ON THE BLOOD OF DECAPOD CRUSTACEA. BY W. D. HALLIBURTON, M.D., B.Sc. (Lond.), Sharpey Physiological Scholar, University College, London. (Pl. VIII.)

(From the Physiological Laboratory, University College, London.)

I HAVE used in my experiments the blood or haemolymph of the more common decapod crustaceans: viz.—the common lobster (Homarus vulgaris), the edible crab (Carcinus maenas), the fresh water crayfish, (Astacus fluviatilis), and the sea crayfish (Nephrops norwegicus). For a large number of specimens of the latter animal, I have to thank Professor Cossar Ewart; and for assistance towards the expenses involved in the research I am indebted to a grant from the British Medical Association.

It will be convenient to divide the subject into five parts.

PART I. The blood as a whole.
PART II. The phenomena of spontaneous coagulation.
PART III. The proteids of the plasma and serum.
PART IV. The colouring matters of the blood.
PART V. The blood from a comparative point of view.
APPENDIX. Tables.

PART I.

The Blood as a whole.

I propose, in this section, to discuss the methods adopted in the research, and to indicate the chief facts that I have ascertained in connection with the blood, and in the subsequent sections to amplify particular points.

The blood is obtained by making cuts in the ventral region in the soft integuments between the abdominal segments, or in the claw. The
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blood gushes out very readily, and from a large lobster nearly half a pint can as a rule be obtained.

**Colour.** The blood which can be seen flowing in the ventral sinus just beneath the skin in this region appears in the vessel to be colourless. The reddish tinge which is present in some specimens when the blood is drawn is so similar to the hue of surrounding parts, that it cannot be perceived through the transparent parts of the skin. The blood when first shed is either nearly colourless, or of a reddish colour from the presence in it of a red pigment presently to be described. It has also an opalescent or milky appearance from the presence of numerous amoeboid corpuscles. The milkiness is more marked in blood coming from the claw, than in that from the tail of the same animal. This is due to the cells being more abundant in blood from the former situation. This appearance is however but momentary, for coagulation begins to occur almost instantaneously. This is especially the case with the lobster and crayfish. In the crab coagulation is not so rapid, nor is the ultimate clot so firm and jelly-like; this fact is also noted by previous observers.

The blood after being a few moments in contact with the oxygen of the atmosphere acquires an indigo-blue tinge; but the readiness with which this is seen varies in different specimens. The blue colour is due to the oxygenation of a proteid body which exists in solution in the blood plasma; in the reduced state it is colourless; in the oxidised condition it is blue. The name haemocyanin was given to it by Fredericq.

The variation in the colour of the blood is owing to the admixture of the tint due to haemocyanin with a varying amount of a red colouring matter. This red pigment has been noted as occurring in the crab by Jolyet and Regnard¹, and in the lobster by Fredericq²; but nothing further has until now been made out about it. I shall show later on that this red pigment is the same as that which exists largely in the exoskeleton and in the hypoderm. It has been called there tetronerythrin, and is one of a class of pigments known as luteins or lipochromes. In Astacus and the lobster, the red colour as a rule predominates; but in Nephrops it is present in so small an amount, that I at first thought it was altogether absent.

Specific Gravity and Reaction. In a few cases in which I took the specific gravity of the blood by weighing, I found it to vary between 1025 and 1030. Its reaction is always faintly alkaline.

Constituents. The blood contains the following classes of bodies:—

1. Proteids.
2. Salts. These resemble those of the water in which the animals live, being more abundant in sea water than in fresh water animals. The ash is also found to contain small quantities of iron and copper, the latter being combined with the proteid haemocyanin. (Fredericq.)
3. Extractives. Among these are tetroneothyrin in variable amount, and fatty bodies, also in variable quantity, which I have not further investigated. There is a small percentage of urea, a fact which has been previously noted in the case of the crab by Rabuteau and Papillon1, and by Jolyet and Regnard2.

The following table exhibits the average percentage proportions of these constituents in the blood of the four animals under investigation.

<table>
<thead>
<tr>
<th></th>
<th>Lobster</th>
<th>Crab</th>
<th>Crayfish3</th>
<th>Nephrops</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>93.49</td>
<td>89.92</td>
<td>95.14</td>
<td>89.06</td>
</tr>
<tr>
<td>Solids</td>
<td>6.51</td>
<td>10.08</td>
<td>4.86</td>
<td>10.94</td>
</tr>
<tr>
<td>Proteids</td>
<td>3.02</td>
<td>6.10</td>
<td>2.19</td>
<td>4.60</td>
</tr>
<tr>
<td>Other organic matters</td>
<td>0.55</td>
<td>1.28</td>
<td>1.54</td>
<td>3.57</td>
</tr>
<tr>
<td>Salts</td>
<td>2.94</td>
<td>2.70</td>
<td>1.13</td>
<td>2.77</td>
</tr>
</tbody>
</table>

The foregoing numbers were the averages obtained from the analyses of the blood of three animals in each case, except that of the nephrops in which six were thus examined. The proteids were estimated by precipitation with alcohol; the blood was allowed to drop direct into alcohol; the constituents of the cells as well as of the blood plasma are therefore included in the foregoing numbers. It is very difficult to estimate the actual dry weight of the cells, because coagulation occurs so rapidly, that it is impossible to obtain them free from the coagulable or fibrin-like substance that is formed: still by quick filtering, an approximate result can be arrived at, and the cells obtained nearly free from fibrin: this can necessarily only be done in cases when a large amount of blood is readily obtained. In the crab the percentage weight of dried cells was found to be 91; and in the lobster 73.

3 Witting (Journal f. pract. Chemie. Bd. lxxiii. p. 128) gives the following numbers for Astacus.

Water 90.69. Salts 1.55. Organic bodies 7.56.
Coagulation. I found that by receiving the blood immediately when shed into very large quantities of neutral salts like magnesium sulphate or sodium chloride coagulation could be prevented; this is in contradiction to what has been previously observed; but a full discussion of this point will come in the section that treats of the phenomena of spontaneous coagulation. I shall then show that the clot is not the so-called plasmodium as described by Mr Geddes, but is due to the formation of a body, almost indistinguishable from the fibrin of vertebrate blood, in which the cells are entangled, and that its formation is due to a ferment action upon a proteid fibrinogenous body which exists in the blood plasma. This ferment is derived from the amoeboid corpuscles of the blood. As is the case in vertebrata, the serum, that is the fluid portion that remains when the clot is removed, differs from the plasma, by not containing the proteid fibrin factor.

PART II.

The Phenomena of Spontaneous Coagulation.

This part of the subject may be conveniently treated of, under the following heads:—

1. The naked eye phenomena of coagulation.
2. The chemical properties of the clot.
3. The fibrin factors.
4. The influence of neutral salts in preventing coagulation.
5. The microscopical characters of the cells and coagulum.
6. The preparation of fibrin ferment.
7. The influence of cold on coagulation.
8. Conclusions.
9. Historical.

1. The naked eye phenomena of coagulation.

Coagulation begins almost immediately the blood is shed, and the coagulum that forms presents the appearance of a network of white fibres throughout the liquid. This soon begins to contract, and squeezes out drops of a perfectly clear liquid; in a few minutes more this liquid, as well as the liquid between the fibres first formed sets into a clear jelly. When the blood is collected in a tube, the portion of the clot which first forms, and which has entangled all the cells, contracts
and occupies the centre of the tube. The liquid in which this clot floats is at first perfectly liquid, then semi-liquid, and finally a firm jelly, so that the tube can be turned upside down without spilling any of the contents. The appearance finally produced is that of a perfectly clear reddish blue or light violet jelly in the midst of which the first clot, composed of filmy fibres, is quite immovably fixed in any position in which it happens to have fallen.

By rather rapid manipulation, the clot which first forms can be filtered off, and the liquid which comes through soon sets into a clear firm jelly. I attempted in several cases to lift the first clot out from the surrounding liquid with forceps, but never successfully; it always breaks, and the forceps come away with only a shred of the material. Stirring is also inefficacious; this process seems but to hasten the jellying of the whole mass. The reason of this I conceive to be that the second process or jellying is in reality only a continuation of the first; and by the time one introduces the forceps to lift out the first clot, the second process, as it may be provisionally called, has already begun in the neighbourhood of the first shreddy clot.

As just said, if the liquid from which the first clot has been removed by rapid filtration be allowed to stand, it soon sets into a firm jelly; this liquid therefore is not serum, or at least not comparable to the serum of vertebrate blood, which does not clot. In the course of a few hours, this second clot also shrinks, squeezes out and floats in a clear liquid, of the same colour as the first liquid. This second liquid however does not coagulate spontaneously; it is in fact serum. The second clot, that is the clot that occurs in the liquid first squeezed out, or filtered off from the first network-like coagulum is, in appearance, when shrunken, like the fibrin which forms in the colourless exudations or lymphs of vertebrata: e.g. hydrocele or pericardial fluids. The difference between the first and second clots is one of degree only; the first which has entangled the cells is more opaque and shreddy; the second contracts much more in proportion and is more filmy: both however possess the same chemical properties, and therefore both are due to the formation of the same material.

Most of the foregoing facts have been observed and recorded before by Fredericq and others; my reasons for dwelling on them thus fully, is that I differ from the conclusions which Fredericq\(^1\) draws from them. He says, "White flocks are rapidly formed that settle to the

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\(^1\) Fredericq. "Note sur le sang de l'Homard." *Loc. cit.*
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bottom of the vessels; by the microscope this coagulation is seen to occur in connection with the blood corpuscles; these shreds having been removed, a second coagulation occurs resembling that of fibrin, viz. the liquid sets into a jelly: moreover the second coagulation can be prevented by admixture with certain salts, the first cannot."

It thus appears that he regards the two coagulations as perfectly distinct processes; the first being the formation of a plasmodium, the second that of a fibrin-like substance.

It seems to me on the contrary that the two processes are not distinct, but that the second is really only a continuation of the first. The grounds on which I make this inference are the following. They will be set forth at greater length in subsequent pages:

1. The clot first formed is not a mere plasmodium of cells, but contains a coagulated substance apart from the cells.
2. The chemical properties of the first clot are identical with those of the second.
3. The process is in reality a continuous one; it occurs first around the cells, and most rapidly in their neighbourhood, since they furnish the ferment which will presently be shown to be necessary for the process; and the difficulty of removing this so-called first clot from the liquid shows that clotting must be proceeding in the surrounding liquid in close connection with the first clot.
4. The formation of both clots, not of one only, can be prevented by the admixture of certain salts, if these be used in sufficient quantity.¹

Moreover an analogous phenomenon of double coagulation may be sometimes observed in vertebrate blood; and here there can be no doubt that the process is only a single one. In blood that coagulates slowly, the first liquid squeezed out by the contracting fibrin is frequently not serum, but a portion of the blood plasma in which the process of clotting has not been completed. This liquid can be poured off, and in a few minutes it sets into a clear jelly.

2. The chemical properties of the clot.

For the investigation of these, the following method has been adopted. Portions of the first and of the second clots were first

¹ Krükenberg also advocates the view that the second is only a continuation of the first coagulation, and is not different from it. He however thinks that the process is one of a material being shed out from the cells merely and is not due to the formation of fibrin as in vertebrate blood. (Vergleichend-Physiologische Studien, 2te Reihe. Erste Abtheilung, pp. 124 and 138. Heidelberg, 1882.)
thoroughly washed with water, and then treated with the following reagents—:

a. **Hydrochloric Acid.** In weak acid (1-2%) the clot becomes swollen but not so markedly as is vertebrate fibrin. Strong acid dissolves it.

b. **Acetic Acid.** The portions of clot become swollen up, but not dissolved.

c. **Nitric Acid.** This dissolves up the clot, forming a yellow solution which turns orange on the addition of ammonia.

d. **Caustic Alkalies (5%o).** These rapidly break up and dissolve the portions of clot.

e. **Solutions of neutral salts.** In 10 and 20%o solutions of magnesium sulphate, sodium chloride, and potassium nitrate the clot is slightly soluble at 40°C. This is reprecipitated by saturating the solutions with magnesium sulphate. It is however less soluble in these solutions than vertebrate fibrin.

f. **Lime water and baryta water.** The first clot is slightly soluble, and a precipitate not soluble in excess occurs on the addition of acetic acid to the solution. This is probably due to the nuclein present in the entangled cells. With the second clot no such precipitate occurs.

g. **Peroxide of hydrogen and tincture of guaiacum.** These reagents give a faint blue colouration.

h. **Peptic digestion.** Peptones and syntonin are formed.

i. **Pancreatic digestion.** Peptones, alkali albumin, leucin and tyrosin are formed.

The clot is thus seen to possess all the chemical properties of vertebrate fibrin. The only differences are:

a. It is not so markedly swollen by weak hydrochloric acid, as is fibrin.

b. It is not so readily soluble in weak solutions of neutral salts.

3. **The fibrin factors of crustacean blood.**

It was before mentioned that Krukenberg looks upon the coagulable material as something shed out from the cells. No doubt

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1 It will be seen that the results were similar for both clots in all cases except that under the heading f: but here the discrepancy is easily explicable.

2 This fact has, in the case of the crayfish, been previously noted by Prof. Lankester in an abstract of a "Report on the Spectroscopic Examination of certain Animal Substances" presented to the British Association at Exeter, 1869. *Journal of Anat. and Physiol.* Vol. iv. 1870, p. 122.

3 Witting (*Journal f. pract. Chemie*, Bd. LXXIII. 1858, p. 121) says that fibrin is present in the blood of Astacus, as a proof of which he adduces the solubility of the clot in potassium nitrate solution.
the cells furnish an important factor in its formation, but the fact that coagulation occurs in the liquid from which the cells are removed by filtration tends to show that it cannot be merely a material shed out from them. The similarity of the chemical properties of this body to fibrin naturally leads one to infer that it may be formed in a similar manner, namely by the solidification of a previously existing soluble proteid or fibrinogen, and this supposition turns out to be a true one. The first point in its favour is that a certain quantity of proteid disappears from the plasma to form the clot; this can be illustrated by the following experiment.

In a specimen of lobster's blood, the proportion of proteids to total solids in the plasma was $1$ to $2:14$; in the serum of the same animal it was $1$ to $2:67$. This loss of proteid was not due to the absence of cells from the serum, for the specimen of plasma taken contained no cells; these had been filtered off with the first clot immediately the blood was shed, and the coagulation that occurred in the plasma was the so-called second coagulation.

A second and more conclusive proof is that a proteid can be precipitated from the plasma by saturating it with magnesium sulphate or sodium chloride. When this is washed and redissolved by adding water, a solution is obtained which is not spontaneously coagulable; but on adding to it a ferment prepared from the cells in the same way as Schmidt prepares his from vertebrate blood, the formation of fibrin takes place. But before this point can be dwelt upon fully it is necessary to consider the action of these salts upon the blood, and upon the process of coagulation.

4. The influence of neutral salts in preventing coagulation.

As already stated, Fredericq asserts that what he calls the second coagulation, but not the first, can be prevented by mixing the blood with solutions of certain salts. Pouchet\(^1\) states that coagulation cannot be prevented in this way (i.e. by solutions of neutral salts). Pouchet however used merely sea water to dilute the blood with, and it was hardly to be expected that he could hinder coagulation by this means. Krukenberg merely quotes Fredericq on the subject. All these observers agree that the liquid is free from globulins, and that no precipitate occurs in the blood by saturating it with magnesium sulphate.

\(^1\) Pouchet, G. "Sur le sang des crustacés." *Journal de l'anatomie et de la physiologie.* Vol. xviii. p. 82. 1882.
In both these points, these observers are mistaken; for in the first place coagulation can be altogether prevented by admixture with a sufficient quantity of certain neutral salts; and in the second place saturation with these salts completely precipitates all the proteid matter from the blood.

A saturated solution of magnesium sulphate is by far the most efficacious agent for preventing coagulation. Sodium chloride acts similarly, but a larger relative amount is required. A saturated solution of sodium sulphate has on the contrary no such action.

For the prevention of coagulation at least four times by volume as much saturated solution of magnesium sulphate solution as blood is required; for instance two hundred cubic centimeters of magnesium sulphate solution will prevent fifty cubic centimeters of blood from coagulating, but not more. Coagulation is thus more difficult to prevent by this means than in vertebrate blood. Coagulation is so rapid, that it is best to put the claw or tail under the liquid before allowing it to bleed. Or, the blood may be allowed to drop direct into the salt solution placed immediately beneath the wound. In some cases however where this latter method is adopted, the blood begins to clot in the passage; in other cases it is not so rapid as this; it is then seen as each drop falls into the solution that its milkiness becomes increased, that is, something is precipitated from the blood. This when thoroughly mixed with the rest of the solution redissolves, but not entirely. The cells and the undissolved portion of the precipitate sink to the bottom of the vessel, and a clear plasma mixed with the saline solution floats above. The sediment at the bottom consists of cells without a trace of fibrinous substance, and of a fine amorphous precipitate as well. The cells remain distinct and do not run together to form a clot. It is possible that it is by the precipitation or at any rate the partial precipitation of the fibrinogenous substance that coagulation is prevented.

With sodium chloride it is necessary to take even a larger quantity of the saturated solution. The blood immediately on being shed must be mixed with at least nine or ten times as much saturated solution of sodium chloride. Sodium chloride is not nearly so efficacious in precipitating the globulins of crustacean blood as is magnesium sulphate. It is perhaps this fact that renders the former less powerful in preventing spontaneous coagulation.

Dilution of this salted blood with water causes coagulation to take place, as in salted vertebrate blood.

This is illustrated by the following experiments:
(1) If some of the sediment, that is cells and precipitated globulin be taken and water added, a nearly clear solution is obtained owing to the re-solution of the precipitated globulin. Then almost immediately, or in the course of a minute a thin diaphanous coagulum is obtained throughout the liquid; this on shaking runs up into a little mass of shrunken clot in which on microscopical examination cells and fibrin are both discernible.

(2) Dilution of the supernatant liquid does not, except in one or two doubtful cases, and that after long standing, produce coagulation.

(3) The liquid is filtered; the cells and precipitated globulin are thus collected; they are washed with saturated solution of magnesium sulphate, suspended in the same solution; and then, on dilution of this, coagulation as in the first case immediately occurs.

5. The microscopical characters of the cells and coagulum.

a. When a drop of blood fresh from the animal is received on a glass slide, covered, and examined with the microscope, the cells are seen in a clear plasma. The blood cells of crustacea have been described and figured by Hewson, Carus, Wharton Jones, Haeckel, Lebert and Robin, and Geddes. Pale cells with amoeboid movements, and long processes are in all cases described. There are however in addition some which contain granules of a yellowish red colour; but these are not constantly present, and are most easily found in those specimens in which the reddish hue of the blood is most marked.

When the drop of blood is examined as above described the cells at first move freely, and exhibit amoeboid movements. They soon become stationary and collect together into irregular clumps. They shoot out exceedingly long processes; these help to bind the cells together. This is accurately described by Mr Geddes as occurring not only in crustacea but in many other invertebrate groups, notably in the

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1 Dilution of the supernatant liquid together with the addition of fibrin ferment always produces coagulation. This point is discussed later on.


3 Carus, C. G. *Von den äussern Lebensbedingungen der weiss- und kalt-blutigen Thiere._ Leipzig, 1824, pp. 85—86.

4 T. Wharton Jones. "The blood corpuscle considered in its different phases of development in the animal series." _Phil. Trans._ 1846, pp. 90—91.


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echinoderms. Mr Geddes however overlooked what very soon makes its appearance; that is an intercellular substance or fibrin-like material. Both Fredericq and Krukenberg have recognised this material outside the cells; both however seem to agree with Geddes in the main points of his plasmodium theory; they imagine the material to be shed out from the cells.

We have already seen that the clot is like fibrin in its chemical properties; under the microscope it has a distinctly fibrinous appearance. The amount of fibrinous material increases as time goes on. The fibres are quite distinct from the long cell processes; they have not such a well defined outline.

b. Microscopical appearance of blood prevented from coagulating by the addition of magnesium sulphate. The sediment examined from such a specimen is found to consist of cells without processes, and quite distinct from one another; there is also some fine granular matter, doubtless the precipitated globulin.

c. Microscopical appearance of clot produced by diluting the last specimen. This is seen to be a mass of cells, mostly without processes, connected by a matrix appearing homogeneous in parts, but in other places distinctly fibrinous.

d. Microscopical appearance of the clot after it has formed spontaneously.

1 Prof. Hacraft has fallen into the same misconception as Mr Geddes regarding the clotting of crustacean blood. In the Proc. Royal Society, Vol. xxxvi. 1883—4, p. 478, he says “Certainly the blood of crabs, crayfish, and lobsters clots in the way described by Mr Geddes, there being no intercellular formation of fibrin.” It was Prof. Schäfer who pointed out Mr Geddes’ mistake in the case of the echinoderms. The material of coagulation in these animals resembles fibrin in one particular, namely in contracting after its formation, but in its other properties it is more nearly akin to mucin. (E. A. Schäfer, “Preliminary notice of an investigation into the coagulation of the Perivisceral Fluid of the Sea Urchin.” Proc. Royal Society. Vol. xxxiv. 1882—3, p. 370—1.)

In connection with the plasmodium theory of Mr Patrick Geddes, it is historically interesting to note that a plasmodium theory was once held with regard to the coagulation of vertebrate blood. In the 18th century the clot was supposed to be simply a running together of the red corpuscles; this view was held in Britain by Keill, Jurin, Thomas Morgan, John Cook, Arbuthnott, William Cowper, Martine, Langrish, William Northcote, and Berdoe; and on the continent by Leeuwenhoek, Boerhaave, Van Swieten, Haller, and Marherr.

Petit, Quesnay, Senac, and Davies were the earliest to have an idea of a coagulable substance in addition to the cells, and the principle of coagulable lymph apart from the red corpuscles was fully recognised and taught by Hewson, Fordyce, and the Hunters.

For the references to the writings of all these authors, see Hewson’s Works, edited by Gulliver, p. xxix. et seq.
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i. Of the clot which first forms. This contains so many cells, bound together so closely that their outlines are indistinguishable in many places; the uniting fibrinous material can however be plainly seen in thinner places.

ii. Of the clot which forms after the removal of the so-called first clot. This, on pressure of the cover glass, shrinks; a large amount of liquid is squeezed out, and a film of fibrinous appearance left.

iii. Of a portion of clot hardened in alcohol, stained and cut with a microtome. Sections show cells imbedded in a fibrinous matrix.

6. Preparation of fibrin ferment from Crustacean blood.

The method I have adopted for the preparation of a ferment from crustacean blood has been that which is known as Schmidt's method.

To the blood after having been shed, or to the serum, absolute alcohol was added in excess, and the precipitate which so formed was kept in contact with the alcohol for about six weeks; it was then filtered; the precipitate was washed with alcohol, squeezed as dry as possible, and then dried in vacuo over sulphuric acid; the resulting mass had a bluish green tinge. It was then powdered, and watery extracts of this powder were found to have coagulating properties. In certain experiments further to be noted this watery extract was used; in certain others pinches of the powder itself were added to the liquid under investigation; the latter method was found to be rather more rapid in its action.

I also had a supply of Schmidt's fibrin ferment prepared from horse serum, with which to compare my crustacean ferment.

The watery extract as above prepared could have contained no lecithin. If lecithin is, as Dr Wooldridge supposes, an agent in bringing about coagulation, it must be admitted that it is not the only one.

In order to test the efficacy of this ferment, specimens of crustacean blood were taken which had been prevented from coagulating by admixture with magnesium sulphate; the subsided cells were filtered off, and the filtrate used as follows:—

a. Dilution with distilled water produced no coagulation.
b. Dilution with watery extract of crustacean ferment produced immediate coagulation.
c. Dilution with watery extract of mammalian blood ferment produced immediate coagulation.

d. Dilution with distilled water, and addition of the dried powder, prepared either from mammalian or crustacean blood, produced coagulation in about five minutes.

These experiments show that coagulation of crustacean blood can be brought about by a ferment, whether that ferment be prepared from crustacean or from mammalian blood.

(1) The following experiments show that the converse is also true, and that coagulation of mammalian fluids can be brought about by ferment prepared from crustacean blood, or from mammalian blood.

Mammalian (cat's) blood was prevented from coagulating by admixture with an equal amount of saturated solution of sodium sulphate. The cells subsided, and the supernatant salted plasma pipetted off and used as follows:

a. 5 c.c. were diluted with 50 c.c. distilled water; this did not begin to coagulate for 23½ hours.

b. 5 c.c. were diluted with 50 c.c. distilled water, and a pinch of the dried powder from lobster's blood added. Coagulation began in 1½ hours.

c. d. e. These were specimens similarly diluted to which the dried powder from crab's, crayfish's, and horse's blood respectively were added; in times varying from 1½ to 3 hours coagulation began. Coagulation in these cases began as a network of fine fibres traversing the liquid and connecting the particles of powder together; after this the whole set into a jelly; subsequently the fibrin contracted.

f. g. h. i. These were specimens each containing 5 c.c. of salted plasma, 40 c.c. distilled water, and 10 c.c. of watery extract from the ferment powder of the same animals. In these coagulation occurred in from three to six hours.

(2) Not only did coagulation occur much sooner in the specimens to which ferment had been added, but after the same lapse of time the quantity of fibrin formed was greater in the latter than in the former case: as instances of this the following may be taken.

Cat's salted plasma; 5 c.c. diluted with 50 c.c. water; weight of fibrin formed after 48 hours = 0.022 grains.

Cat's salted plasma from the same animal; 5 c.c. diluted with 40 c.c. water, and 10 c.c. water extract of dried powder from lobster's blood. Weight of fibrin formed after 48 hours = 0.105 grains.

In another specimen, the numbers in a similar experiment were 0.135 and 0.249 grains respectively.

(3) In other experiments comparisons were instituted between the efficacy of fibrin ferment prepared from mammalian, and that from
crustacean blood. The results showed that the two were about equal; the coagulation began first sometimes in one, sometimes in the other, but the difference in time rarely exceeded half an hour. The quantity of fibrin formed in the two cases, using equal weights of the ferments, was also approximately equal. As instances of this the following experiments may be quoted.

Salted plasma taken in equal quantities; and equal amounts of fibrin ferment, so far as could be told by weighing the dried powder, added: The weight of fibrin which formed was found in one experiment to be:

\[
\begin{align*}
\text{grains} \\
\text{In the case of ferment from lobster's blood } & 407. \\
\text{" } & \text{" horse's } & 392.
\end{align*}
\]

In another experiment similarly conducted:

\[
\begin{align*}
\text{In the case of ferment from crayfish blood } & 1.622. \\
\text{" } & \text{" horse's } & 1.736.
\end{align*}
\]

(4) In other experiments hydrocele fluid was employed: the results will be seen best by giving as an illustration the following experiment:

\begin{enumerate}
\item 10 c.c. of hydrocele fluid were taken, allowed to stand. No coagulation took place.
\item 10 c.c. of hydrocele fluid; a few drops of serum from rabbit's blood were added. This was allowed to stand over night at the ordinary temperature of the air; next morning it had set into a jelly.
\item 10 c.c. of hydrocele fluid; Schmidt's ferment (in powder) was added, prepared from horse's blood. Next morning it had set into a jelly.
\item 10 c.c. of hydrocele fluid; the ferment (in powder) prepared from crayfish blood was added. Next morning it had set into a jelly.
\end{enumerate}

(5) So far the experiments quoted have been performed with either blood or plasma, or with a similar liquid. Now we come to those performed with the assumed proteid precursor of fibrin in these liquids, the fibrinogen.

Fibrinogen is prepared from hydrocele fluid in the usual way\(^1\), by half saturating with sodium chloride; the precipitate so produced is washed, and then redissolved by adding distilled water. Thus a solution of ordinary vertebrate fibrinogen is obtained.

A similar proteid is obtained from the blood plasma of crustacea by saturating it either with magnesium sulphate or sodium chloride, and having the mixture shaken for three hours; the precipitate so obtained

is washed, and then redissolved by adding water; the salt adhering to
the precipitate enables it to dissolve, and a somewhat opalescent
solution of a proteid substance obtained. This we will call crustacean
fibrinogen.

In this way, we obtain two solutions; one of fibrinogen, one of
crustacean fibrinogen: to each of these is added the ferment powder
or a watery extract of the powder, and this both in the case of
crustacean and mammalian blood; and in all cases, after a few hours
at the ordinary temperature, a well marked clot of the ordinary fibrinous
appearance occurs, and then shrinks as usual.

The result of all these experiments is to show most conclusively,
that the white cells occurring in the two groups crustacea and vertebrata
have this property in common; namely that they each furnish a ferment
which brings about coagulation in a coagulable fluid (i.e. a fluid
containing a fibrinogen), whether that fluid be obtained from either one
or the other class of animals.

I have not found that extracting the clot of crustacean blood with
8°/0 solution of common salt, according to Gamgee's¹ method, has
yielded any active ferment.

7. The influence of cold on coagulation.

Another point remains to be discussed in connection with the
spontaneous coagulation of crustacean blood, and that is the influence of
cold upon the process. It is well known that cold hinders or prevents
the coagulation of vertebrate blood. The question naturally arises, does
this hold also with crustacean blood? Previous observers make no
reference at all to this point.

I have found that cold has the same effect on crustacean as on
vertebrate blood.

The first method I adopted was that described by Professor Gamgee
in his Physiological Chemistry².

A small platinum basin was immersed in a mixture on ice and salt;
the blood from a crayfish was allowed to flow into it, when it instantly
congealed. Placing the vessel on the palm of the hand thawed the
blood, which was then perfectly liquid; it was frozen and thawed in
this way several times; and after this was allowed to stand at the

¹ A. Gamgee. "Some old and new experiments on the Fibrin Ferment." Journal
of Physiology. 1879, Vol. ii.
² p. 30.
temperature of the air; in about 10 minutes it had set into a firm jelly. A specimen of the same blood not treated in this way, set into a jelly in two minutes.

But it is not necessary to freeze the blood to prevent coagulation. If the blood be kept at the temperature of melting ice, it remains liquid. In this way, I kept one specimen of crayfish blood liquid for an hour. After this time on removal from the cold it coagulated as usual, only more slowly, the jelly not being firm for some 20 minutes. The apparatus I used in this experiment was on a small scale similar to that described and figured by Dr Burdon Sanderson1.

8. Conclusions.

The spontaneous coagulation of crustacean blood is thus similar in nearly all respects to that of vertebrate blood, the following being the various points of resemblance.

- It does not occur in the living vessels.
- It takes place after the blood is shed.
- It can be prevented by the admixture with the blood of certain proportions of neutral salts.
- It can be hindered by cold.
- The clot consists of fibrin which entangles the blood cells.
- The formation of this fibrin is due to a solidification of a proteid of the globulin class or fibrinogen which exists in solution in the blood plasma.
- This is due to a ferment action.
- This ferment is yielded by the amoeboid corpuscles of the blood.

9. Historical.

All the writers on crustacean blood describe the fact of coagulation. Hewson2 (1770), the earliest writer on the subject, does not discuss the point whether fibrin is formed, but seems to take it for granted that coagulation is essentially the same in both vertebrate and invertebrate animals. Carus2 (1824) describes the clot as glutinous. Carpenter3 (1843) distinctly states that fibrin is formed in invertebrate animals. Wharton Jones (1846) leaves the question open: he says4 "The clot

1 Handbook of the Physiological Laboratory, p. 168.
2 Loc. cit.
4 Wharton Jones, loc. cit. p. 89.
in the crab consists mostly of the aggregated cells, there being but little spontaneously coagulable material;” and again, “The same kind of thing occurs in the corpuscles of the lobster, but a more perfect clot is obtained than in the crab.” Haeckel² (1857) writes, “There is a large quantity of fibrin or fibrinogen (?).” Witting³ (1858) gives distinct proofs that fibrin is formed. Later came Geddes³, Fredericq³, Haycraft³ and Krukenberg³, who all say that fibrin is not formed; the opinions of these observers have been already quoted, and commented upon; to the list must be added Pouchet³ who also supports Geddes’ view of a plasmodium-coagulum.

PART III.

The Proteids of the Blood Plasma and Serum.

The proteids of the blood plasma are two in number, viz. haemocyanin, and fibrinogen. The blood serum contains only the former of these, haemocyanin.

1. Haemocyanin.

This is the only proteid which has hitherto been described in the blood of these animals. It is an interesting body occurring pretty widely distributed in various classes of the invertebrata. The properties that have been hitherto ascribed to it are as follows:—

a. It gives the ordinary proteid reactions.
b. It coagulates by heat at about 68° C.
c. It exists in two conditions analogous to those of haemoglobin; viz. oxyhaemocyanin and reduced haemocyanin; the former having a blue colour, the latter being colourless.
d. On spectroscopic examination, oxyhaemocyanin shows no absorption bands.
e. It always contains a small quantity of copper, which seems to take the place of the iron of haemoglobin.

A good many different observers described the blue colour of, and the presence of copper in the blood of various animals, but it was not until

¹ Wharton Jones, loc. cit. p. 91.
³ Loc. cit.
after Fredericq\(^1\) made his observations on the blood of the Octopus, and proved the existence of this body to which he gave the name of haemocyanin, that it was possible to draw comparisons between the bloods of these various animals.

The blue colour of the blood of certain snails (Helix) is noted by Erman\(^2\) (1817); in Astacus as well as Helix by Carus\(^3\) (1824); in Loligo, Eledone, Sepia, Cancer pagurus, and Helix pomatia by Harless\(^4\) (1847); this observer also showed that the blue colouration was the effect of exposure to the atmosphere; and he showed also the presence of copper in the blood; he also found a trace of iron in the blood. Genth\(^5\) (1852) showed that the blood of Limulus assumed in a similar way a blue tint on exposure to the air, and that it contained copper, and a small amount of iron. Rabuteau and Papillon\(^6\) (1873) showed that in the crab and Octopus similar colour changes occurred. In 1874 Gorup-Besanez\(^7\) added Acanthias and Unio to the above list of animals containing copper.

Jolyet and Regnard\(^8\) (1877) were the first to advance the opinion that the blue colour was united to a proteid; this was fully worked out by Fredericq\(^8\) in the following year (1878). Since then our knowledge concerning the distribution of haemocyanin has been added to by Fredericq himself, by Ray Lankester, and by Krukenberg in numerous papers. A full list of all the animals in which it occurs will be given in the appendix to this paper.

My own observations on haemocyanin have resulted chiefly in confirming those of Fredericq; one or two new points will however be noted. My experiments will come under four headings:—

1. Temperature of heat coagulation.
2. Action of neutral salts.
3. Effect of dialysis.
4. Spectroscopic observations.

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2 Erman, Carus, loc. cit.
3 Harless. “Ueber das blaue Blut einiger wirbellosen Thiere, und dessen Kupfergehalt.” Müller’s Archiv, 1847, p. 48 et seq.
5 Rabuteau and Papillon, loc. cit.
7 Jolyet et Regnard, loc. cit.
8 Fredericq, loc. cit.

The blood of Unio does not however contain haemocyanin.
1. **Temperature of Heat coagulation.** For determining this I have employed Prof. Schäfer's apparatus (described by me in this Journal, Vol. V. p. 154). Rendering the serum faintly acid, I found the temperature of coagulation to be 65°—66°, opalescence occurring at 57°—8° C. This is a degree or two lower than that found by Fredericq\(^1\), who places it at 68° C. This is an unimportant difference, and is to be accounted for, either by the addition of acid in my experiments, or by the fact that haemocyanin of the crustacea and cephalopoda differ in this respect. I obtained similar results in all four animals that I examined. The whole of the proteid in the serum comes down at this temperature, but slowly. In some cases it takes as long as three hours for all the proteid to fall at this temperature. It is a white flocculent precipitate possessing all the properties of coagulated proteid; the first portions precipitated carry down with them the red pigment of the serum. Krukenberg\(^2\) finds by fractional heat coagulation that he can apparently separate the serum-proteid into two, the first coagulation occurring at 68°—70°, the second at 72°—5°. He imagines that the first of these consists of oxyhaemocyanin, and the second of reduced haemocyanin or as he calls it haemocyanogen\(^3\), the reduction being brought about by heating. I do not agree with him here; for the serum retains its blue tinge, even till the last portions of proteid fall down. I should explain his results in one of two ways: either he did not render the serum faintly acid before beginning his experiment; then the precipitation of the first portions of proteid rendered the liquid more alkaline than before, and the increased alkalinity of the liquid raised the temperature of coagulation of the proteid remaining in solution; or secondly, he may not have waited long enough at the lower of the two temperatures for all the proteid to come down, for as above said the precipitation is exceedingly slow.

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3. Haemocyanogen seems to be an objectionable word, as one is apt to confuse with it the Cyanogen compounds of haemoglobin with which it has nothing to do. In the German language however this same objection does not hold, the German for cyanogen (CN) being Cyan. Krukenberg suggests the word (Vergl. phys. Beiträge zur Kenntniss des Respirationsvorgänge bei wirbellosen Thieren. Vergleichend-physiol. Studien. 1\(^{e}\) Reihe, 3\(^{e}\) Abth. p. 87) as being analogous to Haemochromogen, and Haemerythrogen (the reduced product or chromogen of a colouring matter found in the blood of certain Gephyrean worms, which he calls Haemerythrin). I have however retained Fredericq’s words haemocyanin and oxyhaemocyanin.
2. Action of neutral salts on Haemocyanin. It has been above stated that both Fredericq and Krukenberg assert that addition of or saturation with magnesium sulphate or sodium chloride produces no precipitate. These seem to be the only two observers who have tried the experiment. In this point I disagree with them. Just as precipitation by heat is slow, so is also precipitation by salts; to effect complete saturation with either of the above-mentioned salts, the serum must be shaken on an engine, for 12, 24 and in some experiments 36 hours, with the finely powdered salt. The precipitate can be then collected on a filter, washed with saturated solution of the salt and dissolved by the addition of distilled water; the presence of salt adhering to the precipitate enables the water to dissolve the precipitated proteid. In this way magnesium sulphate effects complete precipitation of haemocyanin; sodium chloride only incomplete precipitation. In this haemocyanin is seen to resemble serum globulin. These however are slow methods of precipitating haemocyanin; it can be completely and rapidly precipitated, by saturation with the double sulphate of soda and magnesia1; it can then by re-solution and reprecipitation be obtained in a pure state. The solution of pure haemocyanin in weak sodio-magnesic sulphate solution is found to coagulate at 65°C., and to undergo the same colour changes as noted in the serum.

Addition of crustacean fibrin ferment to such a solution produces no formation of crustacean fibrin.

Sodium sulphate acts in a peculiar manner; it produces, when added to saturation to serum and shaken for a long time, a small amount of a peculiar flocculent precipitate, which does not redissolve on the addition of water, and which possesses the characters of coagulated proteid.

Fredericq has shown that the addition of hydrochloric acid to haemocyanin splits up that body into two products: one of these is a coagulated proteid containing no copper, and the other a colourless solution of a body containing copper. He therefore concludes that haemocyanin consists of two bodies united together, one a proteid, and the other a body containing copper. He compares this to the case of haemoglobin, which can be split up into two bodies; one, globin, a proteid of the globulin class, and the other, haematin, a colouring matter containing iron.

Magnesic sulphate, magnesio-sodium sulphate, and sodium chloride

do not however effect such a separation, but precipitate the haemocyanin in an unaltered condition.

In pure haemocyanin Fredericq describes copper as occurring, but not iron as well. In this I can confirm his observation. The iron which has been described as occurring in the blood by Gorup-Besanez and Harless is doubtless contained in the cells, not in the plasma; that such is the case was shown by Graham, who analysed the cells of crustacea for Wharton Jones.

3. Effect of dialysis. After dialysing out the salts from a solution of haemocyanin, it is found that in three or four days the latter body is precipitated. I have not however been able to obtain complete precipitation in this way.

This observation shows conclusively that haemocyanin is a globulin.

As a further test, specimens of the serum were diluted fifteen or twenty times with water, and a stream of carbonic acid gas passed through them; this produced a slight precipitate of proteid matter. Weak acetic acid added to the serum, whether diluted with water or not but more easily in the former case, also causes an incomplete precipitation of the haemocyanin; strong acetic acid completely precipitates it in a coagulated form.

We see from these observations that haemocyanin resembles serum globulin in certain points, viz.:

a. It is completely precipitable in an uncoagulated condition from its solutions, by saturation with magnesium sulphate, or sodiomagnesic sulphate.

b. It is incompletely precipitable from its solutions by saturation with sodium chloride, or by dialysis.

c. It is incompletely precipitated from its solutions when much diluted by a stream of carbonic acid, or by the addition of acetic acid.

It differs from serum globulin in certain other points, viz.:

a. It contains copper; serum globulin does not.

b. It coagulates at 68° C.; serum globulin coagulates at 75° C.

c. It is more difficult to precipitate from its solutions than is serum globulin by saturation with salts.

d. It is completely precipitated and coagulated by strong acetic acid.

4. Spectroscopic observations. In contact with the air, the colour of haemocyanin is blue; by a stream of carbonic acid, carbonic

1 Wharton Jones, loc. cit. p. 105.
oxide, sulphuretted hydrogen, by the addition of reducing agents like ammonium sulphide, or by the action of a vacuum, it is rendered colourless; in all these cases it again becomes blue by shaking with the air, or by the passage of a stream of oxygen through it. These observations, with the exception of that relating to the action of carbonic oxide, were made by Fredericq.

Fredericq¹, Rabuteau and Papillon², and MacMunn³ agree that on spectroscopic examination of oxy-haemocyanin no bands are seen. Krukenberg⁴ however describes a faint band in the region of the D line in addition to a cutting off of a certain amount of both ends of the spectrum. I have not been able to see with the microspectroscope any such line in the haemocyanin of crustacea; but the cutting off of the ends of the spectrum is well seen, the amount varying with the strength of the solution. Specimens of reduced haemocyanin show no such cutting off of the ends.

This is shown in the figures 1 and 2, Plate VIII.

2. Crustacean Fibrinogen.

The blood serum contains a smaller percentage of proteids than the blood plasma does. A certain amount has therefore gone to form crustacean fibrin. It is not haemocyanin which has thus disappeared; first, because addition of fibrin ferment to haemocyanin produces no fibrin; and secondly, because the fibrin formed contains no copper.

If blood be prevented from coagulating by mixing it with magnesium sulphate solution, the cells can be filtered off; and addition of fibrin ferment to the supernatant liquid causes the formation of fibrin. The supernatant liquid or salted plasma therefore contains the proteid precursor of fibrin.

The methods of investigation of this body which I have adopted are three, viz.:

1. Heat coagulation.
2. Action of neutral salts.
3. Ferment coagulation (already described).

1. Heat coagulation. All the proteid in the salted plasma coagulates as in the serum at 65°—66° C.; and as in the serum it comes down

¹ Loc. cit. ² Loc. cit. ³ Dr MacMunn kindly wrote to me to say he had found no bands in haemocyanin. ⁴ Krukenberg. “Zur Kenntniss des Hämocyanins.” Vergl. phys. Studien, 2ª Reihe, 1ste Abth.
at that temperature slowly. Crustacean fibrinogen therefore coagulates at the same temperature as haemocyanin; it is therefore impossible to separate it from haemocyanin by this method.

2. Action of neutral salts.

a. Sodio-magnesic sulphate. This salt rapidly and completely precipitates all the proteids in the plasma; this precipitate can be washed and then redissolved by the addition of water; subsequent addition of fibrin ferment to this solution causes the formation of crustacean fibrin. This precipitate of proteid therefore contains the precursor of fibrin, but it is impossible to obtain it separate from haemocyanin by this method.

b. Magnesium sulphate. This salt slowly but completely precipitates all the proteids in the plasma. The precipitate which falls first however contains all the crustacean fibrinogen; if the precipitate be collected after the plasma and salt have been shaken together for three hours, washed and redissolved, addition of fibrin ferment to it causes fibrin to be formed. It therefore contains crustacean fibrinogen; it however also contains some amount of haemocyanin, as is seen by its faint blue tinge. After three hours’ shaking in this way, as a rule all the proteid that remains in solution is haemocyanin, addition of fibrin ferment to it causing no coagulation. It is only an imperfect separation of crustacean fibrinogen from haemocyanin that can be brought about by the action of magnesium sulphate.

c. Sodium Chloride. For the study of this salt on the proteids it is best to receive the blood directly on being shed into saturated solution of sodium chloride; the cells and a certain amount of proteid precipitate which forms can then be filtered off, and the filtrate is sodium chloride plasma.

If crystals of sodium chloride be added to such sodium chloride plasma and the mixture shaken for five or six hours, a precipitate of uncoagulated proteid is obtained; this can be washed with saturated solution of sodium chloride. If washed for a short time, and then water be added, the resulting solution contains haemocyanin in addition to crustacean fibrinogen; but if it be washed for a very long time, six or seven days, the whole of the haemocyanin is washed away, haemocyanin being slightly soluble in saturated solution of sodium chloride. The precipitate left on the filter is small in proportion to what was first there; it can be redissolved by adding water; the resulting solution contains crustacean fibrinogen, as is seen by the formation of fibrin on the addition of fibrin ferment, it coagulates at 64°—5°, and it contains
no haemocyanin, as is seen by the fact that the solution is colourless, not blue, and contains no copper.

In this way then a complete separation of the two proteids can be brought about.

Like haemocyanin, crustacean fibrinogen can be precipitated by dialysing out the salts from it. It therefore is a proteid of the globulin class.

Crustacean fibrinogen is thus seen to resemble vertebrate fibrinogen in certain points, viz.:

a. It is a proteid of the globulin class.
b. It forms fibrin on the addition of fibrin ferment, prepared either from vertebrate or crustacean blood.
c. It is completely precipitated by saturation with magnesium sulphate, magnesio-sodic-sulphate, or sodium chloride.

It differs from vertebrate fibrinogen in certain other points:

a. It coagulates at 65°C, vertebrate fibrinogen at 56°C.
b. It is not precipitated by sodium chloride completely unless its solution be saturated with that salt; whereas vertebrate fibrinogen is completely precipitated by half saturation with sodium chloride.

PART IV.

Colouring Matters of the Blood.

These are two in number:

1. The blue colour associated with the proteid haemocyanin.
2. A red pigment, tetronerythrin.

1. Haemocyanin has already been treated of in the preceding part of this paper. The blue colour is due to the combination of oxygen with haemocyanin; in the reduced state it is colourless. This has been shown by Fredericq to occur not only in experiments with haemocyanin outside the body, but also in the body itself: the blood going to the gills being colourless, and that leaving them having a blue colour.

There can be no doubt that haemocyanin plays a part analogous to that of haemoglobin in vertebrate animals: that is, it is the oxygen carrier. It is not however located as haemoglobin in vertebrates is, in special corpuscles, but exists in solution in the blood plasma, as haemoglobin does in the blood of many worms.
I am not aware that haemocyanin has ever been obtained in a crystalline form.

2. Tetronerythrin, the red pigment.

The presence of a red pigment in crustacean blood as well as a blue one, in varying proportions, accounts for the discrepancy between various observers as to the colour of the blood; it having been described as blue, violet, and red. All these colours are seen in different specimens, the difference being due to the fact that the red pigment is present in abundance in some specimens, and absent or nearly so in others. That a red pigment exists apart from the blue was first shown by Jolyet and Regnard\(^1\) in crab’s blood in 1877, and by Fredericq\(^2\) in the lobster’s in 1879. These observers also showed that this red pigment is soluble in alcohol, and that it is not a proteid. Pouchet, writing as late as 1882 however, was still in a state of confusion as to these colours, and judging from the variety of colour exhibited by different animals of the same species, concluded that the two pigments had close affinities, and probably performed the same functions; while the fact is that haemocyanin is always present in about the same proportions, but its blue tinge is often concealed by the red colour of the tetronerythrin when that body is present in abundance; tetronerythrin never replaces haemocyanin, and probably the two bodies have totally different functions.

In the identification of this pigment, and especially in spectroscopic observations upon it, I have received much help and valuable suggestions from Dr MacMunn of Wolverhampton. He has been good enough to send me specimens of tetronerythrin which he prepared from other sources, and with which I have therefore been enabled to compare this red pigment.

It will be convenient to discuss this subject under the following heads:—

2. Chemical properties.
3. Spectroscopic observations.
4. Its occurrence in other parts of the animal, and its probable functions.

1. Mode of preparation. This is exceedingly simple; the blood is extracted with alcohol; this reagent coagulates the proteid matter which is filtered off, and dissolves the red pigment which is contained in the filtrate. This is evaporated down, and when nearly all the

\(^1\) Loc. cit.  \(^2\) Loc. cit.
alcohol has been driven off, the red body separates out in red flakes of non-crystalline amorphous structure, which can be collected or dried or redissolved in alcohol, ether or other reagents shortly to be mentioned. Or the blood may be extracted in the first instance with ether; ether however does not so readily precipitate the proteids as alcohol does.

2. Chemical properties. The red pigment is soluble as just said in alcohol, and in ether; this suggests that it is a pigment associated with a fatty body, or may be itself a coloured fat; the name given to these bodies is that of lutein, or, better, lipochrome (Krukenberg).

That this supposition is correct is shown by the following confirmatory tests.

a. It is soluble in petroleum ether, benzol, chloroform, turpentine, carbon bisulphide, and to a slight extent in olive oil.

The colour of these different solutions were slightly varying shades of orange yellow, with the exception of those in chloroform and carbon bisulphide, which were pink.

b. Colour reactions; these were tried with the solid pigment.

i. Concentrated sulphuric acid; the fragments undergo a series of colour changes, being first a dirty green, then bluish green, and lastly a beautiful violet. Later still this fades and only a brownish colour is left.

ii. Concentrated nitric acid; a distinct bluish green colour is developed which lasts only a few seconds; then the flakes become colourless.

iii. Iodine dissolved in solution of potassium iodide; the solution used is of this composition, iodine 25 grm., potassium iodide 5 grm.; and distilled water 100 c.c. (Capranica). This gives no colour reaction at all with the solid pigment. After the saponification of the pigment by the addition of strong caustic soda to the alcoholic solution, this iodine solution gives a bluish violet colour, which like the nitric acid colour is evanescent.


2 Kuhne and Ayres found that a similar state of things existed in another group of lipochromes, which they found in the cones of the retina and called chromophanes; viz.

PH. VI. 24
3. Spectroscopic observations. So far the chemical properties have only told us that the pigment is one of the class of lipochromes. The spectroscope affords however the means of completely identifying the body.

A strong alcoholic solution is of a deep orange red colour and gives a spectrum in which the red end of the spectrum is cut off up to the C line, and the blue end is also cut off up to the E line or a little on the red side of it (Pl. VIII. fig. 3, C). In a more dilute solution less of the red end is cut off, and a band in the region of the F line becomes detached from the large absorption of the blue end of the spectrum. This is not a very dark band, and in very dilute solutions appears only as an ill-defined shading. It is therefore impossible to measure it in wave lengths with any great exactitude, but so far as can be made out it reaches from 515 to 475. This is shown in Pl. VIII. (fig. 3, B).

After saponification, the yellowish red soap sinks to the bottom of the tube, and on spectroscopic examination of this, the absorption spectrum is found to be similar to that just described.

On comparing this spectrum with that of other lipochromes, it is found to be identical with that of tetronerythrin obtained either from the exoskeleton or hypoderm of the same animals. It is also very similar to that of Kühne's xanthophane and rhodophane, the absorption spectra of which are figured for the sake of comparison (fig. 3, D and E). Rhodophane however is insoluble in petroleum ether and carbon bisulphide, so the two pigments cannot be identical; the absorption spectrum of this body as also of xanthophane show slight differences from that of tetronerythrin. Reducing or oxidising agents produce no change in the colour or spectroscopic properties of tetronerythrin. Its solutions can be boiled without undergoing any change. After exposure for some weeks they become rather fainter in colour; they may be due to the action of light or of the atmosphere. The solid pigment on being dried either in the air or in a vacuum over sulphuric acid, changes first to a dirty green colour, and then becomes colourless.

4. The probable functions of tetronerythrin. The name was first given by Wurm\(^1\) to the red colouring matter, which occurs in the red parts round the eyes of certain birds and known as 'the roses'.

Merejkowski\textsuperscript{1} found the same pigment in 104 species of animals, vertebrate and invertebrate; the list has also been largely added to by Dr MacMunn\textsuperscript{2}.

Merejkowski considers that this pigment plays an important part in the cutaneous respiration of lower animals. He does so on four grounds.

1. Its distribution in the organs. It occurs mostly in tissues that come in contact with the water, especially in the gills. It also occurs abundantly in those organs which need most oxygen for the fulfilment of their functions, e.g. the muscles.

2. Its distribution in the animal kingdom. It is more abundant in sedentary than in freely moving animals.

3. It occurs in very small quantities in animals provided with parasitic algae which produce oxygen in the tissues of these animals.

4. On exposure to the sun it becomes colourless, possibly from oxygen uniting with it to form a new uncoloured product.

It certainly may be the fact that this body plays some important part in respiration, but hardly I fancy in the way that Merejkowski conceives. This I base upon the consideration of the following points:—

1. The colourless product which is formed is probably not the result of oxidation; a stream of oxygen through it produces no such effect; nor does addition of hydrogen peroxide. Moreover it occurs equally well in a vacuum as in the presence of oxygen.

2. It seems to be more probably the result of the loss of water; this is supported by the consideration that the colour changes produced by the addition of sulphuric acid are very similar, and those changes are possibly due to the acid taking the elements of water from the pigment.

3. This body moreover is probably of a fatty nature; and fats are more prone to undergo hydrolytic than oxidative changes.

Dr MacMunn has come to the conclusion that the pigment tetronerythrin is probably built up in the liver, or in the organ that corresponds thereto, in crustacea\textsuperscript{3}. If this is the case, its presence in the blood is interesting; it is being carried by that fluid from the liver where it is probably formed, to the surface and other parts where it plays some function possibly connected with cutaneous respiration.


When describing the blood corpuscles of the crustacea in a former part of this paper (p. 309), I mentioned the fact that some of them in some animals, especially where tetronerythrin is abundant, contained yellowish red granules. We have here most likely to do with the same pigment; tetronerythrin occurs dissolved in the plasma, but it is very soluble in fats; many of the granules of amoeboid cells are fatty in nature, hence they get tinged with the red pigment.

The red pigment is said by Fredericq and also by Krukenberg not to be constantly present in lobster's blood. I have myself come across no specimen where it is absent. It is however possible that it is sometimes absent; it seems to me however more probable that it is always present, though sometimes in so small quantity as to escape detection by a mere inspection of the blood. The alcoholic extract may indeed appear to be perfectly colourless. I found this not only in some specimens of lobster, but also in nearly all the specimens of the sea crayfish I examined. On evaporating the alcoholic liquid to a small bulk, however, it assumes a faint yellow red tinge, and later a few flakes of the pigment separate out.

This is so far as I can discover the first instance in which the presence of a lipochrome\(^1\) has been definitely proved in crustacean or any blood. All the pigments hitherto described in blood seem to be associated with a proteid constituent of the blood.

It is however possibly the case that the granules of varying colour that occur in the blood cells of different groups of animals, especially among the Echinoderms, are in reality lipochromes; this however is only a suggestion, as I have not any proofs or experiments to bring forward in support of the hypothesis.

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PART \(V\).

**Comparative Aspects of Crustacean Blood.**

**Fredericq** pointed out that the plasma of invertebrate blood differs from that of vertebrate blood by fulfilling two functions, a nutritive and a respiratory one: whereas in vertebrate blood the plasma performs only the former of these, the nutritive function, while the respiratory function is delegated to special corpuscles, which do not exist in the blood of the invertebrate.

\(^1\) Unless chlorophyll is to be regarded as a lipochrome. Chlorophyll or a body closely related to it has been found to occur in the blood of many butterflies and moths by \textit{Mr Poulton. Proc. Roy. Soc. 1885.}
This rule on the whole holds good; there are however several exceptions to it.

It seems to be true with regard to the blood of crustacea, or at any rate with the blood of those crustacea that have been hitherto examined. The body that possesses the respiratory function is however not haemoglobin as among the vertebrata, but haemocyanin.

Haemocyanin is not however universally found among crustacea, but haemoglobin has been described in several species, dissolved in the blood plasma.

Haemocyanin again is not confined to crustacea, but is found in several other groups of animals, e.g. Mollusca and Arachnida.

From a comparative standpoint, we may subdivide the consideration of this subject into the following heads:

1. Comparison of the blood of the crab, lobster, crayfish and sea crayfish.
2. Comparison of these with the blood of other crustacea.
3. Comparison of crustacean blood with that of other animals containing haemocyanin.

1. Comparison of the blood in the crab, lobster, crayfish and sea crayfish. The points in which these agree have been the subject of the parts of the paper previous to this. There now only remain to be pointed out, the ways in which they differ from one another.

The differences are of degree not of kind.

The differences in the amounts of various constituents is given on p. 302; there the crab's blood is seen to be richest in proteids, and Astacus the poorest. Astacus is also the poorest in salts, this being probably due to its fresh water habitat. Although the total quantity of proteids is greatest in the crab, the amount of fibrin formed is the smallest, and the clot is not nearly so firm or large as in the other animals. The amount of tetronerythrin varies in different specimens of the same animal, and in Nephrops is always present in exceedingly small quantity, often there being only a trace of it.

In the liver of Astacus, MacMunn found haematin, but not in any other crustacean; this however does not seem to have any effect in the composition of the blood. Astacus blood was at one time stated by Krukenberg to contain no haemocyanin¹, this he corrects in his later papers.

2. Comparison with the blood of other crustacea. Haemocyanin

has been noted by Krukenberg as occurring in the blood of many other decapods, and malacostracous crustacea. Whether crustacean fibrinogen is there also, I have not had an opportunity of discovering. The phenomena of spontaneous coagulation are described as being similar to those in the crab and lobster, and therefore it seems probable that it is there also due to the action of a ferment on a proteid precursor in the blood, as I have just shown to occur in the animals used for my researches.

In other crustacean groups haemoglobin is noted as being present in solution in the blood plasma; in the entomostracous crustacea, Daphnia and Cheirocephalus (Lankester¹), in Apus productus and cancriformis and in Cypris (Regnard and Blanchard²), and in Lernanthropus and Clavella (Van Beneden³). It has also been mentioned as occurring in a marine parasitic crustacean (undescribed) by Van Beneden⁴.

I find no description of the blood in any other crustacean animals.

3. Comparison with the blood of other invertebrate groups.

Haemocyanin is not limited to the blood of crustacea.

a. Cephalopoda.

Haemocyanin here occurs largely, and has been described in several genera by Fredericq and Krukenberg.

The following table, from Fredericq’s memoir on the blood of the Octopus, I quote as being useful to compare with that of crustacean bloods I have given on p. 302.

<table>
<thead>
<tr>
<th></th>
<th>Eledone (Harless)</th>
<th>Sepia (Bert)</th>
<th>Sepia (Schlossberger)</th>
<th>Octopus (Fredericq)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solid matters in 100 parts</td>
<td>7·23</td>
<td>10·9</td>
<td>18·20</td>
<td>12·6</td>
</tr>
<tr>
<td>Salts</td>
<td>2·63</td>
<td></td>
<td>3·205</td>
<td>2·225</td>
</tr>
<tr>
<td>Organic matters</td>
<td>4·6</td>
<td></td>
<td>10·375</td>
<td></td>
</tr>
<tr>
<td>Proteids</td>
<td></td>
<td>3·4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

From the description of the spontaneous coagulation that occurs in these animals, I should be inclined to think that here again we have a ferment action similar to that I have described.

b. Other Molluscan groups.

In the Gastropods haemocyanin is present as a rule.


⁴ Quoted in Gamgee’s Physiological Chemistry, p. 130.
In the Lamellibranchs it seems to be absent.

**c. Arachnida.**

Haemocyanin was first definitely shown to occur in the blood of Limulus by Professor Lankester. Thanks to the kindness of Professors Lankester and Bourne, I have had the opportunity of examining the blood from three specimens of the king crab. In these I found the average percentage of proteids to be 6.12. Haemocyanin here had the same spectroscopic properties, and the same heat coagulation temperature as in crustacea. Moreover all the proteids were precipitated by saturation with magnesium sulphate.

So far as I was able to follow the phenomena of spontaneous coagulation, it presented no differences from that seen in crustacea. First a binding of the cells into shreds occurred, followed by the jellying of the whole mass. This was prevented by the admixture of a large amount of saturated magnesium sulphate solution, but not by sodium sulphate. On dilution of the magnesium sulphate mixture with water, coagulation occurred; by examination under the microscope a fibrinous substance, in addition to the cells, was seen. This possessed the same chemical properties as crustacean fibrin, and almost the same as those of vertebrate fibrin. I did not perform actually the crucial experiment of separating the fibrinogen and the ferment of the blood of Limulus, as I made these observations before my researches in crustacean blood had reached that stage. But from the consideration of the facts above mentioned, it may be taken as certain that the cause of coagulation is the same in Limulus as among crustacea.

That being the case, does this have any bearing on the class relations of Limulus? I fancy not, since similar phenomena are observed in such distant groups as the Cephalopods and Gastropods.

Moreover the blood of Scorpio itself contains large quantities of haemocyanin as Lankester showed: this is the only instance hitherto noted of haemocyanin occurring in an air-breathing animal.

The fact that the same body occurs in various groups of animals is not only instanced by haemocyanin. Tetronerythrin is another example of the same kind. As another, chitin may be mentioned, this body occurring in Cephalopods, and in all the groups of the Arthropods, and as well in other animals which need not be enumerated here.


APPENDIX.

I propose to give here a list of the animals in which haemocyanin has been described as occurring: and also lists of animals in the blood of which other colouring matters have been described.

A. Haemocyanin.

**Crustacea.** Homarus (Fredericq).
Astacus (Krukenberg).
Cancer (Krukenberg).
Carcinus (Jolyet and Regnard).
Nephrops (present paper).
Eriphia (Krukenberg).
Squilla (Krukenberg).
Maja (Krukenberg).

**Arachnida.** Scorpio (Lankester).
Limulus (Lankester).

**Gastropods.** Cassidaria (Krukenberg).
Fissurella (Krukenberg).
Haliotis (Krukenberg).
Helix (Fredericq).
Murex (Krukenberg).
Turbo (Krukenberg).

**Cephalopods.** Octopus (Fredericq).
Sepia (Krukenberg).
Eledone (Krukenberg).
Loligo (Krukenberg).

B. Haemoglobin.

**Vertebrata.** In special corpuscles in all except Amphioxus (Lankester)\(^1\).
Leptocephalus (Lankester)\(^1\).

**Crustacea.** Daphnia (Lankester).
Cheirocephalus (Lankester).
Apus (Regnard and Blanchard).
Lernanthropus (Van Beneden).
Clavella (Van Beneden).
Cypris (Regnard and Blanchard).
Marine parasitic Crustacean (undescribed) (Van Beneden).

Insecta. Cheironomus (Lankester)¹.  
Musca domestica (Mac Munn)².

Mollusca. Planorbis (Lankester).  
Arca (Lankester).  
Solen (Lankester).

Chaetopoda. Lumbricus. Limnodrilus.  
Eunice. Lumbriculus.  
Cirrhatulus. Nais.  
Nereis. Chaetogaster.  
Terebella. Glycera.  
Tubifex. Capitella.  

Gephyrea. Phoronis (Lankester).  
Thallasema neptuni (Lankester).  
Hamingia (Lankester).

Nemertina. Polia (Lankester).  
Other Nemertines (Hubrecht⁴, 1875).

Hirudinea. Nephelis (Lankester).  
Hirudo (Lankester).

Echinodera. An Ophiurid (Fottinger⁴, 1880).

In all invertebrates haemoglobin occurs in solution in the blood plasma, except in the following four where it is contained in special corpuscles: Solen, Glycera, Capitella, Phoronis⁵.

C. Chlorocruorine.

Chaetopoda. Siphonostomum (Lankester)⁶.  
Sabella (Lankester)⁶.  
Chloronema (Quatrefages)⁷.

D. Haemerythrin.

Gephyrea. Phascoloma (Schwalbe)⁸.


³ The observations that Haemoglobin is present in Chaetopods are all of Prof. Lankester. I do not find Aphrodite in his lists however: it is mentioned by MacMunn, *Animal Chromatology*, p. 388.


⁵ Described as Haemerythrin by Krukenberg.


Sipunculus (Krukenberg)\textsuperscript{1}.
Phoronis (Krukenberg)\textsuperscript{2}.

E. Chlorophyll.
\textit{Insecta.} Various Butterflies and Moths (Poulton)\textsuperscript{3}.

F. Tetronerythrin.
\textit{Crustacea.} Homarus (present paper).
Carcinus (present paper).
Astacus (present paper).
Nephrops (present paper).

G. Various coloured granules are described in the corpuscles of Holothurians and Sea Urchins (Geddes)\textsuperscript{4}. The blood of Patella is described as being of an orange colour (Krukenberg).

**EXPLANATION OF PLATE VIII.**

**FIG. 1.**

A. Solar spectrum.
B. Absorption spectrum of haemocyanin (Homarus vulg.).
   Strength of solution 4 – 5\%.

When haemocyanin is kept for some days in a strong solution of magnesium sulphate, the tint becomes more violet. When kept in a strong solution of sodium chloride, it assumes a greenish hue. On spectroscopic examination of these liquids, as before, no bands are seen, but only variations in the amount of the cutting off of the ends of the spectrum.

**FIG. 2.**

For the purpose of comparison with the above, I append Krukenberg’s diagram of the absorption spectrum of haemocyanin prepared from Helix pomatia.

A. Solar spectrum.
B. Absorption spectrum of haemocyanin (Helix pomatia) (Krukenberg).

\textsuperscript{1} Krukenberg. \textit{Vergl. phys. Studien}, 1\textsuperscript{st} Reihe, 3\textsuperscript{rd} Abth. p. 82. The name Haemerythrin is Krukenberg’s.
\textsuperscript{2} Described as Haemoglobin by Lankester.
Fig. 3.

A. Solar Spectrum.
B. Tetronerythrin in alcohol; dilute solution.
C. Tetronerythrin, in alcohol; concentrated solution.
D. Xanthophane (Kühne and Ayres).
E. Rhodophane (Kühne and Ayres).

The spectra D and E are given in the diagrammatic form in which Kühne figured them.