THE RED COLOUR OF SALTED MEAT.

(One Figure in the Text.)

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WHEN fresh meat, or blood, is boiled the colour changes from the red of oxyhaemoglobin to a dull, brownish colour. The change of colour is due to splitting up of oxyhaemoglobin into coagulated proteid and haematin, which are precipitated. The dark colour of haematin, mixed with the white of coagulated proteid, gives the dull brown. When, however, meat has been salted, it has a characteristic red colour when cooked. It is evident, therefore, either that ordinary haematin is not split off from the oxyhaemoglobin, or that the colour of the haematin is masked by the presence of another pigment.

The red colour is well known to be caused in some way by the nitre which is always mixed with the salt used in preserving the meat. It has further been recently shown by Kisskalt[1] that meat becomes red when boiled in water containing nitrite, and he suggests that the red colour of salted meat may be due to the presence of nitrite; the manner in which the change of colour is produced has not hitherto been explained, and the experiments now to be described were undertaken with a view to elucidating the matter.

Endeavours were first made to extract the red pigment from cooked salted meat. The red colour was altogether insoluble in water, but a weak solution was obtained by kneading the meat with alcohol. The red colour faded after a time. On spectroscopic examination an ill-defined absorption band covering the *D* line of the solar spectrum could be distinguished, along with strong absorption of the whole of the blue end of the spectrum. The same appearance was seen on cutting a very

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[1] *Archiv für Hygiene*, vol. 35, p. 11, 1899.

thin slice of the meat and holding it before the slit of the spectroscope. This spectrum did not seem to correspond to that of any known derivative of haemoglobin, and I was therefore led to examine uncooked salted meat.
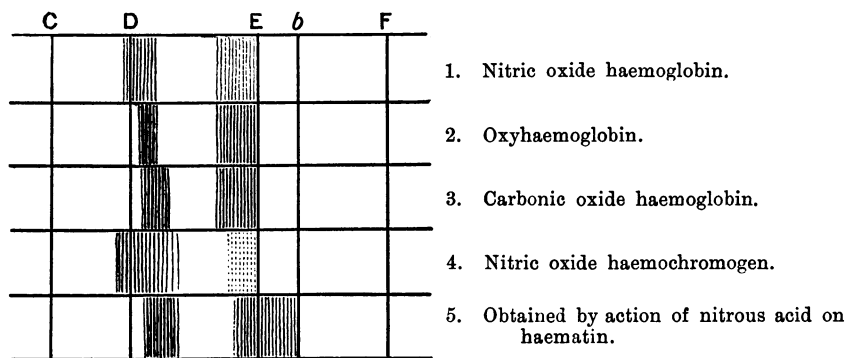
On cutting into pieces of uncooked salted meat, obtained from the butcher, I noticed that the colour of the exposed surface was bright red wherever the salt had penetrated, but slowly became dull on exposure to the air. On the other hand, if the salt had not thoroughly penetrated to the centre of the piece the exposed surface had at first the dull bluish tint of reduced haemoglobin, which soon changed into the red of oxyhaemoglobin on exposure to the air. That this red was really due to oxyhaemoglobin was proved by spectroscopic examination. If the part of the meat which had changed from blue to red was covered with a glass plate so as to exclude air it again became blue from reduction of the oxyhaemoglobin, whereas if the part which had become dull on exposure to air was covered it regained its red colour. It was thus evident that some different pigment from ordinary haemoglobin was present in the parts to which the salt had penetrated.

On extracting the freshly exposed salted part with water the red pigment was found to be quite soluble, and to give a spectrum not altered by warming the solution with ammonium sulphide, and possessing two absorption bands at about the position of the oxyhaemoglobin bands, but not nearly so well defined. This spectrum was found to be identical with that of nitric oxide haemoglobin (no. 1 of figure); and the behaviour of the pigment in all other respects showed that it was nothing else but pure NO-haemoglobin.

This substance, which was discovered by Hermann[1] in 1865, has hitherto not attracted very much attention, as it was supposed only to occur as a laboratory product. It is prepared by passing nitric oxide through blood which has been deprived of oxygen, care being at the same time taken to exclude from the blood the nitric peroxide formed by the nitric oxide as it escapes into the air. The spectrum of NO-haemoglobin was described in general terms by Hermann. As, however, no figure showing the appearance and exact position of the bands has hitherto been published the accompanying sketch will be of service. For comparison the spectra of oxyhaemoglobin and CO-haemoglobin solutions of equal concentrations are also given. It will be seen that in the case of NO-haemoglobin both bands are less well-defined, and that the band in the neighbourhood of the *D* line extends slightly but quite distinctly

[1] *Archiv für Anat. und Physiol.*, p. 469, 1865.

over the D line towards the red. The appearance and position of this band at once distinguish NO-haemoglobin from either oxyhaemoglobin or CO-haemoglobin.

1. Nitric oxide haemoglobin.

2. Oxyhaemoglobin.

3. Carbonic oxide haemoglobin.

4. Nitric oxide haemochromogen.

5. Obtained by action of nitrous acid on haematin.

Undiluted blood has the same florid red colour whether it be saturated with oxygen, carbonic oxide, or nitric oxide, but in a thin layer of blood, or a dilute solution, NO-haemoglobin is slightly more pink, and less yellow, than oxyhaemoglobin, and much less pink than CO-haemoglobin. Solutions of oxyhaemoglobin and CO-haemoglobin may also be distinguished by the fact that on adding ferricyanide and acidifying slightly with acetic acid they at once give the spectrum and colour of pure methaemoglobin, whereas a solution of NO-haemoglobin is only slowly and partially decomposed, the two bands of NO-haemoglobin remaining still visible along with the methæmoglobin band in the red, and the colour of the solution being brownish-red. On exposure to air NO-haemoglobin is slowly converted into methaemoglobin.

On boiling, NO-haemoglobin will be found to give a red coagulum. This fact alone is sufficient to distinguish it from CO-haemoglobin or oxyhaemoglobin. Reduced haemoglobin also gives a red coagulum due to the formation of hacmochromogen ("reduced haematin"), but the colour at once changes to the ordinary dull brown or grey on exposure to the air. The formation by NO-haemoglobin of a permanent red colour on boiling of course accounts for the red colour of cooked salt meat.

A solution of the red pigment formed on boiling may be obtained by slightly diluting and adding alkali or acetic acid to the blood, so that it does not coagulate. The colour of the solution is ruby-red. On dilution it appears brownish-red. On spectroscopic examination of the diluted solution, whether alkaline or acid, the spectrum shown at

no. 4 of the figure is seen. There is a not very sharply defined absorption band covering the D line, and with a centre somewhat to the blue side of the latter: at the same time the whole of the blue end of the spectrum beyond this band is more absorbed than the red end, and a second very faintly defined band can just be distinguished near E, in the position shown.

It is natural to suppose that the substance in solution is a nitric oxide compound of haematin, or rather of haemochromogen. To test this supposition nitric oxide was passed through solutions in water containing ammonia of haematin prepared both by MacMunn's method[1] and by simply boiling blood with caustic soda. The colour of the solutions soon changed to red, and the spectrum described above appeared. The spectrum of haemochromogen was, however, sometimes at first visible, and the action of the nitric oxide was, evidently, first to reduce the haematin to haemochromogen, and then to combine with the latter.

Nitric oxide haemochromogen has already been described by Linossier[2], who obtained it by passing nitric oxide through haematin solutions. He described its spectrum as containing two bands between D and E, and similar to those of oxyhaemoglobin, but with the second band much less sharply defined than the first. This description does not correspond well with that given above, and I was at first much puzzled by the want of agreement. I obtained, however, a spectrum which seems to correspond somewhat better to his description by prolonged bubbling of nitric oxide through slightly alkaline solutions of haematin. This spectrum is shown at no. 5 in the figure. There are two well-defined absorption bands, of which one is near, but quite clear of, the D line on the blue side, and the second, which is more diffuse, extends on both sides of E, reaching nearly to b. The colour of the solution was red. On testing its reaction I found that it had become strongly acid, and unless the reaction became acid the spectrum was not obtained. On the other hand I could not obtain it by acidifying, even with mineral acids, solutions of NO-haemochromogen prepared by boiling NO-haemoglobin with alkali. This spectrum is thus not simply that of an acid solution of ordinary NO-haemochromogen. Finally, I found that on adding nitrite to a solution of NO-haemochromogen or ordinary haematin, and then acidifying strongly, the spectrum was at once obtained. Its appearance on prolonged passage of nitric oxide

[1] *Journ. of Physiol.* vol. 6, p. 22, 1885.
[2] *Comptes Rendus*, vol. 104, p. 1296, 1887.

through haematin is thus apparently due to the presence of free nitrous acid formed in the test-tube by the action of air. Further investigation must decide the nature of the compound in question.

The origin of the NO-haemoglobin present in uncooked salt meat remains to be discussed. A watery extract of the latter, or a sample of the brine in which meat has been salted, gives with potassium iodide, sulphuric acid, and starch solution a very strong blue coloration, indicative of the presence of nitrite, and that the presence of nitrite in some way causes a red colour was, as before mentioned, proved by Kisskalt. The action of nitrites on blood was first observed and investigated by Gamgee[1], who found that the blood assumes a chocolate colour, and completely loses its characteristic power of taking up oxygen and yielding it again to a vacuum. He also described the alteration in spectrum, which was generally assumed until lately to be due simply to the formation of methaemoglobin from the oxyhaemoglobin of the blood. It was, however, pointed out recently by Makgill, Mavrogordato, and myself[2] that the spectrum and colour of oxyhaemoglobin acted on by a nitrite are not those of pure methaemoglobin, but of a mixture with the latter of about a fourth as much of NO-haemoglobin[3].

On further investigation of the action of nitrites on blood I have found that if the blood be deprived of its oxygen previously to the action of nitrite an almost pure spectrum of NO-haemoglobin is obtained, only a little methaemoglobin being formed. To carry out the experiment some fresh blood slightly diluted was introduced into a glass vessel fitted with a stop-cock at both ends. The vessel was connected through one of the taps with an ordinary filter-pump, slightly warmed, and all the oxygen boiled out. The tap was then turned off, and a few drops of a saturated solution of sodium nitrite were allowed to be sucked in, without any air. After a short time the spectrum of reduced haemoglobin at first seen in the blood changed into that of nitric oxide haemoglobin, accompanied by a weak methaemoglobin band in the red. The latter band could only be seen in concentrated solutions.

Nitrites not only act on oxyhaemoglobin and reduced haemoglobin,

[1] *Phil. Trans.* p. 589, 1868.

[2] *Journ. of Physiol.* vol. 21, p. 165, 1897.

[3] The difference in colour and spectrum between pure methaemoglobin, as obtained for instance by adding ferricyanide to diluted blood, and the mixture of pigments obtained with a nitrite is rendered very evident if the solutions be slightly acidified by adding a drop of acetic acid, or simply by blowing expired air through them. The nitrite product has a more or less red colour, and shows the two bands of NO-haemoglobin in addition to the spectrum of acid methaemoglobin, while the other solution has no trace of red in it.

but also on methaemoglobin. If nitrite is added to methaemoglobin prepared from blood with ferricyanide the colour alters to the same reddish-brown tint as is obtained when nitrite acts on oxyhaemoglobin, and the same spectrum is seen, corresponding to a mixture of NO-haemoglobin and methaemoglobin. The explanation of the peculiar behaviour of nitrites towards oxyhaemoglobin, reduced haemoglobin, and methaemoglobin is probably to be found in the facts that (1) haemoglobin readily combines either with nitric oxide to form NO-haemoglobin or with nascent oxygen to form methaemoglobin: (2) nitrous acid easily yields up simultaneously nitric oxide and oxygen: (3) nitric oxide . readily combines with either oxygen or haemoglobin. If no extraneous oxygen is present, as when reduced haemoglobin is acted on by the nitrite, both the nitric oxide and the oxygen yielded up by the nitrite will combine with the haemoglobin; and as far more nitric oxide than oxygen is yielded up, more NO-haemoglobin than methaemoglobin will be formed. On the other hand, when oxyhaemoglobin or methaemoglobin is acted on the nitric oxide will be free either to combine with haemoglobin or with the extra oxygen available; and a balance will be struck depending on the relative strengths of the various affinities.

NO-haemoglobin may be formed by the action of nitrite in a less direct manner than that described above. In the paper just referred to we noted the fact that when the body of an animal poisoned by nitrite is left for a day or two the methaemoglobin which was present at death disappears, and the haemoglobin is completely converted into NO-haemoglobin[1]. This observation is explained by the facts adduced in the preceding paragraphs. Bacteria and possibly other agents reduce the methaemoglobin, and the nitrite present then converts the reduced haemoglobin into NO-haemoglobin. The same change occurs in salted meat, and accounts for the fact that the red colour of the NO-haemoglobin, where it has faded in consequence of the production of methaemoglobin by the action of the air, may be restored by leaving the surface covered up so as to exclude air. In this process bacteria doubtless bring about the reduction, as their growth is only hindered, and not prevented by the action of the salt. Meat left in the brine used for salting was found to become putrid after some weeks.

When oxygenated blood to which nitrite has been added is boiled the coagulum obtained is reddish-brown in consequence of the presence in the clot of NO-haemochromogen produced by decomposition of the NO-haemoglobin formed along with methaemoglobin by the nitrite. If

[1] *Journ. of Physiol.* vol. 21, p. 168, 1897.

the boiling be continued the coagulum becomes redder, and similar in tint to the coagulum from blood saturated with NO. Reducing substances present in the blood appear to favour the further production of NO-haemochromogen. If the blood has first been boiled and the nitrite is then added to the clot the coagulum does not become red.

Nitrites do not act on alkaline solutions of haematin, but if a slightly alkaline haematin solution be reduced with ammonium sulphide in presence of nitrite the haemochromogen spectrum at first seen is replaced entirely by that of NO-haemochromogen. The presence of excess of alkali prevents this change. When reduced haemoglobin is boiled there is formed haemochromogen instead of haematin; and the haemochromogen is of course capable of forming NO-haemochromogen with any nitrite present. It must also be remembered that the reducing substances present in blood or muscle may absorb oxygen energetically when heat is applied, and that in this way even oxyhaemoglobin may yield haemochromogen and be converted by nitrite into NO-haemochromogen. These facts explain the very familiar observation that when a pie is made from fresh meat along with a little bacon, or other salted meat, the fresh meat is red when the pie is cooked.

The nitrite found in salted meat is probably partly formed by the reducing action of bacteria on the nitre which is always mixed with the salt. The action of bacteria is, however, not necessary, as it has recently been shown by Abelous and Gérard[1] that the fresh tissues themselves contain a substance capable of reducing nitrates to nitrites, and that this substance acts even in the presence of antiseptics.

Nitrites are somewhat actively poisonous; and as has been recently shown[2] they cause death by asphyxia through their action on the haemoglobin and the consequent interference with the oxygen supply to the tissues. Their presence in very appreciable quantity in raw salted meat might thus seem to be possibly harmful. On testing cooked salt meat, or the water in which it has been boiled, I have only sometimes found nitrite, however; and it is well known that nitrites are destroyed by prolonged heating. In some samples of ordinary well-preserved tinned meat I have also found no nitrite, although it is probable that the nitrate present would be reduced to nitrite if the sterilisation of the meat were incomplete.

Nitrates are a good deal less poisonous than nitrites, but their presence in salted meat when this is regularly used as food is probably

[1] *Comptes Rendus*, vol. 129, pp. 56 and 124, 1899.
[2] Haldane, Makgill, and Mavrogordato, *Journ. of Physiol.* vol. 21, p. 160, 1897.

not a matter of indifference. In a future paper I hope to give the results of further investigations on this subject.

Chief Conclusions.

1.   The red colour of cooked salt meat is due to the presence of NO-haemochromogen.

2.   The NO-haemochromogen is produced by the decomposition by heat of NO-haemoglobin, to which the red colour of unsalted meat is due.

3.   The NO-haemoglobin is formed by the action of nitrite on haemoglobin in the absence of oxygen, and in presence of reducing agents.

4.   The nitrite is formed by reduction within the raw meat of the nitre used in salting.

5.   The nitrite is destroyed by prolonged cooking.