 cc. reagent solution are added with a pipette, speeding up the delivery by blowing through the pipette. The cylinder is quickly re stoppered and shaken several times slowly. The resulting red colour fades in a few seconds and the methylene blue appears simultaneously. In 5 - 10 minutes the colour reaches its maximal intensity and is stable for a day. After one hour, one cc. alcohol is added, the contents filled to the 50 cc. mark and the colour compared in a Dubosq colorimeter with that of a standard solution.

The apparatus and reagents necessary for the determination of sulphates etc. by this method are described in the original paper. For sulphide estimation only the following are necessary:

1. Zinc solution - 50 gm. zinc acetate, 10 gm. sodium acetate, and 0.05 gm. sodium chloride (all three Merck's analyzed) are dissolved in pure distilled water in a one liter volumetric flask, although this solution becomes turbid on standing for a short time, it is nevertheless serviceable.

2. Reagent solution - 1 gm. dimethyl-p-phenelene diamine sulphate (Merck) is transferred to a 2-litre volumetric flask with 100 cc. water and treated with 400 cc. purest concentrated sulphuric acid of sp. gr. 1.71. Cool while mixing. The flask is then filled to the mark with water. Cool while mixing. The colourless solution is stable for an umlimited time.

3. Iron solution - 25 gm. ferric ammonium sulphate (Merck's analyzed) is treated with 5 cc. purest conc. sulphuric acid sp. gr. 1.71 and the whole made up to 200 cc. in a volumetric flask.

4. Methyl red solution - A. solution: 100 mg. methyl red (Merck's analyzed) dissolved in absolute alcohol in 100 cc. volumetric flask.

B. Solution: 1 cc. sol. A dissolved in absolute alcohol in 100 cc. volumetric flask.

5. Methylene blue solution - 46.7 mg. purest methylene blue (Merck's analyzed) dissolved in distilled water and made up to 1000 cc.

6. Standard solution - 20 cc. zinc solution, 7.5 cc. reagent solution, 2 cc. iron solution, 1 cc. methyl red B solution and 5 cc. methylene blue solution made up to 50 cc. in a 50 cc. measuring flask with distilled water and let stand 5 - 10 minutes. The test solution is serviceable for 24 hours.

Note: 5 cc. H_2SO_4 sp. gr. 1.71 = 3.89 cc. H_2SO_4 sp. gr. 1.845

Calculation -

$$x = \frac{z\ k.}{25} \quad \frac{v}{y} \qquad \text{where}$$

x = Sulphur present in micrograms.
z = 1.455 (x/z = actual amt. of S taking part in Carius reaction)
k = micro-grams methylene blue in 50 cc. test solution.
y = reading of colorimeter with test solution set at 20.
v = volume to which the reaction mixture is made up for the comparison.

For blood work a convenient value for k is 200. The equation then simplifies to $x = 11.6 \frac{v}{y}$.

A. 30.73 ♂ Condition Al. Distilled 10 minutes into 20 cc. zinc solution. On addition of colour reagents a light blue colour developed but comparison with the standard was impossible because the latter was too dark and had less of a yellow tinge than the unknown.
30.71 ♂ Condition Al - stood overnight in flask with buffer solution. Distilled 10 minutes in zinc solution then 10 minutes more into fresh solution.
1 st period. Light blue colour. 2nd period. Slightly darker than 1st. The same difficulty in comparison applied to both these also.

B. This time a standard was prepared containing only 1 cc. of methylene blue and no methyl red and compared with the previous distillate. The depth of blue was slightly lighter than the unknown but of the same order. There was still more yellow in the unknown.
Another standard was prepared this time from hydrogen sulphide gas. The gas was passed from a Kipp generator into sodium hydroxide solution and the latter diluted until approximately the desired strength. This gave the best match with the unknown that had been obtained so far but it also did not contain as much yellow as the unknown.

C. Although unable to obtain a comparison with a known standard and thus get quantitative results by this method, it was decided to compare successive 10 minute distillates one with another and so ascertain the regularity of the production of volatile sulphide.

4.8 - kept alive on ice 18 hours, boiled after death and kept in refrigerator 3 days, 20 gms. tail meat was steam distilled with 10 gms. KH_2PO_4 for successive 10 minute periods into fresh zinc solution each time. At the end of the experiment the contents of the distilling flask were pink to methyl red.

The reagents for colour development were added to each distillate and the colour compared with that of the first distillate in a Dubosq colorimeter.

Sample	Standard set at(No 1)	Reading of Unknown	Remarks
2	30	32	The comparison was not easy. There seemed to be less yellow in No. 1 than in succeeding distillate.
3	30	35	
4	30	32	
5	30	40	
6	30	39	

Discussion:

1. The St. Lorant method is not suitable for direct determination of volatile sulphur in lobster distillates without modification. Probably some other constituent of the distillate is influencing the colour development. In the published account of the method there is some doubt left as to what dye is used along with methylene blue in making up the standard. The author states that it is methyl red but in the theoretical discussion of the method gives a formula of the dye in question which is not that of ordinary methyl red or of any other dye known to the writer.

2. The volatile sulphide is given off continuously in steam distillation and at a roughly constant rate.

Experiment 3

Failing to get satisfactory results with the colourimetric method, it was decided to try the lead sulphide stain of Drushel and Elston (3).

A. A set of standard stains were prepared as follows:
A solution of sodium sulphide was made by passing hydrogen sulphide from a Kipp generator into sodium hydroxide solution. It was standardized by titration with an iodine solution which was itself standardized against a known thiosulphate solution.
1 cc. thiosulphate = 0.01401 gm. I_2 = 8.85 cc. iodine solution.
Therefore 1 cc. iodine solution = 0.20 mg. sulphur.
Now 1 cc. Na_2S solution = 5.41 cc. iodine solution.
Therefore, 1 cc. Na_2S solution (A) = 1.082 mg. sulphur.
1 cc. solution diluted 100 cc. = solution B.
From these two solutions standard stains were prepared by using the following amounts.

1 cc.	solution	A	= 1 mg.	sulphur
0.5cc.	"	A	= 0.5"	"
0.25 cc.	"	A	= 0.25"	"
10.0 cc.	"	B	= 0.1 "	"
5.0 cc.	"	B	= 0.05"	"
3.0 cc.	"	B	= 0.03"	"
1.0 cc.	"	B	= 0.01"	"
0.9 cc.	"	B	= 0.009"	"
0.7 cc.	"	B	= 0.007"	"
0.5 cc.	"	B	= 0.005"	"
0.3 cc.	"	B	= 0.003"	"
0.1 cc.	"	B	= 0.001"	"

This set of standards was permanent and the unknown stains were compared with them.

B. The amount of H_2S evolved by steam distilling lobsters in various conditions was then determined. 10 gms. of the raw minced tail meat was steam distilled with 10 gms. KH_2PO_4 and water for 10 minutes and the distillate caught in 10 cc. zinc solution (same as used in colorimetric method). The lead sulphide stain was prepared by acidifying the distillate in the usual way and the depth of stain compared with the standard. The delivery tube of the distilling apparatus had a detachable tip 6" long which was transferred with the distillate to the flask of the staining apparatus.

No.	Sex	Weight gm.	Conditions	Volatile S. mg.	Time of Dist.	Remarks
26.81	♀	172	A 1	0.01	10	-
26.82	♀	289	A 1	0.015	10	-
26.83	♀	239	A 1	0.025	10	Frothed
26.84	♀	1050	A 1	0.01	10	-
26.85	♂	736	A 1	0.008	10	-
8.91	♀	298	Dead 8 hrs.	lost	-	-
8.92	♂	498	Dead		-	-
14.8	♂	-	Dying	0.02	10	Boiled
8.93	♀	484	Dying	0.007	10	-
8.94	♀	385	Alive but poor condition	0.003 C.	10	48 hrs. in ice box
Blank on 10 gms. KH_2PO_4				0.002	15	-
" " 10 " " "				0.0015	10	-
" " 10 " " " + 0.2 gm. cysteine hydrochloride				0.15	10	-

Discussion:

No significant difference was found between live, vigorous lobsters, and those which having been kept on ice 48 hours, were about at the point of death.

The average amount of S evolved by 10 minutes distillation of perfectly fresh, raw lobster tail meat was 0.014.

The blank determination on the reagents was only 0.0015 for 10 minutes. Cysteine also gives off H_2S gradually on steam distillation but free cysteine is not considered to be present in muscle in any appreciable amount.

There are present, however, other compounds containing the labile SH group . This point will be discussed later.

C In this experiment distillation was carried on for 10 minutes into zinc solution, then for 1 hour into lead acetate solution, then 10 minutes into zinc solution then another hour into lead acetate solution.

No	Sex	Condition	Sample	Time of Dist. mins.	Vol.S. mg.	Remarks
13.8	♀	Caught 12.30pm. Boiled 3.30 " Distilled 10.30"	1	10	0.02	zinc solution
			2	60	0.05	$PbAc_2$ "
			3	10	0.025	zinc "
			4	60	0.150	$PbAc_2$ "

From the above the average evolutions of S in 10 minutes is 0.018 which is fairly close to the amount found for the two 10 min. periods above viz. 0.02 and 0.025 mg. The H_2S is therefore, given off continuously at a nearly constant rate for 2-1/3 hours. How long this continues was not determined as it was not considered necessary to carry on the steam distillation for longer periods.

D. The following experiment was done to find out if the substance causing the evolution of H_2S could be removed by washing with absolute alcohol. A 10 gm. minced sample (1) of tail meat from the same lobster 13.8 was transferred to a Kjeldahl flask and 250 cc. absolute alcohol added. Another similar sample (2) was macerated in a mortar with absolute alcohol until finely pulverized and transferred to a Kjeldahl flask with absolute alcohol to a total of 250 cc. Both flasks were allowed to stand in the freezer at 0° for eight days. On removal sample (1) was steam distilled with 10 gms. KH_2PO_4 and the distillate collected in fractions. Sample (2) was filtered, the residue washed twice with absolute alcohol and after draining the residue was returned to the flask and steam distilled in the same way as sample (1).

No	Sex	Sample	Time of Dist. mins.	Vol. S. mg.	Remarks
13.8	♀	(1)(a)	5	0.25	
		(b)	5	0.05	
		(c)	until alcohol nearly all distilled over.	0.5	
		(d)	10	0.004	
		(2)(a)	10	0.5	
		(b)	10	0.075	
		(c)	60	0.3	lead acetate solution
		(d)	10	0.04	

At the conclusion of the experiment the pH of the contents of the distilling flasks were (1) 4.6 (2) 5.3.

Discussion:

The two sets of results above parallel each other although they do not coincide, owing to the difference in the rate of distillation of an alcoholic and an aqueous suspension. It is definitely shown that such an alcohol extraction does not remove the H_2S producing substance in the lobster muscle.

E. The tail portion of two live male lobsters weighing 665 and 595 gms. respectively were minced and thoroughly mixed. 10 gm. samples were weighed out and steam distilled after being treated as follows:

(1) No treatment.
(2) Added 88 cc. water and 2 cc. 50% trichloracetic. Let stand 30 mins. filtered and washed precipitate once. Distilled precipitate (a) and filtrate (b).
(3) Added 80 cc. water, 5 cc. 10% sodium tungstate and 5 cc. 2N sulphuric. Let stand 30 minutes, filtered and washed once. Precipitate (a) filtrate (b).
(4) Added 90 cc. water and excess sodium chloride. Let stand in freezer over night. Filtered. Distilled residue (a) and filtrate (b).
(5) and (6) are blanks on the reagent used in (2) and (3).

No	Sex	Condition	Sample	Time of Dist. mins.	Amt. H_2S	Remarks.
4.9	♂	Al	1	10	0.01 mg.	-
			2(a)	10	0.03	-
			(b)	10	0.004	-
			5	10	0.001	-
			3(a)	10	0.01	-
			(b)	10	0.005	-
			6	10	0.001	-
			4(a)	10	-	-
			(b)	10	0.005	-

The above figures are not as decisive as could be desired. It would be expected that if the H_2S producing substance is non-protein in nature that the partition of vol. S between precipitate

and filtrate would be in favour of the latter, whereas if it is a protein, there would be no vol. S given off by the filtrate except that due to the impurity of the reagents. In the above experiment while some H_2S producing substance has passed into the filtrate the major portion still remains in the protein precipitate. This could be accounted for in two ways: (1) Incomplete extraction of a non-protein H_2S producing substance or (2) Presence of more than one such substance one of which is non-protein and the other protein. The experimental evidence is very insufficient to bear out either of these conclusions.

II

Canning Experiments

These experiments were conducted in collaboration with Mr. Ernest Hess who performed the canning. The object was to ascertain whether any difference could be observed between lobsters held alive until canning and those which were boiled immediately and then held until canning.

1. 24 lbs. of fresh live lobsters were divided into two lots:
A.- 12 lbs. boiled immediately for 15 mins. and canned after standing in the cold room (temp. about 15°C) for various time intervals.
B - 12 lbs. were kept alive in the cold room and samples taken for canning at various time intervals corresponding to those of lot A.
Each sample was boiled for 15 mins. immediately before canning.

The amount of volatile sulphur given off by steam distilling with 10 gms. KH_2PO_4 for 10 mins. into zinc solution was determined for each sample on the boiled tail meat at the time of canning. The cans were kept for 26 days at room temperature and then opened. The insides of the tins were examined for blackening and the meat for discoloration. A portion of tail meat from each can was then ground up, 10 gms. transferred to a distillation flask, distilled as before 10 mins. with KH_2PO_4, and PbS stains prepared.

Plain ¼ lb. tin cans were used. The meat wrapped in paper and a few cc. of brine added to each can. The cans were then sealed and sterilized for 35 mins. in an autoclave at 10 lbs. pressure.

Sample	Time hrs.	Initial S. mg.	Final S. mg.	Condition on opening Inside Tin	Meat
A 1	0	0.007	0.10	(1 small spot on cover (2 " " " paper	normal -
2	3	0.009	0.15	small black spots on cover.	1 claw discolored X
3	6	0.007	0.18	(black ring around seal (few small spots on cover.	normal -
4	12	0.006	0.12	numerous brown stains.	discolored throughout. XXX
5	24	0.006	0.03	heavy black stains on cover.	discolored throughout. XXXX
6	36	0.007	0.12	black around seal. Tiny specks.	badly discolored blue XXXX
B 10	3	0.006	0.02	slight brown staining.	normal -
11	6	0.007	-	(black stain on cover and (numerous tiny specks.	slightly discolored X
12	12	0.006	0.20	(several small stains, (black ring around seal. (Tiny specks.	discolored in spots. XX
13	24	0.007	dead	(slight stain around seam, (tiny specks.	normal -
14	24	0.009	0.17	(black around seal. Stains (on cover and bottom. Black (specks.	meat and paper badly discolored blue. XXX

N. B. No. 13 died sometime between 6 and 12 hours.

None of these cans were as bad as is sometimes encountered in practice due probably to the short time of storage and thorough sterilization. In all those marked with more than two X'S however, the good appearance of the product was definitely lessened.

2. Effect of Sterilization.

12 lobsters just brought in from traps were divided into three lots:
Lot A - Control - No treatment
Lot B - 1. Boiled 15 minutes and canned. Sterilized 35 min. in autoclave
2. " 30 " " " " "
Lot C - Boiled 15 minutes and canned. Sterilized in water bath.

The volatile sulphur was determined in the usual way on each lot before canning. The plan of this experiment was then to open half of Lots B and C and as soon as cool after the sterilization and test them. The other half were to be kept three or four weeks and then opened. Unfortunately just after the experiment had got under way the gas was turned off in the building owing to the removal of the meter to the new building. On this account we were unable to sterilize any cans by the boiling water bath method and were also unable to test the contents of Lot B cans immediately after the sterilization. This part was therefore omitted and all the cans were kept 23 days, when they were opened and tested.

Sample	Inital Vol. S. mg.	Final Vol. S.	Condition Meat	Condition Can
A	0.035	-	-	-
B 1	0.035	0.10	Sl. discolored X	(black spots on (cover, tiny specks XX
2	0.035	0.18	Discolored XX	black ring around seal. Black specks X

3. Effect of Canning Process.

11.91 ♂ Just caught. Boiled at once and held on ice 12 hrs Then canned.
11.92 ♀ Just caught. Held on ice alive 12 hours. Boiled and canned.

Both cans opened and examined as soon as cool.

No.	Vol. S. mg.	Condition	
		Meat.	Tin.
11.91	0.15	Normal	3 or 4 tiny black specks.
11.92	0.20	Normal	Normal.

Discussion of Canning Experiments:

Comparison of the above with the previous experiment indicates that under our conditions the increase in volatile sulphur took place during the canning process rather than during the subsequent storage. The fact that cans which were opened at once were not discolored may be due to the fact that the dissolving action of the juice on the tin is a slow porcess.

The results of the three experiments show that in all cases the volatile sulphur of the meat was greatly increased by canning and storage over the value found for the freshly boiled meat. No apparent difference was found between those which were stored and those which were opened at once. Bacterial or enzymic action in the can is therefore not indicated. Confirming Reed and MacLeod, lobsters kept longer than 6 hours between boiling and sterilization produced discolored tins. The difference between keeping the lobster alive and holding them after boiling was not marked although the most discolored cans of all were produced by lobsters held 24 and 36 hours after boiling. In this connection it should be noted that the temperature of the cold room was only 15°C which is lower than average factory conditions.

The method used does not show any correlarion between the amount of vol. S in the meat and the amount of discoloration, some of the normal tins giving values just as high or slightly higher than those which were badly discolored. Other factors than the amount of S evidently enter into the production, of the stain. One of these is undoubtedly the pH of the juices and amount of ammonia and amines it contains. The tin must be dissolved off the iron before the sulphur can come into action.

III

Discussion:

The ease with which volatile sulphur is liberated from lobster muscle under such mild treatment points to the presence of a compound with an unstable sulphur linkage such as the sulfhydryl

group. The only sulfhydryl compound which is known to exist free in muscle in appreciable amount is glutathione.

Hopkins (4) has shown that glutathione is much more unstable than even free cysteine or cystine and will lose sulphur when incubated at 37° for 5 hours in 0.3 N BaOH and excess of lead acetate. Under these conditions cysteine and cystine show no trace of blackening.

Pure glutathione undergoes decomposition with loss of both sulphur and nitrogen when aerated at room temperature in the presence of a trace of iron and at a pH of 7.6; while on boiling the pure aqueous solution (pH 3.0) sulphur is lost continuously over a long period. After 40 hours boiling one quarter of the sulphur is eliminated as H_2S and as free sulphur. Free sulphur does not become evident until the 20th hour of boiling. The elimination of sulphur takes place similarly but slightly slower when the tripeptide is boiled in absolute alcohol.

The elimination of sulphur from lobster muscle resembles that from glutathione very closely. Failure to obtain any blackening by aeration of lobster muscle could easily be due to the short time (30 min.) employed.

The only discrepancy lies in the experiments in which the lobster muscle was precipitated with trichloracetic acid and with tungstic acid. It would be expected that the proportion of sulphur eliminated from the filtrate would exceed that from the precipitate. As already pointed out however, this may be due to incomplete extraction. Glutathione is a very probable source of the volatile sulphur of lobster muscle but the evidence is much too scanty for a definite statement to be made.

Another possible source of this volatile sulphur is in unstable sulphur linkages of proteins. Considerable evidence has accumulated (5) that loosely bound sulphur exists in proteins in other forms than cystine. Much of this work, however, was done with impure proteins and before the discovery of glutathione and the results are therefore open to ambiguity. Loss of sulphur on boiling with water is, however, not peculiar to lobster muscle.

Osborne (6) found that volatile sulphur is given off on boiling the gastrocnemius muscle of a guinea pig which had been starved for 24 hours prior to death. He states that he was unable to find any volatile sulphur in the case where the animal prior to death was well fed and in good condition. Sheep muscle also gave off volatile sulphur on boiling. Rettger observed that on heating normal milk above 85° C, milk proteins undergo partial decomposition with liberation of a volatile sulphide (5). Neumeister found that on heating coagulated ovalbumin with water in an autoclave at 160°C hydrogen sulphide and ammonia are evolved (5).

The fact that autoclaving canned lobster increases the volatile sulphur removable by steam distillation to approximately the same value as that of cans which have been stored and found discolored is deserving of further investigation.

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