L.—*Remarks on the Brine of Salt Meat, and on the Distribution of Albumen through Muscular Tissue.*

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ON considering Professor Graham's remarkable discoveries on liquid diffusion, it appeared to me that the process of dialysis might be available to separate the salt from the brine of salt meat, in order to obtain from it a liquid which might be used as soup. I accordingly commenced, in November, 1862, some experiments on the subject, and soon found that by evaporating brine to about one-third of its bulk, decanting the mother-liquor after the crystallization of the salt, and dialysing the liquor for from 18 to 36 hours, the liquid remaining on the dialyser possessed a strong taste of soup, and none but the amount of salt necessary for flavour; in fact, it could hardly be distinguished from soup prepared in the usual way. Under the impression that cheap soup obtained by this method might be used as a means of assisting in the relief of the distressed Lancashire operatives, I communicated on the subject with a friend at Liverpool, who was actively engaged on this benevolent undertaking, and sent him samples of brine-soup. His answer was not, however, so favourable as I had anticipated; moreover, on considering further the process for the preparation of the soup, it became obvious to me that although this liquid possessed the smell and taste of broth, it was necessarily poor in nutritious qualities, being deficient, in a great measure, in the crystalloid constituents of the juice of flesh. Thus phosphates, lactates, kreatine, and kreatinine, crystalloids which are present in flesh, and pass into the brine during the process of salting, must be lost to a great extent while the brine is being dialysed. From these and other considerations I then gave up my intention of publishing the above method for preparing brine-soup. Previously, however, on the 14th February, 1863, an article appeared in the "Family Herald" under the title *Utilization of Brine*, stating

shortly, but clearly, that I had found a method for extracting soup from brine, the process being the application of dialysis to the brine of salt meat. I was much annoyed at this article, which was published entirely without my knowledge or sanction.

It is no way remarkable that during the ordinary process of salting meat, a considerable proportion of its crystalloid constituents should diffuse into the brine, and that the salt of the brine should, from its known diffusibility, readily permeate the meat. Thus it is that brine which has been used for salting meat is charged with phosphates, lactates, kreatine, and kreatinine. It occurred to me that this liquid might be a good source for the preparation of lactic or sarco-lactic acid and kreatinine, when comparatively large specimens of these substances are required. Liebig has demonstrated their presence in brine, but he failed to extract kreatine from it, on account of the difficulty of isolating this substance from chloride of sodium. The following method, I adopted, differs somewhat from that recommended by Liebig for the extraction of the constituents of juice of flesh. It enabled me to obtain kreatine in small quantity from brine in addition to the two other substances.

Brine, obviously rich in juice of flesh, was boiled and filtered to remove the albumen, then concentrated to about one-fourth of its bulk, and allowed to cool, when a considerable proportion of the chloride of sodium crystallized. The liquid was then decanted and mixed with alcohol, which was added as long as it caused in the fluid a precipitate. The liquid filtered from this precipitate contained the lactates, kreatinine, and most of the kreatine. After distilling off the alcohol, the residue was mixed with a concentrated solution of chloride of zinc; and some weeks later a mass of crystals had formed in the fluid, consisting of *lactate of zinc*, the *double chloride of zinc and kreatinine*, and *kreatine*. These crystals were collected on a filter, washed with a very small quantity of cold water pressed between folds of filtering paper, and dissolved in water. The solution filtered from some oxide of zinc contained in suspension was now boiled with hydrated oxide of lead, and again filtered. This last filtrate contained the kreatinine and kreatine; the lactic acid had remained on the filter as an insoluble lead salt. In order to obtain the lactic acid under the form of lactate of lime, I decomposed the lead precipitate with sulphuric acid, then neutralized the fluid with carbonate of lime, and, after filtration, removed the dissolved sulphate of lime by the addition of alcohol.

The fluid yielded on evaporation a mass of crystals, which, on analysis, proved to be nearly pure lactate of lime. In order to separate the kreatine from the kreatinine in the filtrate from the lead precipitate, the fluid was evaporated to dryness, and the residue treated with alcohol which dissolved out the kreatinine and left the kreatine. The latter was obtained crystallized from its subsequent solution in water, and the former from its solution in alcohol.

Another peculiarity in the composition of the brine of salt meat has attracted my attention,—this is the large proportion of albumen it contains. At first sight it may appear very natural that the albumen of the juice of flesh should pass out into the salt water with the other soluble constituents of meat ; but on a further consideration the phenomenon has appeared to me very remarkable, as it does not apparently agree with the known laws of diffusion. A mass of flesh, in so far as its physical properties are concerned, would be classed as a colloid body ; for other parts of the animal body also organised, as membranes, are used as colloid septa, being essentially colloid bodies ; albumen being a colloid substance, we have here apparently the case of a colloid diffusing very readily through a colloid mass, which is in opposition to the laws of diffusion. I thought at first that the albumen of the juice of flesh might perhaps be peculiarly possessed of the property of passing through a membrane like a crystalloid ; but having placed some juice of flesh in a piece of pig's intestine,* and immersed the whole in water, in one experiment I could not observe any diffusion of albumen out of the membrane after 24 hours, and in another there was merely a trace of albumen in the water. I obtained a similar result by placing some juice of flesh in a dialyser supplied with a parchment-paper diaphragm, and floating the dialyser on water. I then instituted the following experiment:—A piece of fresh raw beef was placed in a tall and narrow jar, and allowed to remain undisturbed for two days ; being then examined, it was found that the water up to the level of the upper margin of the meat had turned red ; the water above the meat was divided into four horizontal layers by strips of paper gummed to the glass, and each layer was decanted in succession with a syphon. The first and second layers on being tested, gave no trace of albumen ; the

* The pig's intestine had been dried for its preservation. I had softened it in water at the time the experiment was undertaken.

third layer yielded a very slight haziness by the application of heat and nitric acid; the fourth layer yielded a decided haziness, though no distinct coagulation. The coloured fluid now decanted, on being boiled and with nitric acid, showed that it contained a large quantity of albumen. Consequently in this experiment the albumen had diffused readily out of the meat, but the water, within certain narrow limits, had opposed an invincible obstacle to its further diffusion. In the following experiment, water was replaced by gelatin. I immersed a piece of fresh muscle in a solution of isinglass just before it solidified. The next day there was not the slightest coloration of the jelly, even close to the meat, from which it may be concluded that no albumen had passed out; gradually, however, the gelatin round the meat became slightly coloured, and on the sixth or seventh day, each fragment of meat was surrounded by a red zone which did not however permeate the whole of the jelly. The coloured isinglass was found, on being tested, to contain albumen, but not near so much as if the muscle had been immersed in water; from this experiment it may be concluded that gelatin at first checks completely the exit of albumen from muscle, but after a time this substance passes out, although to a very limited extent; and moreover, it is shown that albumen has but a very slight tendency to travel through gelatin. These results all agree perfectly with those obtained by Graham, who considers albumen as an essentially colloid substance.

The next point was to determine the physical nature of muscular tissue, and for this purpose I transformed a piece of muscle into an unmistakable colloid mass by mincing it finely, and triturating it afterwards in a mortar with a fluid solution of isinglass. The mass was afterwards placed in a wide beaker and allowed to gelatinise; a thin layer of water was then poured carefully over the jelly. Two days later the water was very pale red coloured, and on the application of heat and nitric acid, a haziness took place, changing into a few flakes of coagulated albumen; this quantity of albumen was exceedingly small. After five days the liquid was smelling a little from decomposition; it contained a little more albumen, but the quantity was in no respect to be compared with that which passes out of pure muscle into water. From this experiment it follows, that muscular tissue, when transformed into a colloid, loses, in a very great measure, its property of allowing the albumen it contains to pass out into water, consequently *a muscle is not a colloid mass.*

Muscular tissue consists of bundles of fibres connected together by means of cellular tissue; a fluid being distributed throughout muscle may therefore be considered as having to pass through an immense number of delicate membranes and innumerable cellular spaces. I have shown that a common membrane, such as a piece of intestine preserved in the dry state, does not let albumen through it; but would a very delicate animal membrane have the same property? As I was reflecting over this question, an experiment I had performed shortly before gave me a clue to its answer. I had selected as a dialyser the cellular or fibrous membrane which covers the liver of the ox and the sheep;* this is an exceedingly fine membrane perhaps not thicker than goldbeater's skin; having removed a small quantity of this tissue from the liver of the ox, I had made it into the diaphragm of a dialyser by causing it to adhere to the rim of the wide opening of a glass filtering-funnel; the diameter of this dialysing septum was 7 centimetres; 10 cc. of a fluid extract of flesh had been introduced into the funnel, and the membrane placed in contact with the surface of a small quantity of water. Twenty-four hours afterwards the water was slightly coloured red, and on boiling it a few flakes of albumen appeared; this was certainly an indication that albumen passed through a fine delicate membrane more readily than through that of the intestines.

A similar experiment had been also undertaken with a still finer membrane, that covering the liver of the sheep; in this case the diameter of the diaphragm was 4·5 centimetres; it adhered firmly to the rim of a small funnel, into which I introduced 5 cc. of an aqueous extract of flesh, and the membrane was placed in contact with the surface of a small quantity of water. The experiment was begun immediately after the membrane had been removed from the liver; it was then quite fresh and moist, whilst it had been allowed to dry in the former experiment. Eighteen hours afterwards the water was slightly red; on boiling, it first became muddy, and then flakes of albumen appeared. I determined, approximately, that about one-third of the albumen in the dialyser had passed out into the water. In a last experiment, I made a dialyser with a test-tube, the bottom of which had been broken, and I fastened a small piece of

* The liver has two membranes, one which covers it entirely, and another—the peritoneum—which incloses it but partly. I used indifferently these two membranes, together or separately.

a still more delicate part of the membrane of sheep's liver to the rim of the tube. Some extract of flesh was introduced into the tube, and the membrane placed in contact with a little water; the water began very quickly to turn coloured, and, on testing it four hours after the beginning of the experiment, I found it charged with albumen.

These experiments appear to me very interesting, as showing that albumen passes readily through a very thin animal membrane into water; and we may expect that it is by a similar process that albumen is able to pass through the infinite number of delicate membranes which form the walls of the muscular cells; it can be admitted that the fluid travels inside the cells by capillary motion.

I now applied myself to determine the reason of this passage of albumen through a very thin membrane,* and from a suggestion of Prof. Graham, I was induced to examine very closely the membrane of the liver I had used as a dialysing septum, in order to ascertain whether it exhibited any rupture or breach of continuity through which albumen might accidentally have passed. The result of this inquiry was that after macerating the liver in water for some hours, its membrane can be detached without difficulty, and in a perfectly sound condition, as far as can be ascertained by a very careful ocular examination—that when fastened to a dialyser, water being poured upon it, filters through the membrane, the latter becoming studded on its free surface with minute specks of water gradually running into each other, and after a time forming drops. In one experiment the column of water in the dialyser was 25 millimetres (say one inch) in height, and the diameter of the membrane 24 millimetres; 22 minutes after the water had been poured into the dialyser the first indication of moisture appeared on filtering paper placed in contact with the under surface of the membrane. The first drop fell from the membrane 4 hours later; the fall of the second drop was hastened by an accidental blow to the table; the third drop fell 1 hour and 35 minutes after the second. We may, therefore, conclude that the fine membrane experimented upon had not been

* I stated before the Chemical Society I considered this phenomenon one of liquid diffusion: according to a suggestion from Professor Graham, I instituted the additional series of experiments reported in the present paper (communicated in the following meeting of the Chemical Society), from which it will be observed I have found the process to differ from that described by Graham as liquid diffusion.

torn or otherwise injured, but was *porous*, and on this account allowed a fluid to pass through it.

The rate of transition of albumen through the membrane of the liver into water was ascertained by fixing the membrane to the rim of a common size test-tube, the bottom of which had been cut off, introducing five cubic cent. of an extract of flesh (the albumen of which had been quantitatively determined) into this dialyser, and placing the diaphragm in contact with five cubic cent. of water. After 17 hours, 1.6 cubic cent., or 32% of the albuminous fluid, had passed into the water, and exactly 32% of the albumen was found to have travelled through the membrane into the water. The amount of albumen, and the volume of fluid which had escaped out of the tube, were, therefore, exactly proportional. This shows not only the rate of passage of albumen through the membrane, viz., 32% in 17 hours, but also that the process is one entirely of transition through small apertures, and not of liquid diffusion.

In order to inquire further into the passage of albumen through the membrane of sheep's liver, I made an experiment, which unexpectedly yielded a very interesting result. I thought of turning the liver itself into a dialyser, the tissue of the gland being abundantly supplied with albumen. A surface of the gland enclosed in its membrane was immersed in distilled water, and I observed in this case, that some albumen found its way into the external water, although less than when the membrane had been removed from the gland. A comparative experiment was made by immersing in equal bulks of water a part of liver enclosed in its membrane, and a section of the liver; the latter being consequently not covered by membrane, the surface of the section was about one-half that of the liver immersed with its membrane. The next day, at the same time, I interrupted the experiment and determined the albumen in 20 cubic cent. of each fluid—0.0695 grms. of albumen was found to have passed through the membrane for every 20 cc. of the external fluid, and 0.118 grms. of albumen had exuded directly from the tissue for every 20 cc. of the external fluid. But as the surface of the membrane was about twice that of the section, it follows that for equal surfaces bathed in equal bulks of water, about four times as much albumen would have passed out of the section as out of the surface covered with membrane.

Now why does the membrane let albumen through it more readily when removed from the liver than when adhering to it?

As I hope to have shown satisfactorily that the membrane can be separated from its gland without being torn or injured, and moreover, that this membrane in its sound condition is *porous*, I believe the question only admits of one explanation, namely, that when forming part of the liver, the membrane is coated with a colloid substance, which occupies its pores more or less, and extends into the substance of the liver, causing it to adhere firmly to the tissue of the gland, as if it were pasted upon it. This view is supported by the following facts :—

1st. In its normal condition the membrane adheres strongly to the tissue of the gland, but after soaking in water it can be easily removed from it.

2nd. On peeling off the membrane it is difficult to prevent some of the tissue being torn away with it.

3rd. The membrane itself appears as if containing a substance like glue, which causes it to adhere very firmly to glass when dried in contact with it.

4th. The membrane when dried is much less pervious to water than in the fresh moist condition, as if a substance had dried within its pores, which required to be dissolved, in order to enable liquids to pass through.

I am not prepared to state whether this colloid substance, if its existence be considered as proved, is equally distributed throughout the gland or not ; but its presence under the above conditions appears to me a remarkable circumstance in a physiological and chemical point of view.

Considering the results of these experiments, it now appears little doubtful that the distribution of albumen through muscular tissue takes place by a process of porous imbibition, as in the case of the membrane ; the travelling of albumen through muscle must therefore be considered mainly or entirely as a phenomenon of *physical distribution*, due to *porosity*, and not of liquid diffusion.

In order to judge whether the passage of albumen through muscular tissue is in any degree a phenomenon of liquid diffusion, I determined, in the following experiment, the relation between the quantity of albumen, and of the crystalloid substance *phosphoric acid*, which diffuses from muscle into water within a given time, and compared the result with the relation between the quantity of albumen and phosphoric acid contained in juice of flesh. It was necessary to begin by determining the proportion between albumen and phosphoric acid in juice of flesh,

which was done in the following way. An extract of 200 grms. of beef, well minced, was prepared with 125 cc. of distilled water; I determined the quantity of albumen and phosphoric acid contained in measured samples of the extract, the albumen being coagulated by heat and dried on a weighed filter. The phosphoric acid was estimated by evaporating to dryness a known bulk of the extract, incinerating the residue with spongy platinum, dissolving the ash in acetic acid, and precipitating the phosphoric acid with acetate of peroxide of uranium; precipitation as triple phosphate of ammonia and magnesia could not be adopted, Liebig having shown that phosphoric acid in flesh exists, partly as bibasic phosphate, partly as tribasic phosphate of potash.

On the other hand, I poured 125 cc. of distilled water into a beaker containing 200 grammes of fresh beef, free from fat, and allowed the beaker to remain undisturbed for a certain time, after which the albumen and phosphoric acid were determined in the external fluid in the same way as had been done in the extract. If the result of the experiment showed that the proportion of phosphoric acid to the albumen in the extract was the same as the proportion of the phosphoric acid to the albumen in the water in which meat had been soaked, then it would be obvious that albumen travels through flesh with the same speed as a crystalloid, in which case flesh might be considered exclusively as a porous mass; but if these proportions should respectively differ, there being less albumen in the external fluid in proportion to the phosphoric acid it contained than in the extract (in proportion to the phosphoric acid in the extract), then we would conclude that a muscle allows of a certain amount of *liquid diffusion* throughout its substance, being therefore to some degree a colloid; we shall see that such is the case. The results are as follows:—

In 100 cc. extract.		In 100 cc. external fluid after 26 hours.	
Phosphoric acid..	0·233	} Prop. 1 : 12·5	{ 0·169 } Prop. 1 : 6·3.
Albumen	2·925		

The conclusion from this experiment is, that, at the common temperature, the rate of distribution of the colloid substance, *albumen*, through and out of muscular tissue, is only equal to about one-half the diffusibility of the crystalloid substance, *phosphoric acid*, probably under the form of phosphate of potash, through and

from muscular tissue. Therefore a muscle opposes some obstacle to the ready passage of colloid substances, such as albumen, throughout its tissue, which obstacle does not occur in the case of phosphoric acid—a crystalloid, consequently a muscle, in addition to its porosity, is, to some (a slight) degree, possessed of the characteristic property of a colloid mass.

By means of the ready distribution of albumen from the blood-vessels into every portion of a muscle, nutriment is perfectly supplied throughout the organ.

On considering the preparation of soup from the action of dialysis on the brine of salt meat, it occurred to me that by enclosing meat in bladders, or in the intestines of the slaughtered animals, and immersing the whole in a saturated solution of common salt, the salt would diffuse itself through the membrane of the bladder into the meat, while the latter would retain nearly the whole of its albumen and other colloid constituents which are lost in the ordinary mode of salting. The salting in bladders would, therefore, be an improvement on the process commonly in use, as meat thus preserved would be more nutritive and better flavoured; moreover, the common process of removing the salt from salt meat to prepare it for cooking, which generally entails a further loss of albumen and other colloids, would no longer be thus objectionable, because, instead of soaking salt meat directly in water, it would be soaked while enclosed in the bladder. After experimenting on the subject, I found that in warm weather the diffusion of common salt through an intestinal membrane into flesh, although occurring very readily, is not rapid enough to prevent the centre of the meat from undergoing a slight decomposition. I was, therefore, obliged to modify the process, and on the 12th May, 1863, I instituted the following experiments:—

Some beef was roughly cut up and divided into four quantities, each weighing 250 grammes. I added to quantity No. 1, 25 grammes, or 10% of salt; to No. 2, 50 gms., or 20% of salt; to No. 3, 75 gms., or 30% of salt; and to No. 4, 100 gms., or 40% of salt. These several samples of meat, with their respective doses of salt, were enclosed in pieces of pig's intestines preserved by drying and moistened at the time, weighed, and immersed in a saturated solution of salt. On the following 12th June, or after the lapse of a month, the brine had a slight red colour, and contained but very little albumen. The meat was withdrawn from the brine and examined; with the exception of sample No. 1,

which had become a little lighter, they had all increased in weight. The bladders externally had not the slightest smell of decomposition; on examining their contents, No. 1 and No. 2 had no undissolved salt in them, but there was some in Nos. 3 and 4. There was no smell of decomposition inside the masses. Having secured the bladders, they were soaked in pure water, during which time a little albumen passed out. I tasted the samples at various periods within four days from this date, and found that at least three days were necessary to remove the taste of salt. After four days' immersion in fresh water, although the weather was warm, no smell of decomposition was emitted by the meat; it had a good taste, though much like that of salt meat, and had not so much of the flavour of fresh meat as I had anticipated; it was, I thought, more tender than meat salted by the usual process. It must have been richer in albumen and colloid constituents than commonly salted meat, and, I believe, could not have failed to be more nutritious. The result of these experiments is, that meat mixed with about one-tenth of its weight of salt, and salted in bladders, tastes as well, if not better, than meat salted by direct immersion in brine, and it must be more healthy and nutritive.