CXLII. THE EFFECT OF SHORT PERIODS OF COLD STORAGE ON BEEF AND MUTTON.

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(Received October 22nd, 1925.)

THIS work was started at the request of Dr F. E. Wynne, Medical Officer of Health for Sheffield, who wished to construct a cold room for storage of carcasses in connection with proposed new public abattoirs.

It was stated by local butchers that meat is "spoilt" by being placed in a cold room for any period of time, however short, and that it was necessary to take carcasses straight from slaughter to the butchers' shops. No experiments appear to have been made in this field of research except some on the autolysis of beef and mutton by Fearon and Foster [1922] and by Callow [1925] on the freezing of cod and its effect on autolytic change. These workers used antiseptics in their experiments, and therefore statements with regard to preserving meat for food cannot be based on their results. Work on the carnosine content of beef kept at 25° F. by Clifford [1922] showed a fall in this extractive, which, however, did not take place for several weeks, and would not affect the question in point.

The experiments to be described in this paper were carried out to show the effect, if any, on beef and mutton kept in cold storage for periods up to 13 days, and duplicate estimations of the total nitrogen, soluble nitrogen, amino nitrogen, carnosine and creatine were made at approximately 3 day intervals.

EXPERIMENTAL METHODS.

The meat was obtained on the day of slaughter and sent straight to the National Institute for Medical Research at Hampstead, where, by the kind permission of Dr Dale, it was placed in the cold rooms. For the experiments on mutton, a leg was taken and cut in half across the muscles. Half was then put into the inner room at a temperature of 25° F., and the other half in the outer room at a temperature of 35° F. Evaporation was avoided by suspending the joints in large glass filtrate jars, covered with clock glasses. At intervals, samples of muscle weighing 200–300 g. were cut off the joints and put into separate thermos flasks, previously left unstoppered in the chambers for 5 to 6 minutes to attain the same temperature as the room where the meat was kept. These flasks were then corked, brought to the laboratory, and

samples weighed as rapidly as possible for the various estimations which were always duplicated. Whenever possible the same muscle was used from both halves of the leg, so that the results from meat kept at 35° F. and at 25° F. are strictly comparable.

With beef exactly the same method was adopted, but in Exp. 1 meat from the shin was used, and in Exp. 2 a thick joint of fillet steak.

Total nitrogen. This was estimated by Kjeldahl's method as described by Cole [1920], about 1-2 g. meat being taken. Care was exercised to see that pieces from the same muscle were used for this and all other estimations on the same day, and when possible throughout the whole experiment. However, on one or two occasions it was difficult to identify the special muscles in a piece of frozen meat cut to a suitable size to go into the neck of a thermos flask.

From the tables of Exps. 1 and 2 it will be seen that there is a slight rise in total nitrogen suggesting that some water is lost by evaporation when beef is kept under the experimental conditions described; this loss, however, is slight. With Exps. 3 and 4 on mutton no such rise was seen and evaporation must have been negligible.

Soluble nitrogen. For estimations of non-protein soluble nitrogen the following method was used. 5-10 g. muscle were weighed and cut up very finely with scissors on a watch glass. This mince was washed into a beaker with about 100 cc. distilled water and covered with a watch glass. The beaker was then heated slowly till the water just came to the boil. The liquid was then poured off the meat, and a second boiling with 50 cc. distilled water carried out in a similar way. The first extract was then added to the meat and second extract, and 5 cc. of 20 % metaphosphoric acid added to precipitate the protein. The mixture was allowed to stand overnight and was then filtered into a 250 cc. measuring flask, the residue was well washed with distilled water, the washings being allowed to run into the flask, and finally, the liquid was made up to the mark with distilled water. Kjeldahl estimations were then carried out on 50 cc. portions of this filtrate to give the amount of soluble nitrogen present.

In the results shown in the tables, besides the actual experimental figures obtained, the ratio of soluble nitrogen to total nitrogen is given, since this is the means by which Fearon and Foster [1922] and Callow [1925] measure the rate of autolytic change. It will be seen that no change takes place in the ratio in short periods of time with beef at 35° F. or 25° F. In Exp. 3 on mutton the same is true, but in Exp. 4 there is a slight rise throughout the experiment showing a very small amount of autolysis at 25° F. in 10 days.

Amino nitrogen. The amino nitrogen content of the meat was determined on portions of the same filtrate employed for the estimation of soluble nitrogen. Van Slyke's apparatus was used.

It can be seen from the tables that there was no rise or fall in the amino nitrogen content of beef or mutton during the experimental period.

Carnosine. Estimations of this extractive were made according to a method described by Clifford [1921]. Again the estimations were carried out on portions of the filtrate prepared as stated for soluble nitrogen. Carnosine remained constant except in one case, that of Exp. 2 on beef, kept at 35° F., when on the sixth day there was a marked fall. This was probably due to an incipient putrefaction, as on the eighth day the meat was definitely decomposed.

Creatine and Creatinine. For the estimation of creatine 1-1.5 g. muscle was weighed into a flask, 20 cc. of 3 % hydrochloric acid were added, the flask plugged with cotton wool, and the whole autoclaved at 130-140° for 30 minutes. The creatinine thus formed from the muscle creatine together with any preformed creatinine present was estimated by Folin's method as described by Plimmer [1920].

There was no change in this extractive during the experimental periods either at 35° F. or at 25° F.

EXPERIMENTAL RESULTS.

The results of the experiments are given in the following tables:

Exp. 1. <i>Shin of beef.</i>							
G. per 100 g. meat							
Day	G. per 100 g. meat		Sol. N	Creatine expressed as creatinine			
	Total N	Sol. N	Total N %	Amino N	Carnosine	as creatinine	
0	A. 2.82	0.349	12.02	0.731	0.980	0.359	
	B. 3.10	0.376	12.11	0.765	0.976	0.370	
3	A. 2.84	0.360	12.7	0.760	0.981	0.378	
	B. 2.81	0.359	12.8	0.737	0.980	0.375	B exactly same in appearance as A
6	A. 3.33	0.420	12.3	0.764	0.980	0.362	Large spicules of ice in frozen meat. Copious drip when thawed. Outer room meat very sticky and smells slightly musty
	B. 3.38	0.422	12.5	0.751	0.979	0.370	
8	B. 3.58	0.458	12.5	0.764	0.983	0.411	Meat in outer room definitely bad, therefore Exp. A stopped. Copious drip on thawing B meat
10	B. 3.56	0.438	12.3	0.760	0.980	0.370	" "
13	B. 3.56	0.436	12.2	0.746	0.984	0.365	" "

A = outer room 35° F.

B = inner room 25° F.

DISCUSSION.

These experiments were carried out in July 1925 when the weather was exceptionally hot and sultry with frequent thunder-storms, and therefore in all probability represent the maximum *post-mortem* change. The weather conditions were rather worse in Exps. 2 and 4 than in 1 and 3.

With such atmospheric conditions it appears impossible to keep meat in a condition fit for human food at a temperature slightly above freezing point (35° F.) for longer than 3-4 days. This is shown by the fact that on the sixth

day of these experiments there was a decidedly musty smell, and an appearance of incipient decomposition in both beef and mutton, and on the eighth day putrefaction had definitely started in three out of the four experiments. It is true that in these experiments the meat was shut up in glass jars, and therefore did not have the benefit of air currents, but, to balance this, in a cold room connected with an abattoir whole or half carcasses would be stored instead of comparatively thin pieces of muscle and consequently they would not cool so rapidly.

Exp. 2. *Beef (fillet steak).*

Day	G. per 100 g. meat		G. per 100 g. meat				Creatine expressed as creatinine	
	Total N	Sol. N	Sol. N %	Amino N	Carno-sine			
0	3.44	0.397	11.2	0.915	0.981	0.450	Day of slaughter	
	B. 3.50	0.404	11.2	0.897	0.980	0.456		
3	A. 3.64	0.403	11.5	0.820	0.979	0.413		
	B. 3.66	0.421	11.1	0.890	0.981	0.452		
6	A. 3.77	0.423	11.2	0.835	0.814*	0.452	A with faint musty smell and sticky. Spicules of ice in B very red and dripping on thawing	
	B. 3.86	0.439	11.3	0.847	0.990	0.452		
8	B. 3.67	0.430	11.8	0.947	0.982	0.409	Meat in outer room quite bad; experiment therewith abandoned. Very copious drip on thawing B	
	B'. 3.66	0.403	11.2	0.976	0.987	0.430		
10	B. 3.58	0.413	11.5	0.908	0.987	0.419	" "	
	B'. 3.57	0.425	11.3	0.909	0.980	0.425		

A=outer room 35° F.

B=inner room 25° F.

Exp. 3. *Leg of mutton.*

Day	G. per 100 g. meat		G. per 100 g. meat				Creatine expressed as creatinine	
	Total N	Sol. N	Sol. N Total N %	Amino N	Carno- sine			
0	3.80	0.478	12.2	0.759	0.338	0.460	Day of slaughter	
	—	0.469	—	0.784	0.332	0.465		
3	A. 3.58	0.444	12.4	0.848	0.341	0.476		
	B. 3.53	0.448	12.7	0.858	0.336	0.455		
6	A. 3.64	0.454	12.4	0.872	0.344	0.433	Frozen meat with ice spicules. Very slight musty smell in A	
	A. 3.80	0.473	12.4	0.826	0.346	—		
	B. 3.76	0.462	12.3	0.862	0.339	0.416	Slight musty smell in A and meat sticky	
8	A. 3.76	0.473	12.3	0.870	0.340	0.468		
	B. 3.81	0.457*	12.1	0.865	0.335	0.455	* Some NH ₃ lost	
10	A. 3.68	0.451	12.3	0.928	0.331	0.478	Meat more sticky and smell stronger in A	
	B. 3.63	0.446	12.3	0.919	0.330	0.465		
13	A. 3.38	0.411	12.2	0.886	0.320	0.470	" "	
	B. 3.18	0.361	11.3	0.860	0.320	0.465		

A=outer room 35° F.

B=inner room 25° F.

Exp. 4. *Leg of mutton.*

Day	G. per 100 g. meat		G. per 100 g. meat				Day of slaughter
	G. per 100 g. meat		Sol. N	Creatine expressed as creatinine			
	Total N	Sol. N	Total N	Amino N	Carno-sine	as creatinine	
0	3.52	0.394	11.2	0.815	0.323	0.495	
	3.47	0.390	11.2	0.814	0.338	—	
3	A. 3.37	0.415	12.3	0.827	0.320	0.491	
	B. 3.45	0.484	14.1	0.799	0.328	0.540	
6	A. 3.31	0.423	12.7	0.744	0.306	0.540	Slight musty smell in A. Spic- ules of ice in B. B very wet on thawing
	B. 3.43	0.414	12.1	0.823	0.330	0.499	
8	B. 3.55	0.448	12.3	0.783	0.330	0.452	A quite bad; therefore aban- doned and two muscles in B used. Very copious drip on thawing of B
	B'. 3.20	0.405	12.6	0.821	0.325	0.462	
10	B. 3.19	0.444	13.9	0.788	0.328	0.311*	*Some drip lost
	B'. 3.28	0.445	13.2	0.767	0.331	0.488	

A = outer room 35° F.

B = inner room 25° F.

A=outer room 35° F.

B=inner room 25° F.

This decomposition of freshly killed meat kept at 35° F. is superficially surprising. That this happens whenever the outside weather conditions are hot and sultry is borne out by the experience of the engineer in charge of the chilling rooms at the Islington Cattle Market. He states that in hot English weather it is impossible to keep carcasses in good condition at 35° F. for more than a few days though with cool outside conditions they can be kept for weeks at that temperature. For any prolonged period of storage during the hot weather in England the temperature must be below freezing point. This difference in the storage properties of a room at a constant temperature of 35° F. according to varying outside atmospheric conditions may possibly be due to one or both of two reasons. The first is that in hot weather *post-mortem* changes take place more rapidly in the meat during the period between slaughter and the time when it attains the temperature of the chilling room, than is the case in cold weather. The second explanation is that the outside air drawn into the freezing chamber is more heavily laden with bacteria in hot weather than in cold, and consequently the organisms are able to decompose the meat by sheer numbers, even at a temperature where bacterial action is necessarily retarded. If this is so, the storage properties of a chilling room should be greatly increased by some method of filtering the air before it is allowed to enter the storage chamber.

The likelihood of there being no change in the composition of meat stored at 35° F. given cool outside conditions is supported by Exp. 3 on mutton where comparatively slight changes occurred, viz. a musty odour from the eighth to the thirteenth days and a sticky condition of the muscle without any definite and unmistakable putrefaction. Chemical estimations carried out on this meat showed no measurable change in composition.

Meat stored below freezing point (25° F.) kept indefinitely as would be expected, and no change in the appearance of the meat could be seen up to

the third day of storage. To the naked eye it looked like fresh meat, or meat chilled to 35° F. By the sixth day, however, the meat at 25° F. was full of ice spicules, and had turned the characteristic bright red colour of frozen meat. There was also a copious drip on thawing this "iced" meat.

Both the frozen and chilled meats show a little surface sweating on being brought into warm air even as early as the third day of storage, but this is purely the result of bringing a cold object into a warm humid atmosphere.

The changes in frozen meat up to 13 days appear to be physical, since no chemical alteration could be detected in the total nitrogen, soluble nitrogen, ratio of soluble nitrogen to total nitrogen, amino nitrogen, carnosine or creatine contents of beef or mutton. The "drip" of frozen meat is probably due to the rupture of the muscle cells by ice spicules.

SUMMARY.

1. Beef or mutton kept at 25° F. and 35° F. appear identical in appearance with freshly killed meat up to the third day of storage.
2. Beef and mutton kept at 25° F. show ice spicules and the red colour characteristic of frozen meat on the sixth day of storage.
3. In hot English weather beef and mutton will not keep for 6 days in a room at 35° F.
4. There is no change in total nitrogen, soluble nitrogen, amino nitrogen, carnosine or creatine in meat kept at 35° F. for 3 days, or at 25° F. for 13 days.

Thanks are due to Dr Dale for kindly allowing the meat to be placed in the cold rooms at the Medical Research Institute at Hampstead.

The expenses of this work were defrayed by the Sheffield Corporation.

REFERENCES.

- Callow (1925). *Biochem. J.* **19**, 1.
Clifford (1921). *Biochem. J.* **15**, 400.
—— (1922). *Biochem. J.* **16**, 341.
Cole (1920). *Practical Physiological Chemistry*, 324.
Fearon and Foster (1922). *Biochem. J.* **16**, 564.
Plimmer (1920). *Practical Organic and Biochemistry*, 555.