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RESPIRATORY PROTEIDS

GRIFFITHS

To Prof. W. D. Halliburton, M.D., F.R.S.,
with the compliments of
A. B. Griffiths.

ATORY PROTEIDS

RESEARCHES IN BIOLOGICAL CHEMISTRY

BY

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PREFACE.

> "*The elements of the living body have the chemical peculiarity of forming with each other most numerous combinations and very large molecules.*"
>
> PREYER.

THE mere accumulation of hard and dry facts is not by any means the sole, nor indeed the principal, work of scientific investigation. The facts may sometimes be turned to some useful account, though probably the majority never prove of any value whatever; but it is only when their relationship to each other is understood, and a system and harmony between them are perceived, that they become truly interesting. The alchemists got to know with a fair approach to accuracy the combining equivalents of the elements they worked with, and the knowledge thus acquired had a certain degree of utility; but it was only when Dalton wove all these facts into his famous theory that their full philosophical significance was realised.

This is only another way of saying that the imagination is, after all, the most precious faculty with which a scientist can be equipped. It is a risky possession, it is true, for it leads him astray a hundred times for once that it conducts him to truth; but without it he has no chance at all of getting at the meaning of the facts he has learned or discovered.

The present work gives an account of recent researches in biological chemistry, and special details are given of the respiratory proteids (coloured and colourless) in the blood of animals. I am of the opinion that there are many proteids of a respiratory function in the blood of animals, and my own investigations (communicated to the *Académie des Sciences de Paris*) support that opinion.

It appears that the advance which we have already accomplished in chemico-biology, instead of narrowing actually expands the fields which remain for us to occupy. If Science means the interpretation of the universe, its scope must widen with our comprehension of the almost infinite nature of its task.

The speculations which the present work may give rise to cannot but afford an interesting intellectual exercise to those who concern themselves with the philosophy of living matter.

In conclusion, the investigations detailed in the following pages have occupied a great deal of my attention for the past six years, and I have now the pleasure in presenting to students of science the following account of recent advances made in our favourite study—biological chemistry.

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RESPIRATORY PROTEIDS.

CHAPTER I.

INTRODUCTION.

THE blood of the invertebrata, like that of the vertebrata, is not homogeneous. It consists of a transparent or semi-transparent liquid, and a number of small, solid corpuscles, which float in it.

In the higher animals the corpuscles are of two kinds—red and colourless; but in the invertebrata there are, as a rule, only colourless corpuscles. The red blood of annelids is different from the red blood of vertebrates, inasmuch as the plasma is coloured and the corpuscles are colourless in the former,* while in the latter the plasma is colourless, and there are present coloured and colourless corpuscles.

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* There are exceptions to this general statement.

The corpuscles in the blood of the invertebrata are of different sizes, and the size varies greatly in the same individual. Their form, however, is generally spherical, and their surface has a raspberry appearance.

In the higher invertebrata the blood clots after a variable period of time. Haycraft and Carlier* have examined the coagulation of the blood in certain invertebrates. According to their investigations the clot is formed, at any rate for the greater part, by the welding together of blood corpuscles. These throw out processes which interlace to form a solid mass.

Although the blood of the invertebrata contains corpuscles its composition greatly varies. For instance, the blood of the lower, and some of the higher, invertebrates is a watery fluid containing proteids. This kind of blood has been termed hydrolymph. But in the majority of the higher invertebrates the blood is less watery, and consequently much richer in proteids. This variety of blood is called hæmolymph. It is generally stated that the distinction between these two varieties lies not only in their composition but also in the physiological functions which they perform.

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* Haycraft and Carlier, *Proc. Roy. Soc. Edin.*, vol. xv. p. 423.

The hydrolymph carries nutriment to the tissues and organs, and removes waste products. It is generally stated that, as a rule, it has no respiratory function, for in those animals which possess blood of the nature of hydrolymph the gaseous exchanges occur directly between the tissues and the surrounding medium. In certain cases the hydrolymph has, however, a respiratory function, and contains respiratory proteids.

The hæmolymph has not only a nutritive, but also a respiratory function, and it frequently contains respiratory pigments.

In the blood of the vertebrata there is only one respiratory pigment—hæmoglobin. In the blood of the invertebrata there is not only hæmoglobin but several pigments and colourless proteids of a respiratory nature; for instance, hæmocyanin, hæmerythrin, chlorocruorin, pinnaglobin, the achroglobins, and probably other proteids which may have a similar function. The isolation of these proteids is extremely difficult, and requires the greatest skill on the part of the investigator.

The Blood of Echinoderms.—The blood of these animals is a true hydrolymph. It is a watery liquid, holding in solution mineral matter and a small quantity of proteids, and in it float numerous amœboid corpuscles. These corpuscles have been described by Geddes,[*] and many of them contain variously coloured globules. Among these pigments is MacMunn's echinochrome,[†] which has a definite composition and a respiratory function; but the majority of these pigments appear to be lipochromes, and consequently have no respiratory function.

Foëttinger[‡] has found hæmoglobin in the blood of *Ophiactis virens* (one of the Ophiuridea), and the same respiratory pigment has been found by Howells[§] in the blood of *Thyonella gemmata* (belonging to the Holothuridea). In the integument of many echinoderms, MacMunn[||] has found hæmatoporphyrin, which is well known to be a decomposition product of hæmoglobin; and there is no doubt that tissue-respiration plays an important part in the respiration of these animals. Whether there are other respiratory proteids, besides hæmoglobin and echinochrome, it is difficult to say; but it is generally stated that the blood of echinoderms has no respiratory function. Nevertheless, the investigator who conscientiously

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[*] Geddes, *Archives de Zoologie Expérimentale et Générale*, t. viii.

[†] MacMunn, *Quart. Journ. Micros. Sci.*, 1885.

[‡] Foëttinger, *Zool. Anzeiger*, 1883, p. 416.

[§] Howells, *Studies from Biol. Lab. Johns Hopkins University*, vol. iii. p. 284.

[||] MacMunn, *Journ. Physiol.*, vol. vii. p. 240.

undertakes to solve problems of the nature recorded in these pages must not be blinded by preconceived ideas. It is always satisfactory to feel that one has helped to advance truth and upset falsehood, even should the effort prove painful by undermining some pet theory or idea.

We now consider the composition of the mineral or saline matter contained in the blood of the following echinoderms:—*Spatangus*, *Echinus*, *Uraster*, and *Solaster*. The percentages of saline matter contained in the blood of these animals have been ascertained as follows:[*]

	I.	II.	III.	IV.	V.	Average.
Spatangus . .	1·820	1·834	1·862	1·841	1·836	1·832
Echinus . .	1·786	1·752	1·761	1·706	1·772	1·755
Uraster . .	1·924	1·936	1·922	1·941	1·932	1·931
Solaster . .	1·973	1·962	1·983	1·985	1·968	1·974

The author has also submitted to analysis the ashes of the blood of these animals. The ashes were obtained by incinerating the blood, partially covered in a platinum dish, at a very low temperature. By so doing the alkaline metals are not volatilised as they are when a high temperature is used.

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[*] Griffiths, *Proc. Roy. Soc. Edin.*, vol. xix. p. 118.

The following results represent the averages of three analyses in each case:

	Spatangus.	*Echinus.*	*Uraster.*	*Solaster.*
Iron oxide (Fe_2O_3)	trace	trace	trace	trace
Lime (CaO) . .	3·62	3·78	3·02	3·16
Magnesia (MgO) .	1·05	1·12	1·38	1·36
Potash (K_2O) .	4·81	4·76	4·52	4·63
Soda (Na_2O) . .	43·78	43·82	44·22	44·03
Phosphoric acid (P_2O_5) .	4·62	4·53	4·24	4·32
Sulphuric acid (SO_3) .	2·31	2·36	2·22	2·23
Chlorine . .	39·81	39·63	40·40	40·22
	100·00	100·00	100·00	100·00

The following table represents the complete analyses of the blood of these echinoderms:

	Spatangus.	*Echinus.*	*Uraster.*	*Solaster.*
Water . . .	95·769	95·907	95·576	95·667
Solids . . .	4·231	4·093	4·424	4·333
Fibrin . .	0·046	0·043	0·042	0·049
Albumin, &c. .	2·365	2·298	2·460	2·322
Salts . .	1·820	1·752	1·922	1·962

The coagulation of the blood of echinoderms has been investigated by Geddes,* Schäfer,† and Haycraft and Carlier.‡

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* Geddes, *Proc. Roy. Soc. Lond.*, 1880.

† Schäfer, *ibid.*, 1883, p. 370.

‡ Haycraft and Carlier, *Proc. Roy. Soc. Edin.*, vol. xv. p. 423.

Geddes states that the amœboid cells coalesce into irregular masses and shoot out processes which bind the cells together. But, according to Schäfer, the clot is not a mere plasmodium: there is also a fibrin-like substance which separates from the plasma; and the author's own analyses prove the presence of fibrin in the blood of echinoderms.

According to Haycraft and Carlier, the blood of the sea-urchin varies very much in the number of corpuscles present in the different specimens. In most cases, when allowed to coagulate, the clot is very small, and not easy to demonstrate in a few drops of blood.

The Blood of Annelids.—The blood of these animals is of the nature of hæmolymph; and in many of them it has been investigated.* The following is a list of the different annelids investigated, arranged in their several classes, along with the names of the respiratory pigments contained in the blood:

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* Griffiths, *Proc. Roy. Soc. Edin.*, vol. xix. p. 120.

	Class.	Respiratory Pigment.	Pigment in the—
Sipunculus . .	Gephyrea	Hæmerythrin	Corpuscles
Hirudo . .	Hirudinea	Hæmoglobin	Plasma
Hæmophsis . .	”	”	”
Lumbricus . .	Oligochæta	”	”
Sabella . .	Polychæta	Chlorocruorin	”
Serpula . .	”	”	”
Arenicola . .	”	Hæmoglobin	”
Aphrodite . .	”	”	”
Glycera . .	”	”	Corpuscles
Terebella . .	”	”	Plasma
Nereis . .	”	”	”

The percentage of saline matter contained in the blood of these annelids are given in the following table:

	I.	II.	III.	IV.	Average.
Sipunculus . .	3·22	3·20	3·25	3·27	3·27
Hirudo . .	3·23	3·40	3·32	3·34	3·32
Hæmophsis . .	3·64	3·59	3·62	3·61	3·61
Lumbricus . .	3·89	3·91	3·83	3·82	3·86
Sabella . .	3·51	3·56	3·61	3·55	3·55
Serpula . .	3·26	3·19	3·24	3·24	3·23
Arenicola . .	3·92	3·91	3·87	3·84	3·88
Aphrodite . .	3·77	3·72	3·71	3·80	3·75
Glycera . .	3·65	3·59	3·62	3·60	3·61
Terebella . .	3·44	3·41	3·45	3·39	3·42
Nereis . . .	3·81	3·83	3·79	3·82	3·81

	Sipunculus.	*Hirudo.*	*Hæmophsis.*	*Lumbricus.*	*Sabella.*	*Serpula.*
Iron oxide (Fe_2O_3)	0·13	0·25	0·22	0·26	0·18	0·17
Lime (CaO) .	3·00	3·33	3·31	3·21	3·42	3·40
Magnesia (MgO)	1·65	1·52	1·60	1·54	1·22	1·26
Potash (K_2O) .	5·02	4·99	5·10	5·00	4·03	4·10
Soda (Na_2O) .	44·31	43·98	44·11	44·10	45·23	45·26
Phosphoric acid (P_2O_5) .	4·78	4·89	4·80	4·76	4·56	4·55
Sulphuric acid (SO_3) .	2·86	2·92	2·82	2·85	2·10	2·14
Chlorine . .	38·25	38·12	38·04	38·28	39·26	39·12
	100·00	100·00	100·00	100·00	100·00	100·00

The ashes of the blood of these animals have been analysed, and the preceding and following results represent the averages of two analyses in each case.

	Arenicola.	*Aphrodite.*	*Glycera.*	*Terebella.*	*Nereis.*
Iron oxide (Fe_2O_3) .	0·20	0·24	0·22	0·22	0·23
Lime (CaO) . .	3·63	3·21	3·32	3·64	3·20
Magnesia (MgO) .	1·25	1·52	1·55	1·20	1·50
Potash (K_2O) . .	4·05	5·06	5·01	4·08	5·04
Soda (Na_2O) . .	45·20	44·32	44·20	45·21	44·36
Phosphoric acid (P_2O_5) .	4·50	4·80	4·79	4·56	4·82
Sulphuric acid (SO_3)	2·11	2·81	2·82	2·00	2·79
Chlorine . .	39·06	38·04	38·09	39·09	38·06
	100·00	100·00	100·00	100·00	100·00

These animals possess blood which is much richer in solid constituents than echinoderms; and the next table gives the composition of the blood of the eleven annelids investigated:

	Sipunculus.	*Hirudo.*	*Hæmophsis.*	*Lumbricus.*	*Sabella.*	*Serpula.*
Water	91·65	90·75	90·40	90·12	90·82	91·22
Solids	8·35	9·25	9·60	9·88	9·18	8·78
Fibrin	0·13	0·18	0·16	0·19	0·15	0·14
Albumin	5·02	5·73	5·82	5·80	5·48	5·40
Salts	3·20	3·34	3·62	3·89	3·55	3·24

	Arenicola.	*Aphrodite.*	*Glycera.*	*Terebella.*	*Nereis.*
Water	90·24	90·34	90·48	90·60	90·26
Solids	9·76	9·66	9·52	9·40	9·74
Fibrin	0·17	0·19	0·18	0·19	0·17
Albumin	5·72	5·70	5·75	5·80	5·74
Salts	3·87	3·77	3·59	3·41	3·83

Besides the fact that the blood of worms contains certain pigments, very little is known concerning it. There is no real coagulation, although it contains fibrin, but a heat-coagulum is formed at 65° C. When this coagulum is filtered off—*i.e.*, in the case of those worms whose blood contains hæmoglobin and chlorocruorin—no proteid remains in solution; but in the case of those whose blood contains hæmerythrin there is an additional proteid present which coagulates at 70° C.

In *Sipunculus* the blood-corpuscles contain a coloured fluid between the external wall and the central nucleus. It may be stated that this is probably the first appearance of a true coloured corpuscle, but it differs essentially from the coloured corpuscle of the mammalia, for in the latter the colouring matter is distributed throughout the corpuscle.

The hæmoglobin present in the blood of worms is identical in chemical composition (as well as spectroscopically) with that present in the higher animals.

The blood obtained from five hundred earthworms (*Lumbricus terrestris*) was treated with benzene, which readily dissolves the colouring matter. The mixture was allowed to stand for twenty-four hours at 0° C., when it separated into two distinct layers. The one containing the colouring matter was then separated from the other, and about one-sixth of its volume of pure absolute alcohol was added. After filtration the alcoholic extract was exposed to $-12°$ C., when red crystals were obtained. These crystals yielded the following results on analysis :

	Blood of *Lumbricus.*			Blood of Dog.
	I.	II.	III.	
Carbon	53·91	53·86	—	52·85
Hydrogen	7·02	7·10	—	7·32
Nitrogen	—	—	—	16·17
Sulphur	0·41	0·37	—	0·39
Iron	—	—	0·39	0·43
Oxygen	—	—	—	21·84

The foregoing analyses prove that the colouring matter of the blood of *Lumbricus* is comparable chemically to that of a vertebrate animal like the dog.*

It may be stated, *en passant*, that in *Glycera* and a few other annelids, the hæmoglobin is present in special corpuscles, but in the majority of these animals it is dissolved in the plasma.

The gases of the blood of the eleven annelids (previously mentioned) have been investigated by using the mercurial air-pump. The mercurial air-pump used for this purpose was that of Gautier slightly modified (Fig. 1),† and the method allows the collection of the blood *in vacuo* (from the time of leaving the body, vein, &c.) without any alteration in its composition. The glass receiver A C D (left-hand figure), in

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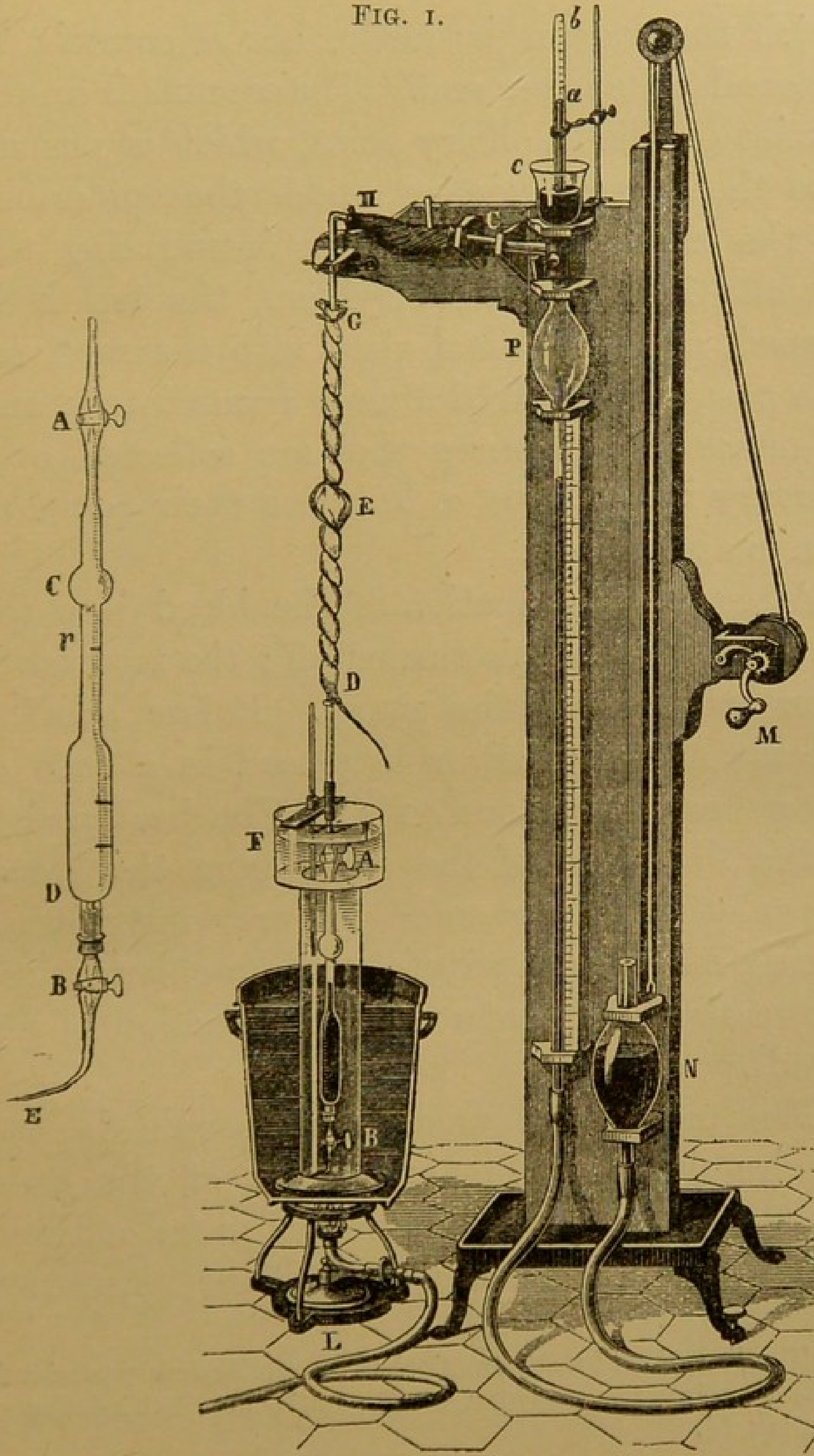
* Griffiths, *Proc. Roy Soc. Edin.*, vol. xviii. p. 294.

† *Ibid.*, p. 288.

FIG. I.



Apparatus for extracting, &c., the gases of the blood.

which the vacum is made, has a cannula E fastened to its lower end. The cannula is drawn out into a fine capillary point, which is pushed into the artery, vein, or under the epidermis, as the case may be. After introducing the cannula into the blood system, the tap B is opened, and the blood rises into the receiver. The gases are evolved almost immediately, and by means of the pump they are collected over mercury in the tube *a b*, where their composition is ascertained.

After the introduction of the blood into the receiver the tap B is turned off; the receiver is then attached to the pump. Before opening the tap A, the receiver is placed in a bath of water heated to about 40° C. The heat assists in the liberation of the gases from the blood. Coagulation is prevented by previously introducing a small quantity of sodium chloride into the receiver (*i.e.*, before the introduction of the blood).*

The pump and pneumatic trough do not require description as they are of the usual kind. The volume of the mixed gases collected at *a b* having been ascertained, the percentage

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* The liberation of carbonic anhydride is accelerated by previously introducing into the receiver a small quantity of a hot solution of tartaric acid.

of each gas is estimated by the ordinary methods of gas analysis. The carbonic anhydride is absorbed by potash, the oxygen by pyrogallic acid, whilst the amount of nitrogen is represented by what remains. By this method the following results have been obtained:

	Oxygen.	Carbonic anhydride.	Nitrogen.
Sipunculus .	12·31	28·29	1·82
Hirudo . .	12·93	29·62	1·91
Hæmophsis .	12·99	30·00	1·94
Lumbricus . .	13·02	30·15	1·96
Sabella . .	12·50	28·30	1·89
Serpula . .	12·46	28·04	1·83
Arenicola . .	12·89	30·12	1·90
Aphrodite . .	11·99	28·20	1·87
Glycera . .	12·87	29·24	1·89
Terebella . .	12·90	29·55	1·76
Nereis . .	12·82	29·02	1·84

The foregoing figures represent volumes of the gases per 100 volumes of blood (the volumes being reduced to 0° C. and 760 mm.), and they also represent the averages of three determinations in each case.

The nitrogen (probably containing argon (?)) is simply dissolved in the blood, but the oxygen and carbonic anhydride are partly dissolved and partly in a state of loose chemical combination with certain constituents of the blood. The oxygen is united to the hæmoglobin, hæmerythrin, or chlorocruorin, as the case may be; and possibly the greater part of the carbonic anhydride is united to certain salts contained in the blood. The respiratory pigments or proteids have a strong affinity for oxygen, becoming oxy-compounds, and this oxygen may be set free by various reducing agents. It is possible that reducing agents may exist in the blood, in certain conditions, which, by their action, will convert it into venous or deoxygenated blood. It is, however, more probable that all active tissues act as reducing agents so that oxygenated blood passing through them gives up its oxygen, and becomes deoxygenated.

Invertebrate blood which contains hæmoglobin has a greater power of combining with and absorbing oxygen than blood which contains either hæmerythrin or chlorocruorin. Although hæmerythrin and chlorocruorin are not so active as respiratory proteids as hæmoglobin, the former are as important as the latter. It is probable that by a process of physiological selection the respiratory proteids may have become more complex, and their molecular instability,* therefore, greater as the animal

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* For instance, hæmoglobin is less stable than hæmocyanin. See Griffiths in *Comptes Rendus de l'Académie des Sciences*, t. cxiv. p. 496.

body became more elaborated, and a necessity arose for the setting apart of respiratory proteids for the abstraction of oxygen from the air.

The Blood of Insects.—In a large number of insects the blood is colourless, although sometimes it is of a green, yellow, or red hue. This colour is not due to the amœboid corpuscles, but to the plasma in which they float.

The blood-plasma of *Musca domestica* (house-fly),[*] and that of the larva of *Chironomus*[†] (both belonging to the Diptera), contains hæmoglobin.

Poulton[‡] has made a number of observations on the blood of the Lepidoptera. The colour of the blood in these insects is principally green; but it varies, to a certain extent, with the food. This pigment (chlorophyll) has no respiratory function; and, in fact, no respiratory *pigment* appears to be present in the blood of the Lepidoptera. The existence of a respiratory pigment is doubtful, especially when one bears in mind the anatomical disposition of the respiratory apparatus in these animals—*i.e.*, the penetration of the air by the tracheæ through all the

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[*] MacMunn, *Proc. Birm. Phil. Soc.*, vol. iii. p. 385.

[†] Lankester, *Journ. Anat. and Physiol.*, vol. ii. p. 114.

[‡] Poulton, *Proc. Roy. Soc. Lond.*, 1885, p. 270.

living tissues. Nevertheless, the blood of the Lepidoptera is rich in proteids (especially in globulines), and it is capable of abstracting and retaining a considerable amount of oxygen. The gases of the blood of certain insects, investigated by the use of the mercurial air-pump, gave the following results:[*]

	Oxygen.	Carbonic anhydride.	Nitrogen.
Acherontia atropos .	16·21	32·92	1·09
Pontia brassicæ . .	16·34	34·21	1·92
Noctua pronuba . .	17·24	33·19	1·72
Vanessa io . . .	16·56	34·00	1·81
Smerinthus tiliæ . .	17·10	33·09	1·76
Lucanus cervus . .	16·28	34·97	2·31
Dytiscus dimidiatus .	16·22	34·86	2·35

The foregoing figures represent volumes of the gases per 100 volumes of blood (the volumes being reduced to 0° C., and 760 mm.). Although the blood of the Lepidoptera appears to be devoid of respiratory *pigments* (it may, however, contain colourless respiratory proteids) it has a respiratory function. In fact, the want of respiratory *pigments* in this case confirms the idea that the mere *colour* of hæmoglobin, for example, is of no use. It may be remarked however, that myohæmatin[†] (which is connected

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[*] Griffiths, *Proc. Roy. Soc. Edin.*, vol. xviii. p. 291; vol. xix. p. 125.

[†] MacMunn, *Phil. Trans. Roy. Soc.*, 1886, pt. i. p. 272.

with hæmoglobin and its derivatives) occurs in the muscles of insects; and tissue-respiration by means of this pigment is well developed in this class of animals.

It appears that in the Lepidoptera the transport of oxygen from the surrounding medium to the living tissues is made, to a considerable extent, by means of *colourless* proteids of the blood. These substances probably form oxygenised combinations which are unstable, and which are carried by the blood across the tissues and are there dissociated, yielding the oxygen to the elements of those tissues which require it.

It may be stated, *en passant*, that Regnault, Reiset, Munk and others have experimentally proved that insects take as much oxygen in proportion to their weight as the highest vertebrates.

The author * has ascertained the percentages of saline matter contained in the blood of the larvæ of certain Lepidopterous insects, &c. These percentages are as follows:

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* Griffiths, *Proc. Roy. Soc. Edin.*, vol. xix. p. 124.

		I.	II.	III.	Average.
Lepidoptera.	*Pontia brassicæ*	3·62	3·71	3·68	3·67
	Noctua pronuba	3·84	3·77	3·81	3·80
	Vanessa io	3·91	3·98	4·00	3·96
	Smerinthus tiliæ	3·92	3·99	3·87	3·92
Coleoptera.	*Lucanus cervus*	3·51	3·49	3·55	3·51
	Dytiscus dimidiatus	3·48	3·50	3·46	3·48

The blood was obtained from a large number of individuals in each case.

The ashes of the blood of these insects yielded the opposite results (*see* p. 21).*

The composition of the blood of these insects is represented in the next table:

	Pontia brassicæ.	*Noctua pronuba.*	*Vanessa io.*	*Smerinthus tiliæ.*	*Lucanus cervus.*	*Dytiscus dimidiatus.*
Water	88·49	88·09	87·94	88·21	88·29	88·35
Solids	11·51	11·91	12·06	11·79	11·71	11·65
Proteids, &c.	7·89	8·10	8·08	7·92	8·20	8·17
Salts	3·62	3·81	3·98	3·87	3·51	3·48

Fredericq† examined the blood of the larva of *Oryctes nasicornis* (one of the Coleoptera),

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* The averages of two analyses in each case.

† Fredericq, *Bulletins de l'Académie Royale de Belgique*, 3^e série, t. i.

	Pontia brassicæ.	*Noctua pronuba.*	*Vannesa io.*	*Smerinthus tiliæ.*	*Lucanus cervus.*	*Dytiscus dimidiatus.*
Iron oxide (Fe_2O_3) . . .	0·10	0·09	0·11	0·09	0·10	0·08
Lime (CaO)	2·02	2·96	2·06	2·43	2·25	2·21
Magnesia (MgO) . . .	1·00	1·12	1·09	1·13	1·16	1·09
Potash (K_2O)	4·12	4·09	4·06	4·10	4·20	4·17
Soda (Na_2O)	43·48	44·03	43·62	43·29	44·00	43·62
Phosphoric acid (P_2O_5) . .	3·21	3·61	3·50	3·40	3·32	3·41
Sulphuric acid (SO_3) . .	2·91	2·80	2·87	2·92	2·83	2·80
Chlorine	43·16	41·30	42·69	42·64	42·14	42·62
	100·00	100·00	100·00	100·00	100·00	100·00

and found that it is a colourless fluid containing corpuscles, and that it gives a precipitate with magnesium sulphate, which proves that it is rich in globulines. This blood coagulates at 55° C.; and when exposed to air it becomes a dark brown colour. This browning is due to oxidation, but has nothing to do with the function of respiration. In fact, the brown pigment is not decomposed by acids or alkalies, and it is not decolorised or deoxygenated *in vacuo*. It is merely the colour of the clot formed on exposure to air.

Concerning the coagulation of the blood of insects, it may be stated that Poulton* has shown that the blood clots after a variable period of time, and he has known samples of blood that have not clotted at all. Larval blood coagulates far more rapidly than pupal blood. The author† has entirely confirmed these observations of Poulton.

The colourless blood of the cockroach (*Periplaneta orientalis*) is rich in large amœboid corpuscles. So-called crystals may be obtained by evaporating a drop of the blood; they form radiating clusters of prismatic needles. The fresh blood is slightly alkaline, but in the

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* Poulton, *Proc. Roy. Soc. Lond.*, 1885, p. 294.

† Griffiths, *Proc. Roy. Soc. Edin.*, vol. xix. p. 125.

Lepidoptera it is acid—in fact this is the only known instance in the animal kingdom of the occurrence of acid blood, and according to Poulton the acid appears to be formic acid. The formic acid is probably produced by the oxidation of formic aldehyde, which is formed in the chlorophyll of the food, and which is oxidised to formic acid in the body of the insect.

The Blood of Arachnids.—The composition of the ashes of the blood of a few of these invertebrates has been ascertained. The ashes gave the following results (the averages of two analyses in each case) : *

	Epeira.	*Tegenaria.*	*Pholcus.*
Copper oxide (CuO) . .	0·20	0·23	0·26
Lime (CaO)	3·56	3·62	3·50
Magnesia (MgO) . . .	1·94	2·03	1·98
Potash (K_2O)	5·00	4·91	4·89
Soda (Na_2O)	44·12	44·63	43·92
Phosphoric acid (P_2O_5) . .	4·83	4·92	4·85
Sulphuric acid (SO_3) . .	2·82	2·78	2·80
Chlorine	37·53	36·88	37·80
	100·00	100·00	100·00

The blood of these animals contains hæmocyanin, which is the well-known respiratory

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* Griffiths, *Proc. Roy. Soc. Edin.*, vol. xix. p. 126.

pigment containing copper. Lankester[*] has also shown that the blood of the scorpion and of the king crab[†] becomes blue on exposure to air, and that it contains hæmocyanin. So far as is known, there are only a few air-breathing animals whose blood contains hæmocyanin.

Tissue respiration also plays an important part in these animals, as myohæmatin is present in the muscular tissues of the Arachnida.

Concerning the uses of these two pigments, it may be stated that the hæmocyanin receives oxygen from the air, and carries it to the tissues; and the myohæmatin receives the oxygen from the blood, &c., and retains it until tissue elements are in need of it.

The Blood of Crustaceans.—The blood or hæmolymph of these animals contains respiratory proteids and corpuscles. Hæmocyanin is the respiratory proteid which is present in the blood of the majority of crustaceans, but in a few genera it is replaced by hæmoglobin dissolved in the plasma. The blood of the following genera contains hæmoglobin: *Daphnia*, *Cheirocephalus*, *Apus*, *Lernanthropus*, *Cypris*, and *Clavella*.

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[*] Lankester, *Quart. Journ. Micros. Sci.*, vol. xxiv. p. 151.

[†] Howells found 6·12 per cent. of proteids in the blood of *Limulus*.

In most crustaceans there is also present in the blood a red pigment (tetronerythrin) or lipochrome, which has no respiratory function.

The coagulation of the blood of crustaceans has been investigated by Geddes, Halliburton and others. It is due to the formation of a fibrin-like substance in which the cells are entangled. The formation of this substance "is due to a ferment action upon a proteid fibrinogenous body which exists in the blood plasma. The ferment is derived from the amœboid corpuscles of the blood." The respiratory proteids (hæmocyanin and hæmoglobin) pass into the serum after the separation of the clot.

We now consider the composition of the mineral or saline matter contained in the blood of certain crustaceans. The percentages of saline matter contained in the blood of these animals were ascertained by Fredericq* to be the following:

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* Fredericq, *Bulletins de l'Académie Royale de Belgique*, 3^e série, t. iv.; and *La Lutte pour l'Existence*, p. 33.

	Blood.	
	Density.	Saline matter.
Astacus fluviatilis (fresh water)	1·029[*]	0·940
Carcinus mœnas (brackish water)	—	1·480
„ „ (sea water)	1·034[*]	3·007
Homaris vulgaris (sea water)	—	3·040
Platycarcinus pagurus (sea water)	1·036	3·104
Palinurus vulgaris (sea water)	1·040[*]	2·900
Maja squinado (sea water)	—	3·370

Fredericq has shown that the percentage of saline matter in the blood of crustaceans varies with that in the habitat of the animal. It is greater in marine than in fresh-water animals. In fact, the percentage of saline matter in the blood varies in direct proportion to the density of the habitat or *milieu extérieur*.

The ashes of the blood of certain crustaceans have also been analysed, and the results on page 27 represent the averages of three analyses in each case.[†]

The composition of the blood of four crustaceans is represented in the table[‡] on p. 28.

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[*] Determinations of A. B. Griffiths.

[†] Griffiths, *Proc. Roy. Soc. Edin.*, vol. xviii. p. 293.

[‡] Determinations of Halliburton (*Chem. Physiol.*, p. 326).

	Cancer pagurus.	*Carcinus mænas.*	*Astacus fluviatilis.*	*Palinurus vulgaris.*	*Homarus vulgaris.*
Copper oxide (CuO) .	0·22	0·19	0·20	0·18	0·18
Iron oxide (Fe_2O_3) .	trace	trace	—	—	trace
Lime (CaO) . . .	3·55	3·57	3·58	3·79	3·54
Magnesia (MgO) . .	1·91	1·89	1·88	1·90	1·89
Potash (K_2O) . .	4·97	4·78	4·82	4·92	4·77
Soda (Na_2O) . .	43·90	44·91	44·96	43·98	44·99
Phosphoric acid (P_2O_5) .	4·90	4·86	4·81	4·87	4·84
Sulphuric acid (SO_3) .	2·90	2·81	2·75	2·86	2·81
Chlorine . . .	37·65	36·98	37·00	37·50	36·96
	100·00	99·99	100·00	100·00	99·98

	Homarus vulgaris.	*Carcinus mœnas.*	*Astacus fluviatilis.*	*Nephrops.*
Water . . .	93·49	89·92	95·14	89·06
Solids . . .	6·51	10·08	4·86	10·94
Proteids, &c. .	3·57	7·38	3·73	8·17
Salts . . .	2·94	2·70	1·13	2·77

The gases of the blood of certain crustaceans gave the following results :*

	Oxygen.	Carbonic anhydride.	Nitrogen.
Cancer pagurus . .	14·79	28·62	1·01
Palinurus vulgaris .	14·62	30·00	1·82
Homarus vulgaris .	14·99	31·11	1·76

The foregoing figures represent volumes of the gases per 100 volumes of blood (the volumes being reduced to 0° C. and 760 mm.).

In concluding our remarks on the blood of crustaceans, it may be stated that although hæmocyanin and hæmoglobin appear to be the only respiratory proteids present in the blood, MacMunn's† myohæmatin is present in the muscles of these animals. Myohæmatin is a respiratory pigment, retaining the oxygen

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[*] Griffiths, *Proc. Roy. Soc. Edin.*, vol. xviii. p. 290.

[†] MacMunn, *Phil. Trans.*, 1886, p. 272.

brought to the tissues by the blood until it is required by the tissues.

The Blood of Molluscs.—The blood of the lower mollusca (Lamellibranchiata and Gasteropoda) is corpusculated, but the nuclei (which are generally present) are sometimes very indistinct. The blood of the majority of the Lamellibranchiata is a hydrolymph, although there are several exceptions—*i.e.*, the blood of *Pinna*, *Mytilus* and *Anodonta* is a true hæmolymph. The blood of the Gasteropoda is a hæmolymph. Hæmocyanin is present in the blood of most molluscs, although it is replaced by hæmoglobin* in the blood of *Solen*, *Arca* and *Panorbis*, and there is a respiratory proteid in the blood of *Pinna* containing manganese in its molecule. In addition to hæmocyanin, hæmoglobin and pinnaglobin, there are certain colourless globulins in the blood of *Patella*, *Chiton* and *Doris*, which will be described later in the present volume.

The percentages of saline matter contained in the blood of certain Lamellibranchiates have been ascertained to be as follows:†

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* Present in special corpuscles and not dissolved in the plasma (Lankester, *Proc. Roy. Soc. Lond.*, vol. xxi. p. 71).

† Griffiths, *Proc. Roy. Soc. Edin.*, vol. xviii. p. 292; vol. xix. p. 127.

	I.	II.	III.	IV.	Average.
Mya . . .	0·921	0·963	1·021	0·987	0·973
Solen . .	0·987	0·989	1·000	1·032	1·902
Pecten . .	1·003	0·998	0·989	1·006	0·999
Lima . .	0·986	0·989	0·992	0·995	0·990
Anodonta . .	1·002	0·998	1·006	0·996	1·000
Mytilus . .	1·796	1·799	1·810	1·800	1·801

The ashes of the blood of these animals yielded the following results on analysis (*see* p. 31).

The next table represents the complete analysis of the blood of five Lamellibranchiates:

	Mya.	*Solen.*	*Pecten.*	*Lima.*	*Anodonta.*[*]
Water . .	98·356	98·250	98·270	98·273	99·146
Solids . .	1·644	1·730	1·730	1·7	0·854
Fibrin . .	0·036	0·040	0·038	0·035	0·033
Albumin .	0·621	0·721	0·689	0·700	0·565
Salts . .	0·987	0·989	1·003	0·992	0·256

Lankester proved the presence of hæmoglobin in the blood of *Solen*, and MacMunn[*] has proved that the brown colour of *Solecurtus strigillatus* (belonging to the family Solenidæ) is due to the presence of hæmatoporphyrin, and it is very probable that the blood of this mollusc also contains hæmoglobin.

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[*] Schmidt's analysis, Lehmann's *Physiol. Chem.*, vol. iii. p. 256; the other four analyses are by A. B. Griffiths, *loc. cit.*

[†] MacMunn, *Journ. Physiol.*, vol. viii. p. 384.

	Mya.	*Solen.*	*Pecten.*	*Lima.*	*Anodonta.*	*Mytilus.*
Copper oxide (CuO) *	trace	—	trace	trace	0·23	0·22
Manganese oxide (MnO_2)	—	—	—	—	—	trace
Iron oxide (Fe_2O_3)	—	0·20	—	trace	—	—
Lime (CaO)	3·59	3·46	3·70	3·58	3·61	3·72
Magnesia (MgO)	1·86	1·79	1·80	1·85	1·82	1·86
Potash (K_2O)	4·89	4·90	4·87	4·90	4 90	4·80
Soda (Na_2O)	44·20	44·03	44·11	44·08	44·18	43·90
Phosphoric acid (P_2O_5)	4·89	4·80	4·76	4·83	4·89	4·82
Sulphuric acid (SO_3)	2·75	2·73	2·80	2·77	2·80	2·76
Chlorine	37·82	38·09	37·96	37·99	37·55	37·92
Lithium	—	—	—	—	trace	—
	100·00	100·00	100·00	100·00	99·98	100·00

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* Paul and Cownley (*Pharm. Journ.*, 1896, p. 442) found from 0·0181 to 0·0303 per cent. of metallic copper (= 0·0226 to 0·0379 per cent. of CuO) in certain species of *Ostrea*.

The orange-coloured blood or hæmolymph of *Chiton** (one of the Polyplacophora) and *Patella*† (one of the Branchiogasteropoda) contains no hæmocyanin (but colourless proteids, which will be described at a later period), and its composition is represented in the following table :

	Chiton.	*Patella.*
Water	90·572	89·972
Solids	9·428	10·028
Proteids, &c.	7·820	8·322
Salts	1·608	1·706

The gases of the blood of these two molluscs, investigated by means of the mercurial air-pump, gave the following results :

	Chiton.		*Patella.*	
	I.	II.	I.	II.
Oxygen	12·92	12·87	13·21	12·99
Carbonic anhydride . .	30·46	31·29	31·52	31·02
Nitrogen	1·36	1·86	1·76	1·81

The above figures represent volumes of the

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* Griffiths, *Comptes Rendus*, t. cxv. p. 474 ; *Journ. Chem. Soc.*, vol. lxiv. p. 60.

† Concerning the physiology of *Patella*, see Griffiths's papers in *Proc. Roy. Soc. Lond.*, vol. xlii. p. 393 ; vol. xliv. p. 328 ; and his book, *The Physiology of the Invertebrata*, pp. 108, 284 (Reeve & Co., London).

gases per 100 volumes of blood (the gaseous volumes being reduced to 0° C. and 760 mm.)*

The blood of these molluscs readily coagulates, forming a gelatinous coagulum, and this coagulum rapidly becomes fluid again. Similar observations have been made by Krukenberg on other molluscs.

The percentages of saline matter contained in the blood of other molluscs are given in the following table: †

		I.	II.	III.	Average.
Pulmogasteropoda.	*Helix pomatia* . .	1·065	1·072	1·069	1·068
	Helix aspersa . .	1·079	1·080	1·062	1·077
	Limnæus stagnalis .	1·200	1·203	1·210	1·204
	Limax flavus . .	1·122	1·100	1·115	1·112
	Limax maximus .	1·119	1·127	1·114	1·120
Branchiogasteropoda.	*Buccinum undatum* .	1·699	1·710	1·698	1·702
	Patella vulgata .	1·706	1·721	1·719	1·715
	Murex	1·826	1·820	1·818	1·821
Cephalopoda.	*Sepia officinalis* .	2·840	2·862	2·851	2·851
	Octopus vulgaris .	3·004	3·032	3·020	3·018
	Loligo	3·216	3·201	3·208	3·208

The blood of the majority of the Gasteropoda and Cephalopoda contains hæmocyanin.

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* Vernon (*Journ. Physiol.*, vol. xix. p. 18) has proved that the respiratory quotients of marine invertebrates are higher than in warm-blooded animals. Calculated on the amount of solid in their bodies, the respiration of the lower animals is enormous.

† Griffiths, *Proc Roy. Soc. Edin.*, vol. xviii. p. 292; vol. xix. p. 129.

Analyses of the ashes of the blood of certain molluscs gave the following results :

	Murex.	*Loligo.*	*Sepia.*	*Octopus.*
Copper oxide (CuO) .	0·24	0·21	0·24	0·21
Lime (CaO) . .	3·71	2·32	2·31	2·40
Magnesia (MgO) .	1·82	1·54	1·51	1·55
Potash (K_2O) . .	4·86	4·90	4·92	4·90
Soda (Na_2O) . .	44·26	45·23	45·40	45·31
Phosphoric acid (P_2O_5)	4·53	4·80	4·90	4·88
Sulphuric acid (SO_3) .	2·66	2·84	2·81	2·83
Chlorine . . .	37·92	38·16	37·90	37·92
	100·00	100·00	99·99	100·00

The blood of these animals is rich in solid constituents, as the following table shows :

	*Murex.**	*Loligo.**	*Sepia.*†	*Octopus.*‡
Water . . .	88·557	86·154	81·800	86·311
Solids . . .	11·443	13·846	18·200	13·689
Proteids, &c. .	9·623	10·645	14·995	10·675
Salts . .	1·820	3·201	3·205	3·014

One hundred volumes of the blood of each of these molluscs contained the following volumes of the three gases—the volumes being reduced to 0° C. and 760 mm. :

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* Determinations of A. B. Griffiths, *Proc. Roy. Soc. Edin.*, vol. xix. p. 130.

† Determination of Schlossberger.

‡ Determination of Fredericq.

	Murex.		*Sepia.*					
	I.	II.	I.	II.	III.	IV.	V.	VI.
O	14·21	13·98	13·26	12·91	13·14	14·62	14·21	14·34
CO_2	30·16	29·76	30·12	31·21	32·10	30·14	29·12	29·89
N	1·82	1·65	1·60	2·00	1·51	1·41	1·73	1·23

	Octopus.			*Loligo.*	
	I.	II.	III.	I.	II.
O	13·33	13·28	13·65	13·46	13·22
CO_2	30·23	31·29	31·22	30·02	32·31
N	1·45	1·30	1·29	1·46	1·23

The nitrogen is simply dissolved in the blood, but the oxygen and carbonic anhydride are partly dissolved and partly in a state of loose chemical combination with certain constituents of the blood. The oxygen, with the hæmocyanin, and probably the greater part of the carbonic anhydride, is united to certain salts contained in the blood. The oxygen and carbonic anhydride in the blood of the invertebrates do not behave according to the law of Dalton (the law of partial pressures) in regard to the absorption of a mixture of gases by a simple fluid. A portion of each gas combines chemically with certain constituents of the blood. It was

Magnus* who first demonstrated that the oxygen and carbonic anhydride of the vertebrate blood did not obey the law of Dalton; and the same is true concerning the gases of the blood of the invertebrata.

The blood of the Cephalopoda coagulates very soon after it is shed, and Halliburton states that coagulation is probably due to the action of a ferment which converts a soluble fibrinogen into a fibrin-like substance.

The Blood of Vertebrates.—The peculiarities or properties of the blood of these animals have been studied by numerous investigators, consequently we do not intend to offer more than a few remarks on the subject.

The blood plays an important part in respiration, nutrition, and in all the other functions of the body. The respiratory proteid present in the blood of vertebrates is hæmoglobin; but this red pigment is absent in the blood of *Amphioxus* and *Leptocephalus*. The blood consists of a fluid portion or plasma and corpuscles (red and white). "Corpuscles are contained in the blood of all the vertebrata. In *Amphioxus* they are all of one kind, colourless and nucleated. The genus *Leptocephalus*, among the Teleostei, is said to possess the same peculiarity; but, in

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* Magnus, *Poggendorff's Annalen*, vol. xl. p. 583.

all other known vertebrata the blood contains corpuscles of two kinds. In the Ichthyopsida and Sauropsida, both kinds are nucleated ; but one set are colourless, and exhibit amœboid movements, while the others are red, and do not display contractility. Except in the Marsipobranchii, which have round blood-corpuscles, the red corpuscles are oval. They attain a larger size in the perennibranchiate amphibia than in any other vertebrates. In the mammalia the blood-corpuscles are also of two kinds, colourless and red, the colourless possessing, and the red being devoid of, nuclei. It is but very rarely that a nucleated corpuscle, with a red colour especially developed about the nucleus, is seen in mammalian blood ; but such cases do occur ; and, from this and other circumstances, it is probable that the mammalian corpuscle is a free-coloured nucleus. The colourless corpuscles of the mammalia are spheroidal, and exhibit amœboid movements ; the red corpuscles are flattened, usually circular, but sometimes oval (Camelidæ) discs, devoid of contractility."

Vertebrate blood is always alkaline; and in the higher forms coagulation commences in from half a minute to thirteen minutes after the blood is shed.

The composition of the plasma and corpuscles, and the percentages of the gases of the blood of the higher vertebrates are too well known to need description in these pages; but as far as the author knows, the composition, &c., of the blood of the lower vertebrates have not yet been investigated from a chemical standpoint.

CHAPTER II.

ECHINOCHROME.

In 1883, MacMunn[*] discovered a brown pigment in the perivisceral fluid of certain echinoderms (*Echinus esculentus*, *Strongylocentrotus lividus*, *Echinus sphæra*, &c.). This pigment, which MacMunn named echinochrome, possesses a respiratory function. It exists in two conditions, which are analogous to those of hæmoglobin, viz., oxyechinochrome (charged with oxygen) and deoxygenated echinochrome.

The author[†] has determined the approximate composition of echinochrome. When a sufficient quantity of the perivisceral fluid had been obtained it was allowed to clot at the ordinary temperature of the air. The fresh clot was extracted with chloroform, benzene or carbon disulphide, in which the respiratory pigment is

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[*] MacMunn, *Proc. Birm. Philos. Soc.*, vol. iii. p. 380; *Quart. Journ. Micros. Sci.*, 1885.

[†] Griffiths, *Comptes Rendus de l'Académie des Sciences*, Paris, t. cxv. p. 419.

soluble; and on spontaneously evaporating the solution echinochrome remains as an amorphous residue. The mean of four analyses corresponds with the formula:

$$C_{102}H_{99}N_{12}FeS_2O_{12}.$$

Echinochrome is only slightly soluble in water and alcohol; by prolonged boiling in contact with mineral acids it is converted into hæmatoporphyrin, hæmochromogen and sulphuric acid:

$$C_{102}H_{99}N_{12}FeS_2O_{12} + 5H_2O + 3O_2 =$$
$$2C_{34}H_{34}N_4O_5 + C_{34}H_{37}N_4FeO_5 + 2H_2SO_4.$$

As MacMunn* discovered hæmatoporphyrin in the integuments of *Asterias rubens* and other echinoderms, it may be derived from echinochrome, which exists in the perivisceral fluid of these animals. The spectroscopic characteristics of echinochrome are given in Fig. 2, spectrum 1. Fresh echinochrome shows no distinct bands, but when the clot is treated with sodium hydroxide a purple colour is produced; and the deepening of the colour on exposure to the air is due to the absorption of oxygen or the formation of an oxygenated compound. Echinochrome is soluble in absolute alcohol, ether, chloroform, benzene, glycerine (glycerol), carbon disulphide, &c. By means of the micro-spectroscope, the serum shows two

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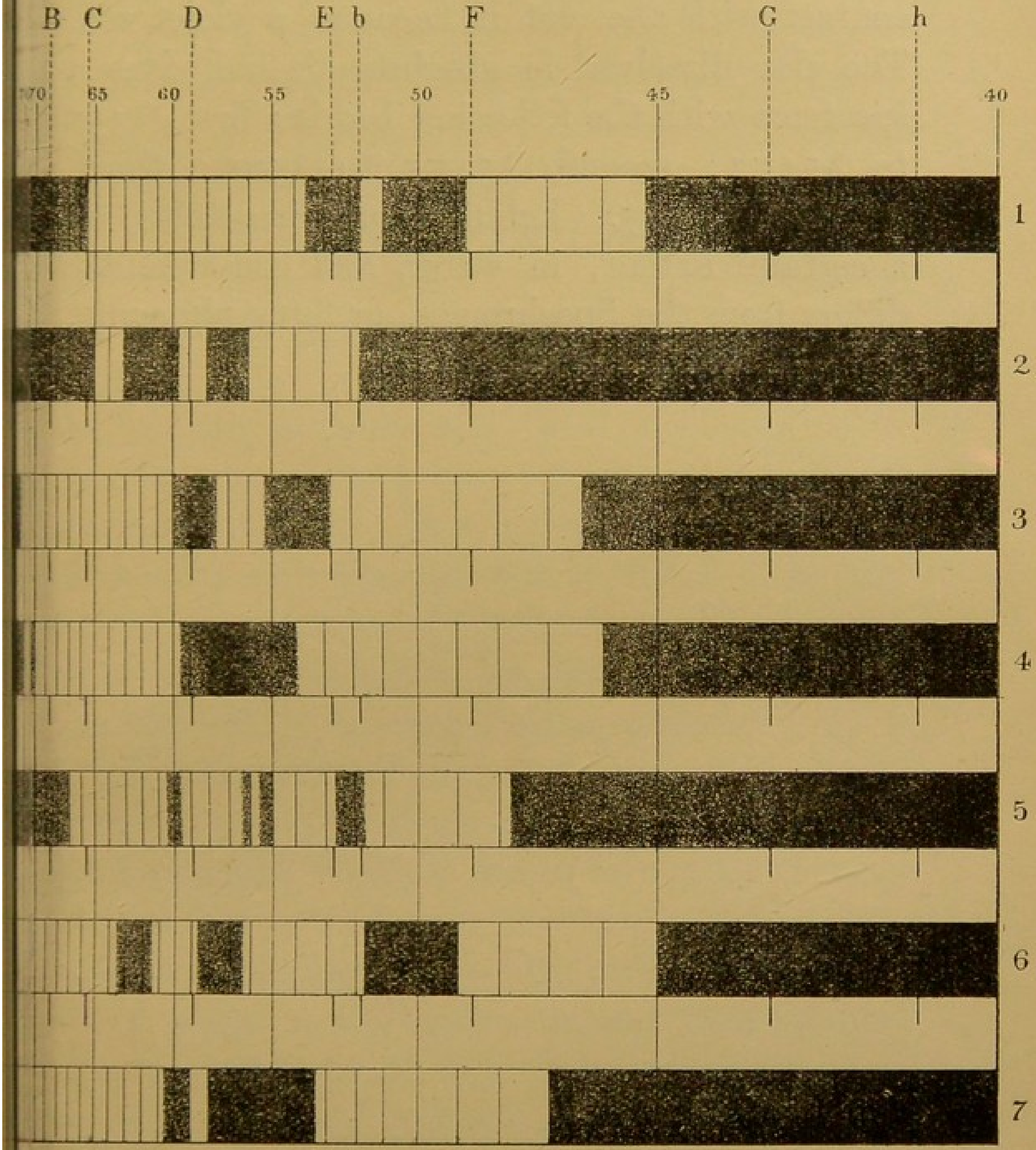
* MacMunn, *Journ. Physiol.*, vol. vii. p. 242.

FIG. 2.



SPECTRA OF RESPIRATORY PIGMENTS.

1. Echinochrome. 2. Oxychlorocruorin. 3. Oxyhæmoglobin.
4. Hæmoglobin. 5. Myohæmatin. 6. Alkaline Hæmatoporphyrin.
7. Acid Hæmatoporphyrin.

bands in the green, but if allowed to stand in contact with the clot it becomes a violet red. The clot dissolved in absolute alcohol gives a spectrum with the following bands: first, λ557 to λ545·5; second, λ524·5 to λ501; third, λ494·5 to λ475. Echinochrome is soluble, to a certain extent, in water, and consequently differs from the lipochromes, or fatty pigments, which are insoluble in water.

CHAPTER III.

HÆMERYTHRIN.

In certain annelids the blood corpuscles contain a coloured fluid between the external wall and the nucleus. This is the first appearance of a coloured corpuscle, but it differs from the coloured corpuscles of mammals as the pigment is localised, whereas in the higher animals it is distributed throughout the corpuscle.

Ray Lankester* has shown that the perivisceral cavity of *Sipunculus nudus* contains a pink pigment, which is due to corpuscles varying in size from $\frac{1}{3500}$ to $\frac{1}{4000}$ of an inch in diameter, and that this pigment, which is also present in other parts of the worm, is not hæmoglobin. Krukenberg† proved that the pink colour is due to the presence of oxygen, and that the colour is removed by carbon dioxide. The pigment gives no absorption bands either in the oxidised

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* Ray Lankester, *Proc. Roy. Soc.*, vol. xxi. p. 71.

† Krukenberg, *Vergleich. Physiol. Studien*, p. 85.

or reduced condition. Krukenberg calls this pigment "hæmerythrin" and the chromogen belonging to it "hæmerythrogen." The pigment is decomposed by hydrogen sulphide. Oxyhæmerythrin appears to be a more stable compound than oxyhæmoglobin. It may be stated, *en passant*, that the body fluid of other gephyreans contains hæmerythrin or pigments closely allied to it.

The approximate composition of hæmerythrin has been determined,* and the following method was used to extract this respiratory pigment from the blood of *Sipunculus*: When a sufficient quantity of blood had been obtained, it was allowed to coagulate, and after having removed the clot, the serum, which contains the whole of the hæmerythrin, was treated with alcohol; this reagent dissolves out urea and fatty bodies which are present in the blood of *Sipunculus*. The precipitate from the alcohol was dissolved in a dilute solution of magnesium sulphate; the solution was then saturated with magnesium sulphate and filtered. The precipitate so obtained was washed with a saturated solution of magnesium sulphate, and then dissolved in water. The precipitate dissolves owing to the

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* Griffiths, *Comptes Rendus de l'Académie des Sciences*, Paris, t. cxv. p. 669.

presence of a small quantity of magnesium sulphate which adheres to it. The solution was heated to 56° C., so as to coagulate any proteids coagulating at that temperature, and alcohol added to the filtrate as long as a precipitate was formed. After filtration, the precipitate was repeatedly washed with water, then carefully dried at 60° C., and finally *in vacuo*, without decomposition. The average of three analyses gave the following formula for hæmerythrin:

$$C_{427}H_{761}N_{135}FeS_2O_{153}.$$

This pigment exists in two conditions, oxyhæmerythrin (red) and reduced or deoxygenated hæmerythrin (brown), and it is a true respiratory pigment.

Hæmerythrin is a highly-complex molecule, although its molecular weight is not as high as that of starch, $C_{1200}H_{2000}O_{1000}$.*

This formula was proved by Raoult's method, which is extremely valuable for ascertaining the molecular weights of highly-complicated substances. Raoult proved that the lowering of the freezing point is proportional to the weight of substance dissolved in a constant weight of the solvent. Hence the formula :

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* Brown and Morris, *Journ. Chem. Soc.*, 1889, p. 462.

$$\frac{\text{T} \times \text{Wt. of substance}}{\text{Wt. of solvent} \times \text{depression of freezing point}} = \text{Mol. Wt.}^{*}$$

In fact, Raoult states, as a general law, that every substance in dissolving in a definite liquid capable of solidifying, lowers the solidifying (or freezing) point of the liquid, and that there are no exceptions to this law. The law is of great value, because of the power it gives of attacking problems—such as we have under consideration—from an altogether different standpoint. This *physical* method is a means of confirming the results obtained by the combustion furnace and the ordinary methods of chemical analysis.

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* T is the "abaissement moléculaire vrai" (true molecular depression).

CHAPTER IV.

CHLOROCRUORIN.

In 1838, the eminent zoologist Milne-Edwards[*] discovered that the blood of certain worms (*Sabella*) contained a green substance; and in 1868 Ray Lankester[†] proved that this green substance, which he named chlorocruorin, is a respiratory pigment. It exists in two conditions, as oxychlorocruorin and reduced chlorocruorin. The spectrum of oxychlorocruorin (Fig. 2, spectrum 2) consists of two absorption bands, one between C and D, and the other between D and E:

(1) The band between C and D = λ 618 to λ 593.
(2) The band between D and E = λ 576 to λ 554·5.

The spectrum of reduced chlorocruorin consists of one band between C and D, but it is not well defined.[‡] The author[§] has determined

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[*] Milne-Edwards, *Annales des Sciences Naturelles*, 2e série, t. x. p. 190.

[†] Ray Lankester, *Journ. of Anat. and Physiol.*, vol. ii. p. 114; vol. iii. p. 119.

[‡] MacMunn, *Quart. Journ. Miscros. Sci.*, 1885.

[§] Griffiths, *Comptes Rendus de l'Académie des Sciences*, Paris, t. cxiv. p. 1277; *Journ. Chem. Soc.*, vol. lxii. p. 1256.

the approximate composition of chlorocruorin, and the method of extracting the pigment has been already described in Chapter III. The mean of three analyses gave the following results :

Carbon	54·23
Hydrogen	6·82
Nitrogen	16·16
Iron	0·45
Sulphur	0·78
Oxygen	21·56
	100·00

The above figures correspond with the empirical formula

$$C_{560}H_{845}N_{143}FeS_3O_{167}$$

for chlorocruorin.

By the action of acids and alkalies chlorocruorin yields hæmatin, an albuminoid, and fatty acids.

Ray Lankester[*] states that this pigment and hæmoglobin have a common base in cyanosulphæm, and perhaps in Stokes' reduced hæmatin (hæmochromogen). Chlorocruorin is an unstable compound and is dissolved in the plasma. It occurs in the blood of the following genera of the Chætopoda ; *Sabella*, *Siphonostomum*, *Chloronema*, *Branchiomma*, *Spirographis*, and probably others as well. Ray

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[*] Ray Lankester, *loc. cit.*

Lankester and MacMunn examined the blood of *Serpula*, and found that though the blood was red it gave a spectrum similar to that of chlorocruorin. The red pigment has been considered to be an intermediate one between chlorocruorin and hæmatin, or it may possibly be an isomer of chlorocruorin.

The metal present (in organic combination) in the blood of worms is iron, and it occurs in at least three different pigments, namely, hæmerythrin, chlorocruorin and hæmoglobin.

CHAPTER V.

HÆMOCYANIN.

Hæmocyanin is the chief oxygen carrier in the blood of the higher invertebrates. The blood contains colourless corpuscles, but the pigment or hæmocyanin is diffused through the serum. This important respiratory proteid is of a blue colour in the oxidised state, but is colourless in the reduced state.

During the past seventy years many workers have shown that the blood of certain invertebrates has the power of becoming blue on exposure to air, and although Fredericq in 1878 proved that the blood of the mollusca contains a substance allied to hæmoglobin, the composition of hæmocyanin (as Fredericq[*] termed this substance) remained unknown until it was investigated from a chemical standpoint.

The author[†] has determined the approximate

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[*] Fredericq, *Bulletins de l'Académie royale de Belgique*, sér. ii. vol. xlvi., vol. xlvii.

[†] Griffiths, *Comptes Rendus de l'Académie des Sciences*, Paris,

composition of hæmocyanin. The hæmocyanin derived from the blood of *Homarus*, *Sepia*, and *Cancer* respectively was submitted to chemical analysis. The blood of *Homarus* and *Cancer* was readily obtained by making incisions in the chelæ, carapace (so as to expose the heart), and in the posterior ventral region of the body. The blood of *Sepia* was obtained from the heart and branchiæ. When a sufficient quantity of the blood of each animal had been obtained, it was allowed to clot. After removing the clot the serum which contains the whole of the hæmocyanin was treated with alcohol; this reagent dissolves out the red pigment (tetronerythrin), urea, and fatty bodies which are present in the blood of *Homarus* and *Cancer*.* The precipitate from the alcohol was dissolved in a dilute solution of magnesium sulphate and the pigment extracted by the process already described in Chapter III.

The analyses of the hæmocyanin derived from the blood of *Homarus*, *Sepia*, and *Cancer* repectively gave the following results:

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t. cxiv. p. 496; *Bulletins de l'Académie royale de Belgique*, sér. iii. t. xxiii. p. 842; *Journ. Chem. Soc.*, vol. lxii. p. 898.

* Although tetronerythrin is absent in the blood of *Sepia*, urea and fatty bodies are present; alcohol was, therefore, used as in the case of *Homarus* and *Cancer*.

	Homarus.		*Sepia.*		*Cancer.*		Average.
	I.	II.	I.	II.	I.	II.	
Carbon	54·12	54·23	54·06	54·18	54·20	54·14	54·155
Hydrogen . . .	7·00	7·14	7·08	7·13	7·10	7·12	7·095
Nitrogen . . .	16·35	16·23	16·31	16·21	16·26	16·25	16·268
Copper	0·36	0·31	0·34	0·33	0·31	0·32	0·328
Sulphur . . .	0·69	0·65	0·62	0·60	0·67	0·65	0·647
Oxygen . . .	21·48	21·44	21·59	21·55	21·46	21·52	21·507
	100·00	100·00	100·00	100·00	100·00	100·00	100·000

The above figures correspond with the empirical formula

$$C_{867}H_{1363}N_{223}CuS_4O_{258}$$

for hæmocyanin.

The analyses, from their close resemblance one to another, seem to point to the fact that hæmocyanin is a true chemical unit; in this respect it differs somewhat from hæmoglobin, and it is much more stable than the latter. Although Lehmann, Struve, and others have stated that hæmoglobin is not a true chemical unit, it should be borne in mind that Zinoffsky* has shown that the discordant results of several investigators were due to imperfect methods of preparing and purifying the hæmoglobin for analysis; and he and many others hold the opinion that hæmoglobin is a definite chemical molecule. There is no reason why extremely complex molecules should not exist, any more than highly-complicated animals or plants—both are explainable if we accept the doctrine of evolution.

As the analyses of hæmocyanin are comparable with each other, although the pigment was derived from three distinct animals, it appears that we are justified in calculating the above empirical formula for hæmocyanin, but, as in

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* Zinoffsky, *Zeitschrift für Physiologische Chemie*, vol. x. p. 16.

the case of other proteids, we are unacquainted with its constitutional or rational formula. The molecular weight of hæmocyanin is 18762; but chemists need not be surprised at such a figure, if they bear in mind the molecular weight assigned to starch (32400) by Brown and Morris; that assigned to copper hæmol ($C_{648}H_{1030}N_{178}FeCu_5S_2O_{177} = 14565$) by Merck;* and that assigned to hæmoglobin ($C_{636}H_{1025}N_{164}FeS_3O_{189} = 15129$) by Hüfner.

Hæmocyanin exists in two conditions which are analogous to those of hæmoglobin—viz., oxyhæmocyanin and reduced or deoxygenated hæmocyanin. The former has a blue colour, while the latter is colourless.

No definite combination takes place between hæmocyanin and carbon monoxide; in this respect it differs from hæmoglobin, which forms the well-known CO-hæmoglobin. Hæmocyanin, unlike hæmoglobin, does not not combine with nitric oxide, but it combines with methane, acetylene, and ethylene, forming definite compounds when these gases are passed through invertebrate blood containing hæmocyanin.

CH_4—hæmocyanin is an amorphous compound of a violet colour.
C_2H_2 ,, ,, ,, ,, ,, greenish ,,
C_2H_4 ,, ,, ,, ,, ,, brownish ,,

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* Merck, *Apoth. Zeit.*, April 17, 1895. Copper hæmol is a definite compound of copper and hæmoglobin.

These compounds appear to be fairly stable, but they require further investigation.

By using the vacuum of a mercurial air-pump it was estimated that 1 gramme of hæmocyanin absorbs 1·52 c.c. of oxygen (at 0° C. and 760 mm.)—this result being the average of six determinations. Hæmocyanin acts upon polarised light, and by means of the polariscope it was found that hæmocyanin (dissolved in a dilute solution of magnesium sulphate) gave a specific rotation for yellow light $[\alpha]_D = -58{\cdot}3^\circ$.

The author has confirmed Fredericq's* statement that when hæmocyanin is preserved in a closed vessel, even though putrefaction may occur, it remains unaltered in its characteristic properties for at least six months. In hermetically sealed tubes hæmocyanin may be preserved for an indefinite period, for after the lapse of two years it was found that it still preserved its characteristic properties. And even the microbes of putrefaction simply converted oxyhæmocyanin into reduced hæmocyanin.

The blood of the following animals contains hæmocyanin :

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* Fredericq, *Travaux du Laboratoire de Léon Fredericq* (Liège), vol. iii. p. 194.

Arachnida.	Crustacea.	Lamelli-branchiata.	Pulmogas-teropoda.	Branchio-gasteropoda.	Cephalo-poda.
Scorpio	Homarus	Mytilus	Helix	Fissurella	Octopus
Limulus	Astacus	Anodonta	Limnæus	Paludina	Sepia
Epeira	Carcinus	Unio	Arion	Haliotis	Loligo
Tegenaria	Cancer	Mya		Turbo	Eledone
Pholcus	Squilla	Pecten		Murex	
	Nephrops				
	Palinurus				
	Maja				
	Eriphia				
	Callinectes				

The properties of hæmocyanin are the following:

(*a*) It is a globulin, and is completely precipitated from its solutions by saturation with magnesium sulphate. It is incompletely precipitated by dilute acetic acid, sodium chloride, and a current of carbon dioxide.

(*b*) It is precipitated by alcohol, ether, tannic acid, mineral acids, lead acetate, and the salts of copper. The acids, as well as alcohol, while precipitating also decompose it.

(*c*) Millon's reagent produces a rose colour with hæmocyanin.

(*d*) Hæmocyanin *always* contains a small quantity of copper, which is its chief characteristic.

(*e*) When examined spectroscopically oxyhæmocyanin shows no absorption bands, but only a cutting off of both ends of the spectrum; and with reduced hæmocyanin the amount of shading is greatly diminished.

(*f*) Hæmocyanin is a respiratory proteid or the carrier of oxygen to the tissues. By means of the oxygen thus conveyed oxidations are carried on in the tissues, by which the latent energies there present are converted into heat and the so-called "vital" forces.

CHAPTER VI.

PINNAGLOBIN.

THE blood of *Pinna squamosa* is a white fluid, which exposed to air assumes a brownish colour. There is a globulin in the blood of this mollusc, which possesses the same properties of oxygenation and deoxygenation as those of hæmocyanin and hæmoglobin. The author* has named this respiratory proteid *pinnaglobin,* and the method of extracting pinnaglobin has been described in Chapter III.

The average of six analyses of this globulin gave the following results :

Carbon	55·07
Hydrogen	6·24
Nitrogen	16·24
Manganese	0·35
Sulphur	0·81
Oxygen	21·29
	100·00

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* Griffiths, *Comptes Rendus de l'Académie des Sciences,* Paris, t. cxiv. p. 840 ; *Bulletin de la Société Chimique de Paris,* 3 sér., t. vii. p. 397 ; *Journ. Chem. Soc.,* vol. lxii. p. 1016 ; *Chem. News,* vol. lxv. p. 203.

The above figures correspond with the empirical formula

$$C_{724}H_{985}N_{183}MnS_4O_{210}$$

for pinnaglobin.

Pinnaglobin exists in two conditions as oxy-pinnaglobin of a brownish colour, and reduced pinnaglobin which is white. This pigment combines with methane, acetylene and ethylene :

CH_4—pinnaglobin is a greenish compound.
C_2H_2 ,, ,, greyish ,,
C_2H_4 ,, ,, reddish ,,

These compounds appear to be fairly stable, but they require further investigation. Pinnaglobin does not combine with nitric oxide and carbon monoxide.

These amorphous compounds are dissociated in a vacuum. By using the vacuum of a mercurial air-pump, it was estimated that 1 gramme of pinnaglobin absorbs 1·62 c.c. of oxygen at 0° C. and 760 mm. Pinnaglobin and oxy-pinnaglobin give no absorption spectra. By means of the polariscope it was found that pinnaglobin (dissolved in a dilute solution of magnesium sulphate) has a specific rotation for yellow light $[\alpha]_D = -60°$.

The analysis of the ash of the blood of *Pinna* gave the following results :

Manganese dioxide (MnO_2)	0·19
Lime (CaO)	3·70
Magnesia (MgO)	1·83
Potash (K_2O)	4·86
Soda (Na_2O)	44·02
Phosphoric acid (P_2O_5)	4·79
Sulphuric acid (SO_3)	2·73
Chlorine	37·88
	100·00

Pinnaglobin is a respiratory proteid containing the metal manganese. The metals found in the respiratory proteids of animals are iron, copper and manganese—metals whose atomic weight (Mn=55, Fe=56, Cu=63,) do not differ very much from each other. Have these weights any important meaning? What bearing has Prout's hypothesis or Crookes' theory of the genesis of the elements on the subject? Why should the metals present in respiratory proteids have practically the same atomic weight, and be located about a quarter the distance from the lowest weight (H=1) to the highest weight (U=240)? Has the subject any bearing on the doctrine of evolution, the theory of natural selection and the action of the environment? These problems require solution. We have here much ground yet uncovered; but we can only work on, "unresting," "unhasting," trusting that in the end our work will throw a new light upon this deeply interesting department of biological chemistry.

> Labour with what zeal we may,
> Something still remains undone.

CHAPTER VII.

a–ACHROGLOBIN.

According to Aristotle, fishes and all higher animals were "blood-containing," and thus distinguished from the lower animals (invertebrates), which he regarded as "bloodless." He was mistaken as to the absence of blood in the lower animals, for in most it is present.

The globulin to be described in the present chapter was discovered in the blood of *Patella vulgata.** Unlike the respiratory proteids already described, this proteid does not contain a metal in its molecule. It is a colourless substance, which possesses the properties of oxygenation and deoxygenation—in other words, it is a respiratory proteid, being the carrier of oxygen in the system.

The presence of colourless globulins in the blood of invertebrates possessing a respiratory

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* Griffiths, *Comptes Rendus de l'Académie des Sciences*, Paris, t. cxv. p. 259; *Chem. News*, vol. lxvi. p. 122.

function is remarkable, but it ought not to surprise any one whose mind is free from bias. The idea of colourless globulins, devoid of a metallic basis like iron or copper, and possessing a respiratory function, is feasible as well as consistent with the fact that albuminous matter is capable of absorbing gases, especially oxygen, without any change occurring in the albuminous molecules. It may be stated that even in the blood of the higher animals part of the oxygen is simply dissolved or absorbed, and the other part is in combination with the hæmoglobin of the coloured blood corpuscles. Ordinary albumin absorbs oxygen, the gas being evolved *in vacuo.* This is an important fact, because something of a similar nature occurs in the blood of those animals with colourless respiratory proteids. Oxygen is absorbed by the colourless respiratory proteids, and is given up when required to the tissues, &c.

When a sufficient quantity of the blood had been obtained,* it was treated with alcohol, in order to dissolve the yellow pigment † and fatty substances which are present in the blood of

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* Which was extremely difficult, although very many animals were used for the purpose.

† This pigment is a lipochrome, and has no respiratory function.

Patella.[*] The residue, insoluble in alcohol, was dissolved in a dilute solution of magnesium sulphate, and the globulin isolated by the process described in Chapter III.

The average of three analyses gave the following formula

$$C_{523}H_{761}N_{196}SO_{140}$$

for α–achroglobin.

This globulin exists in two conditions, oxygenated and deoxygenated, but in both conditions it is colourless; hence the reason that it was called achroglobin (from ἄχροος, *colourless*), and as other colourless globulins possessing a respiratory function have been discovered, the prefix α is used to distinguish it from the others.

By using the vacuum of a mercurial air-pump it was estimated that 1 gramme of this globulin absorbs 1·32 c.c. of oxygen and 3·15 c.c. of carbonic acid at 0° C. and 760 mm. pressure. When dissolved in a dilute solution of magnesium sulphate it was ascertained that α–achroglobin has a specific rotation for yellow light $[\alpha]_D = -48°$.

This new globulin absorbs oxygen in the

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[*] Concerning the physiology of *Patella*, see papers by A. B. Griffiths, *Proc. Roy. Soc.* Lond., vol. xlii. p. 393; vol. xliv. p. 327.

respiratory organs (branchiæ), the oxygen being conveyed by the blood to the tissues, &c., of the animal. In addition to this mode of carrying oxygen within the system, it may be stated that tissue-respiration is well developed in the mollusca. Histohæmatins, and even hæmoglobin, are present in various parts of the bodies of these animals. MacMunn states that myohæmatin (Fig. 2, spectrum 5) is the pigment of muscle, and the histohæmatins are the colouring matters of the tissues and organs; both may be reinforced or replaced at times by hæmoglobin when extra activity of internal respiration is required; probably the same radicle may be made use of for building up these pigments, for they seem to be related, since the same decomposition product, hæmatoporphyrin (Fig. 2, spectra 6 and 7) is probably yielded by all of them. In the words of the late Professor A. Würtz: "Les organes des animaux sont constitués par d'innombrables substances qui se forment et se modifient par les procédés de la vie, et auxquelles M. Chevreul a donné le nom de *principes immediats.*"

CHAPTER VIII.

β-ACHROGLOBIN.

THE blood of the Chitons is a yellow liquid, but the pigment is a lipochrome, and has no respiratory function. The blood of these animals also contains a respiratory globulin. This globulin, like the one described in the last chapter, does not contain a metal in its molecule. It is a colourless substance, which possesses the properties of oxygenation and deoxygenation. This respiratory proteid was isolated* by the method described in Chapter III., and its approximate composition is represented by the formula

$$C_{621}H_{814}N_{175}SO_{169}.$$

It is a carrier of oxygen within the system. One gramme of this globulin absorbs 1·2 c.c. of oxygen and 2·81 c.c. of carbonic acid at 0° C.

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[*] Griffiths, *Comptes Rendus de l'Académie des Sciences*, Paris, t. cxv. p 474; *Chem. News*, vol. lvi. p. 208; *Journ. Chem. Soc.*, vol. lxiv. p. 60.

and 760 mm. pressure. It has a specific rotation for yellow light $[\alpha]_D = -55^\circ$.

The oxygenated compound (oxy-β-achroglobin) which is formed in the branchiæ is carried by the circulating blood to the different organs and tissues, where the gas is absorbed or retained.

CHAPTER IX.

γ-ACHROGLOBIN.

The globulin to be described was discovered in the blood of the Tunicata.* The blood consists of a clear plasma containing colourless corpuscles. The globulin present in the blood does not contain a metal in its molecule, although it possesses the properties of oxygenation and deoxygenation. It is a colourless substance, and was isolated by the method described in Chapter III. The achroglobins are soluble in alkaline carbonates and phosphates, and they are precipitated by an excess of either magnesium sulphate or sodium chloride.

The average of four analyses gave the following formula

$$C_{721}H_{915}N_{194}SO_{183}$$

for γ-achroglobin.

This globulin is the carrier of oxygen within

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* Griffiths, *Comptes Rendus de l'Académie des Sciences*, Paris, t. cxv. p. 738; *Journ. Chem. Soc.*, vol. lxiv. p. 236.

the system, and it is, therefore, a true respiratory proteid. One gramme of it absorbs 1·49 c.c. of oxygen at 0° C. and 760 mm. pressure, and it has a specific rotation for yellow light $[\alpha]_D = -63^\circ$.

It may be mentioned that in certain Tunicates (viz., the Ascidians) the function of circulation differs entirely from other invertebrates. The peculiarity of this circulation is the *reversal* at regular intervals of the direction of the blood current. The heart is devoid of valves, and contracts with a wave-like movement. If the wave is from below upwards, "the blood passes into an abdominal vessel, thence into transverse ascending canals that lead to the extraordinary network of vessels connected with the respiratory structures, into a dorsal vessel, and thence by a connecting branch to the posterior end of the heart. After a certain period, the wave of contraction through the heart, and the course of the blood, are generally reversed in direction, and the blood now flows from the ventral heart into the dorsal vessel, down through the branching network into the abdominal or ventral vessel, and so to the anterior end of the heart." In the ascidians the blood is colourless and contains colourless corpuscles and a respiratory proteid. In *Appendicularia flabellum*, Huxley states that there are no corpuscles, and "the direction of the pulsations of the heart is not reversed at intervals, as it is in the ascidians in general. Fol, however, states, that, in other *Appendiculariæ* the reversal of the contractions of the heart takes place. . . . . There are no distinct vessels, but the colourless fluid, which takes the place of blood, makes its way through the interspaces between the ectoderm and endoderm and the various viscera."

The ash of the whole body of an ascidian yielded the following results on analysis:

Weight of ash taken.			
5·36 grms.	Weight of $Mg_2P_2O_7$ obtained 0·45 grm.	= P_2O_5 .	5·37
5·36 „	„ $BaSO_4$ „ 0·025 „	= SO_3 .	0·16
7·43 „	„ CaO „ 0·065 „	= CaO .	0·87
7·43 „	12·8 c.c. $\frac{N}{20}$ $K_2Cr_2O_7$ used	= Fe_2O_3	4·13
7·43 „	Precipitate obtained with NH_4HO weighed 1·15 grm. 1·15 − 4·13 % Fe_2O_3 − 5·37% P_2O_5	= Al_2O_3.	7·32
7·43 „	Weight of MgO obtained 0·01 grm.	= MgO .	0·13
7·43 „	„ K_2PtCl_6 „ 0·03 „	= K_2O .	0·077
0·18 „	„ NaCl „ 0·005 „	= Na_2O .	1·53
9·88 „	0·45 c.c. $\frac{N}{10}$ $AgNO_3$ used	= Cl .	0·016
Silica (by difference)		= SiO_2 .	80·39
			100·000

The body of an ascidian yielded 2·98 per cent. of ash or mineral matter.

CHAPTER X.

δ-ACHROGLOBIN.

The study of respiratory proteids is of much importance, and their discovery has thrown much light on the respiratory processes in animals. The narrow view, that all or nearly all the pigments of the vertebrates are directly or indirectly formed from hæmoglobin, held by many human physiologists up to very recent times, is shown to be erroneous by a study of the chromatology, &c., of the invertebrates. Thus, there are other substances which are of as great importance to many invertebrates as hæmoglobin is to the higher forms of animal life; and these substances (coloured or colourless) certainly are not metabolites of hæmoglobin. The proof of the existence of these respiratory proteids no longer rests on the observation of their spectra, as several of them have been isolated and their approximate compositions ascertained by means of the combustion furnace and other methods of chemical analysis. These respiratory proteids are as important as hæmoglobin, if not more so in some animals, and some of them have the right of priority in time, as they were developed at an earlier period than hæmoglobin, speaking from a phylogentic point of view.

Considering the present defective state of our knowledge of the respiratory proteids as chemical compounds or units, the researches recorded in Chapters II. to X. may be of some assistance to those biological chemists who care to investigate the subject for themselves. It is a misfortune there are so few English chemists who undertake researches in *biological* chemistry, although there are vast regions yet unexplored. But biological chemistry is not the fashion in England, and it does not pay! It is outside her schools; hence some of the reasons that in this country little progress has been made in the subject.

Having prefaced the present chapter with certain incidental remarks, we have now to describe the respiratory proteid which has been termed δ-achroglobin.* This proteid occurs in

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* Griffiths, *Comptes Rendus de l'Académie des Sciences*, Paris, t. cxvi. p. 1206; *Journ. Chem. Soc.*, vol. lxiv. p. 615; *Chem. News*, vol. lxvii. p. 288.

the blood of several species of *Doris*, and its approximate composition is represented by the formula

$$C_{659}H_{792}N_{165}SO_{153}.$$

This globulin does not contain a metal in its molecule. It is a colourless substance, which possesses the properties of oxygenation and deoxygenation—in other words, it is the carrier of oxygen within the system. One gramme of δ-achroglobin absorbs 1·25 c.c. of oxygen at the normal temperature and pressure. It has a specific rotation for yellow light $[\alpha]_D = -54^\circ$. This respiratory proteid combines with methane, acetylene, and ethylene:

$CH_4 - \delta$-achroglobin is a yellowish compound.
C_2H_2 „ „ greenish „
C_2H_4 „ „ brownish „

These compounds are dissociated in a vacuum.

CHAPTER XI.

CHLOROPHYLL.

It has been shown in Chapters VII. to X. that there are *colourless* respiratory proteids which prove that oxygenation and deoxygenation are not dependent upon a coloured or even a metallic basis. Hæmoglobin in the blood of the higher animals, and chlorophyll in the cells of most plants, are coloured bodies, but the mere fact of being coloured does not, as far as we know, aid their respective physiological functions. Nevertheless, the bodies to be described in the present and next chapters are coloured bodies and play important parts in the vegetable and animal kingdoms. It may be stated, *en passant*, that the greater number of organic compounds which mostly contain hydrogen and oxygen, and frequently nitrogen, as well as the indispensable carbon within the molecule, are colourless; but, on the other hand, carbon frequently forms with the same elements compounds that far excel those of other elements in the character and intensity of their colour. As such coloured carbon compounds in many instances do not differ in percentage composition from other bodies that are colourless, it may be safely inferred that this feature of coloration or non-coloration is intimately connected with the arrangement of the atoms within the molecule. Coloured carbon compounds are met with in the life history of animals and plants, and several have been known from ancient times; yet little is known concerning the fundamental cause of the coloration of carbon compounds, but sufficient information has been accumulated to allow some general conclusions to be drawn concerning the connection between colour and chemical structure. Reviewing the numerous organic compounds, it will be observed that the compounds of carbon with one element or with several elements of similar nature are, a sa rule, colourless. For instance, few coloured hydrocarbons[*] are known, and the substitution products of the hydrocarbons by any one mono-

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[*] Graebe (*Berichte*, 1893, p. 2354) states that acenaphthylene ($C_{12}H_8$) is a *yellow* hydrocarbon, and further that its colour (and also that of the red hydrocarbon ($C_{26}H_{16}$) from fluorene) is due to the presence of the group $> C : C <$.

valent element are, with the exception of a few iodine derivatives, colourless. Coloration is first occasioned by the entrance of certain compound—frequently polyvalent—radicles into the molecule, and the presence of *several atoms of carbon* in the molecule is also a necessary condition for the production of a true pigment. Owing to the latter requirement nearly all the organic pigments (with known *constitutional* formulæ) belong to the aromatic series or contain the benzene nucleus, and it is possible that both chlorophyll and hæmoglobin belong to the same series. At any rate, a knowledge of the facts mentioned in the present chapter may help biological chemists to ascertain the constitutional formulæ of chlorophyll (or "chlorophylls"), hæmoglobin, and other vegetable and animal pigments.

The radicles that are capable of the production of a coloured substitution product of a hydrocarbon also show a characteristic behaviour towards "nascent" hydrogen, in that coloured carbon compounds are converted by this reagent with more or less facility into colourless bodies. The reaction that occasions this change of optical behaviour is not, however, always the same. For instance, the nitro (NO_2) group is reduced to the amido (NH_2) group, which latter can be reconverted into the nitro group by oxidation. Compounds containing an azo ($-N=N-$) group in the molecule are frequently decomposed under the same conditions, the azo group being reduced to two amido groups; but intermediate products containing the hydrazo ($-HN-NH-$) group have also been isolated, and these bodies may be regarded as prototypes of a series of colourless substances termed leuco-bodies. A large number of pigments are converted by reduction into such leuco-bodies, which generally contain two more hydrogen atoms in the molecule than the pigment, while the coloured products can be regenerated from them by oxidation.

A knowledge of these facts induced Witt to formulate a theory concerning the nature of pigments (organic), which is partly summarised in the following sentences. The coloration of a body is due to the presence within the molecule of a certain group, the colour-giving group or chromophore, and by the introduction of the chromophore a more or less coloured body, the chromogene, is obtained. It may be that chlorophyll and hæmoglobin* are formed by the

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* It is well known that hæmoglobin is a complex derivative of pyrrol; and Schunck and Marchlewski (*Proc. Roy. Soc.*, vol. lix. p. 234) have recently shown that chlorophyll can be made to yield pyrrol (C_4H_4 NH). It is also well known that indigo (from different

introduction of chromophores (containing iron) into albuminoid molecules. Witt maintains that the coloration of carbon compounds is due to the presence of a heterogeneous ring in the molecule, though it must be admitted that the existence of such a ring in many coloured organic bodies cannot at present be understood. It may be mentioned that the "quinonoid" hypothesis of Dr. Armstrong* is, so far, the only satisfactory attempt to formulate a theory of chromogenesis. In fact, among complex carbon compounds colour is conditioned by a quinonoid structure. "A quinonoid compound may be defined as a *hexaphene*—*i.e.*, an unsaturated cycloid composed of six 'elements,' two 'elements' of which are $\mathrm{C}\overset{\frown}{\smile}\mathrm{R}''$ groups in either para- or ortho-positions. Coloured substances generally appear to fall within this definition," and a change of colour is indicative of a change of structure.

From what has already been said it may be gathered that in order to arrive at the constitutional formulæ of such complex molecules

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plants) is a derivative of indol, the pyrrol of the aromatic or benzene series; and that indol ($C_6H_4\ [CH]_2\ NH$) may be obtained by the pancreatic decomposition of *albuminoids*, and by the fusion of the latter with potassium hydroxide.

* Armstrong, *Proc. Chem. Soc.*, 1888, p. 27; 1892, p. 101, p. 189; 1893, p. 52, p. 206.

as phyllocyanin, phylloxanthin, hæmoglobin, hæmocyanin, &c., it will be necessary to study their reactions, at the same time bearing in mind Armstrong's far-reaching theory of coloration.

Chlorophyll is the green substance in nearly all plants and in some of the lower animals. In several of the lower plants it is diffused through the protoplasm (*e.g.*, *Protococcus*), but in the higher plants it is located in special granules (chloroplastids).*

Chlorophyll does not appear to be a chemical unit, as Stokes, Sorby, Fremy, Hoppe-Seyler, and others have decomposed it into at least two pigments—phylloxanthin (yellow) and phyllocyanin (blue). Pure chlorophyll, however—*i.e.*, the substance which has been extracted by alcohol from the green parts of plants, has not yet been isolated. The pure, so-called crystallised chlorophyll, described in chemical literature, consists probably of *derivatives* of true chlorophyll.

Foremost among such derivatives stands *chlorophyll* as obtained by the action of weak,

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* The same remark applies to hæmoglobin. In most of the lower animals (*e.g.*, *Lumbricus*) it is diffused through the plasma of the blood, but in the higher animals it is located in the red blood corpuscles.

especially organic, acids upon chlorophyll or leaf-green. By treatment with stronger acids it is transformed into phylloxanthin and phyllocyanin. Phyllocyanin by treatment with alkalies or strong acids is converted into the phyllotaonin of Schunck, which is one of the most beautiful derivatives of chlorophyll or leaf-green.

Leaf-green or unmodified chlorophyll may be transformed by alkalies into alkachlorophyll, which latter compound on treatment with acids in the presence of alcohol, yields an alkylether of phyllotaonin; and on heating phyllotaonin with alkalies at a high temperature we obtain phylloporphyrin. Such is an outline of Schunck's important researches on leaf-green, which we have now to consider more in detail.

Phyllocyanin.

Schunck has obtained several compounds of phyllocyanin (one of the acid derivatives of leaf-green), among these being phyllocyanin zinc acetate, phyllocyanin cupric acetate, phyllocyanin ferrous palmitate, phyllocyanin zinc carbonate, phyllocyanin ferrous malate, and a combination between phyllocyanin, tin, and hydrochloric acid. The colour of the last compound resembles in some respects that contained in the petals of red flowers. The copper compound of phyllocyanin is a very stable substance, and, on analysis, yielded results which correspond to the formula

$$C_{67}H_{71}N_5Cu_2O_7.$$

Phyllocyanin has an indigo-blue colour.

Phylloxanthin.

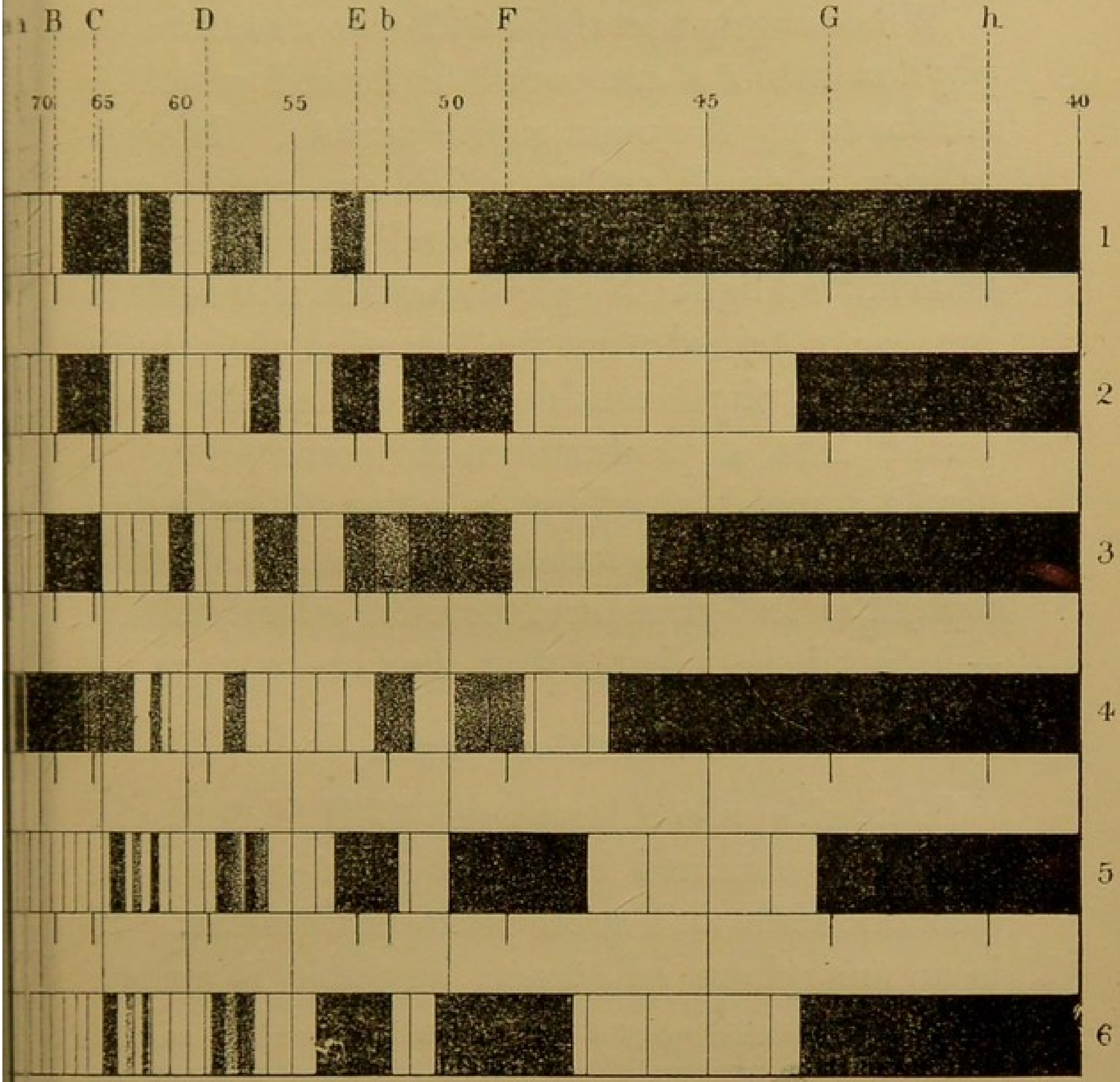
Phylloxanthin is the other acid derivative of leaf-green; and it is difficult to prepare it in a state of purity. It is soluble in ether—the phyllocyanin passing into the lower acid layer of the decomposed leaf-green. After evaporating off the ether the phylloxanthin is first dissolved in a small quantity of chloroform, and the solution mixed with an excess of alcohol; on standing the phylloxanthin is deposited (fatty matter remaining in solution). The deposit is washed, then dissolved in boiling glacial acetic acid. The solution on cooling and standing yields a deposit of phylloxanthin; which is dissolved in hot ether. The ethereal solution is evaporated, and the process repeated several times. The product ultimately obtained is not absolutely pure as it still contains fatty matter.

The properties of phylloxanthin resemble those of phyllocyanin, and it is stated that they may be isomers.

Phylloxanthin is a dark green amorphous substance, although under the microscope it may be seen to resolve itself into pseudo-crystalline needles. It is soluble in boiling absolute alcohol; but is more soluble in ether, carbon disulphide, benzene, and aniline than in alcohol. Its best solvent is chloroform. Its solutions are less distinctly green and more brown than those of phyllocyanin; they are fluorescent, and when dilute exhibit a reddish tinge, of which nothing is seen in the case of phyllocyanin. The spectra of phylloxanthin and phyllocyanin are given in Fig. 3.

Phylloxanthin, like phyllocyanin, may be heated for several hours at 130°C. without undergoing any change, but at 160°C. decomposition commences and at 180°C. the substance is completely decomposed. When incinerated in a platinum dish, phylloxanthin leaves an ash which consists of ferric oxide. Schunck has proved that phylloxanthin always leaves an ash of ferric oxide after incineration. This appears to prove that iron in some form or other is an essential constituent of chlorophyll or leaf-green, and that the strong acids used in the prepara-

FIG. 3.



SPECTRA OF CHLOROPHYLL, ETC.

1. Chlorophyll (in alcohol). 2. Phyllocyanin (in ether).
3. Phylloxanthin (in ether). 4. Alkachlorophyll (in ether).
5. Phylloporphyrin (in ether). 6. Hæmatoporphyrin (in ether).

tion of phylloxanthin do not interfere with the iron which is in organic combination.*

When cupric acetate is added to a solution of phylloxanthin a compound is formed which is similar to phyllocyanin cupric acetate. It crystallises in small scales, which are purple and lustrous by reflected light and pale green by transmitted light. No phylloxanthin zinc acetate has been obtained; but by the action of alcoholic potash on phylloxanthin it is ultimately converted into a product which corresponds to phyllotaonin obtained by the action of alkalies on phyllocyanin. The two acid derivatives of chlorophyll or leaf-green are, therefore, closely allied—in fact it is probable that they are isomers.

Alkachlorophyll.

Schunck's alkachlorophyll ($C_{52}H_{57}N_7O_7$) is a substance obtained by treating leaf-green with alcoholic soda and subsequent purification. It is an amorphous substance, insoluble in water,

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* Cells rich in chlorophyll always contain an appreciable amount of iron, and the presence of iron (soluble) in a soil increases the amount of chlorophyll in the leaves of those plants grown in such soil; see papers by A. B. Griffiths in *Journ. Chem. Soc.*, 1883, p. 195; 1885, p. 54; and Molisch's book *Die Pflanze in ihren beziehungen zum Eisen* (1892) contains valuable information on the subject. Whether iron enters into the complex molecules of chlorophyll and its derivations or not, we must admit in the words of Boussingault that "le fer parait tout aussi indispensable à la vie végétale qu'à la vie animale."

soluble in alcohol, ether, chloroform, benzene, aniline and carbon disulphide. Its solutions have a green colour with a bluish tinge, and its spectrum is given in Fig. 3. By the action of acids on alkachlorophyll, phyllotaonin is formed thus:

$$\underset{\text{[alkachlorophyll]}}{C_{52}H_{57}N_7O_7} + H_2O = \underset{\text{[phyllotaonin]}}{C_{40}H_{40}N_6O_6} + \underset{\text{[x]}}{C_{12}H_{19}NO_2}$$

Phyllotaonin.

Phyllotaonin ($C_{40}H_{40}N_6O_6$) is obtained by the action of alkalies on phyllocyanin, and by the action of acids on alkachlorophyll. It is obtained in flattened crystals of a fine peacock or steel-blue colour. Phyllotaonin melts at 184° C. and on ignition leaves no ash. It is soluble in boiling alcohol and ether; and its solutions have the same colour and show the same absorption-bands as solutions of phyllocyanin. It is also soluble in benzene, carbon disulphide, chloroform and aniline, but insoluble in ligroin.

Schunck has obtained certain compounds of phyllotaonin, viz.: methylphyllotaonin, ethylphyllotaonin, and acetylphyllotaonin. When ethylphyllotaonin is distilled with zinc dust, an oil is obtained which answers to the tests of pyrrol.

Phylloporphyrin.

Phylloporphyrin ($C_{32}H_{34}N_4O_2$) is prepared by the action of alcoholic potash on phyllotaonin in sealed tubes. It forms lustrous crystals of a reddish-violet colour, which are soluble in acids, alcohol and ether. Phylloporphyrin possesses also acid properties though these are not very pronounced. It is insoluble in aqueous alkalies. Schunck and Marchlewski have shown that by the prolonged action of hydrochloric acid gas on phylloxanthin the latter is converted into phyllocyanin. This fact tends to support the view held by some observers that only one chlorophyll really exists, and that there are not two or more chlorophylls, as has sometimes been maintained. "Under the action of acids, chlorophyll yields phylloxanthin, which is then converted into phyllocyanin. The latter when treated with alkalies gives phyllotaonin, and this, by the more energetic action of alkalies at a higher temperature, yields phylloporphyrin."

Nencki and Sieber have shown that the anhydride of hæmatoporphyrin (a derivative of hæmoglobin) possesses the formula $C_{32}H_{34}N_4O_5$; and this formula differs from that of phylloporphyrin by only three atoms more of oxygen. Their physical properties, spectra (see Fig. 3), colour of their solutions, etc., are almost alike; and it may be stated, *en passant*, that both hæmoglobin and chlorophyll are derivatives of pyrrol (C_4H_4NH). "That the two series of derivatives, that of chlorophyll and that of hæmoglobin, each playing an important part in the vegetable and animal organism respectively should so nearly touch at one point is certainly a most remarkable circumstance,"* but what the significance of this may be it is difficult to say in the present state of our knowledge of the subject.

Animal Chlorophyll.

The green pigments present in many animals have been described as chlorophyll; but there is little doubt that in some cases the pigment is not chlorophyll. In *Bonellia viridis* (one of the Gephyrea) the green colour is due to a pigment named bonellein; and in *Æolosoma tenebrarum*† the green pigment (æolosomin) behaves with acids and alkalies entirely different from chlorophyll. The green pigment of *Phyllodoce viridis*

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* For further information see Schunck's papers in the *Annals of Botany*, vol. iii. p. 65; *Proc. Roy. Soc.*, vol. xlii. p. 184; vol. xliv. p. 448; vol. l. p. 302; vol. lv. p. 351; vol. lvii. p. 314; vol. lix. p. 233; *Annalen*, vol. cclxxxviii. p. 209; *Berichte*, vol. xxix. p. 1347.

† Beddard, *Proc. Zool. Soc.*, 1889, p. 51, states that æolosomin is respiratory and may be capable of oxygenation and deoxygenation.

is also not chlorophyll, The pigment present in *Pontobdella* bears a remote resemblance to chlorophyll. There are some cases, however, where chlorophyll is present in and formed by the animal itself. Ray Lankester[*] has proved that the green pigment of *Spongilla fluviatilis* and *Hydra viridis* is due to chlorophyll present in the cells, and not to parasitic algæ; and MacMunn's[†] enterochlorophyll is formed in the so-called livers of many invertebrates. Enterochlorophyll gives a spectrum and reactions similar to those of plant chlorophyll.

Poulton[‡] has examined, by means of the spectroscope, the blood of lepidopterous larvæ and pupæ, and has proved that the presence of chlorophyll or modified chlorophyll, which he maintains is derived from the food, and is used as a protective colour.

MacMunn[§] found true chlorophyll in seventeen marine sponges, and states that it is built up synthetically by them. It has a respiratory function, but the union between oxygen and chlorophyll cannot be a loose combination, as it is in the case of hæmoglobin (and other respira-

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[*] Ray Lankester, *Quart. Journ. Micros. Sci.*, vol. xxii. p. 229.

[†] MacMunn, *Proc. Roy. Soc.*, vol. xxxv. p. 370.

[‡] Poulton, *Proc. Roy. Soc.*, vol. xxxviii. p. 283; and *Colours of Animals.*

[§] MacMunn, *Journ. Physiol.*, vol. lx.

tory proteids) the oxygen must enter into an intra-molecular combination with the chlorophyll, if at all, as the action of reducing agents teaches this fact.

Animal chlorophyll has also been found in a diffused state in certain *Infusoria*, and they appear to have formed it from its elements.

Becquerel and Brongniart* extracted a green pigment from the *Phyllia* (belonging to the Orthoptera) which has all the properties of plant chlorophyll ; but there are green pigments present in certain insects which have nothing in common with chlorophyll.†

The chief function of animal chlorophyll and allied pigments is that of respiration ; but it is probable that these pigments play an important part in sexual selection, in mimicry, or act as "screens" for the protection of underlying cells, for protective purposes. Whatever may be the true function or functions of animal chlorophyll one thing is certain—that the pigment is manufactured in the body of the animal containing it. In fact "investigators should pause before they decide that when an animal chlorophyll is met with, it has been eaten by the animal and deposited unchanged in its tissues ; they should

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* Becquel and Brongniart, *Comptes Rendus*, tome cxviii. No. 24.

† Griffiths, *Comptes Rendus*, tome cxv. p. 958.

remember that the radicle of chlorophyll, like the radicles of other pigments, may be furnished by the action of the digestive juices of the animal or some substance furnished by the plant, and that *the animal laboratory is capable of building up molecules quite as large as that of chlorophyll.* Our own hæmoglobin is not the unchanged hæmoglobin of our food; what is derived from it is broken up and then regenerated, and it shows an ignorance of physiology to suppose that chlorophyll should be an exception to a general rule" (MacMunn).

Gautier's Chlorophyll.

Gautier* obtained crystallised chlorophyll from spinach by using neutral solvents, such as alcohol and ether along with animal charcoal. The formula given by Gautier for his chlorophyll is $C_{40}H_{64}N_2O_4$; and Hoppe-Seyler's chlorophyllan derived from grasses is represented by the formula $C_{30}H_{46}N_2O_3$. These formulæ show that there may be several chlorophylls, as there are many chromophylls (chromoplastids). In fact Gautier says, "en admettant que chez toutes les dicotylédonées la chlorophylle soit identique à celle des chénopodées, et chez toutes les mono-

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* Gautier, *Comptes Rendus*, tome lxxix. p. 861; *Chimie Biologique*, p. 19.

cotylédonées à celle des graminées de nos prairies, on voit donc qu'il existe déjà deux chlorophylles ; celle des monocotylédonées diffère de celle des dicotylédonées par $C_{10}H_{18}O$ en moins, c'est-à-dire par les éléments d'un bornéol. A son tour la chlorophylle des acotylédonées est fort différente de celles des végétaux phanérogames, tout en jouissant de leurs propriétés générales."

Etard* also states that there are several chlorophylls, and he gives to each of these pigments isolated chemically a name derived from the botanical name of the plant. Lucern (*Medicago sativa*) contains among other chlorophylls, α-medicagophyll ($C_{28}H_{45}NO_4$) and β-medicagophyll ($C_{42}H_{63}NO_4$). Certain chlorophylls soluble in pentane are the agents of the chemical production of essences and oils ; while others, insoluble in the hydrocarbons, tend on splitting up, to produce carbohydrates, tannins and extracts.†

There are certain remarks concerning chlorophyll and hæmoglobin which appropriately form a conclusion to the present chapter. Chlorophyll and hæmoglobin are complex substances,

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* Etard, *Comptes Rendus de l'Académie des Sciences*, Paris, tome cxx.

† Schunck and Marchlewski (*Liebig's Annalen der Chemie*, vol. cclxxxviii. p. 209) do not accept Etard's observations and remarks.

and they both exercise an elective action on a mineral gas (CO_2 or O_2). They both are fashioned, as a rule, into globules. Hæmoglobin absorbs oxygen*, and chlorophyll absorbs carbon (in some form or other) as well as oxygen. Chlorophyll and hæmoglobin equally appear to form only a temporary association with the mineral element which with special avidity they seek. Respiration (or absorption of oxygen) is essential to life;† and it is indispensable even to the chlorophyll cells, which became incapable of reducing the carbon dioxide of the air when they lack oxygen, and this is the reason why they are asphyxiated in an atmosphere of pure carbon dioxide, hydrogen or nitrogen.

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* It is not generally known that Voltaire was on the verge of anticipating Priestley in the discovery of oxygen (1774), as he came to the conclusion that the increased weight of iron after being heated in air was due to its absorption of something in the air: "Il est très possible que cette augmentation de poids soit venue de la matière répandue dans l'atmosphere" (see Voltaire's paper in *Recueil des Pièces qui ont remporté les Prix de l'Académie royale des Sciences*, Paris, 1752, tome iv. p. 171).

† There are a few microbes (anaërobic) which appear to live without *free* oxygen (See Griffiths, *Manual of Bacteriology* and *Researches on Micro-Organisms*).

CHAPTER XII.

HÆMOGLOBIN.

“Our whole life is based on iron.”—J. A. THOMSON.

HÆMOGLOBIN is the crystalline, indiffusible substance present in the blood of the higher, and some of the lower, animals. The metal iron is always present in the molecule of this respiratory proteid. The percentage composition of it, according to Preyer and others, is the following:

Carbon	54·2
Hydrogen	7·2
Nitrogen	16·0
Oxygen	21·5
Sulphur	0·7
Iron	0·4
	100·0

and the formula $C_{600}H_{960}N_{154}FeS_3O_{179}$. This formula gives the molecular weight of hæmoglobin as 13332; and as one molecule of hæmoglobin requires three molecules of soda to form a non-coagulable combination, therefore

$$13332 \div 3 = 4444,$$

or the equivalent weight of hæmoglobin.

Hæmoglobin is widely distributed in the animal kindom. The table on p. 95 gives the names of the animals whose blood contains it.

Hæmoglobin is also present in certain muscles (both of vertebrates and invertebrates), and in the nerve cells of *Aphrodite.*

Hæmoglobin can be separated from the corpuscles (of the higher animals) by the passage through it first of a current of oxygen and then of carbon dioxide, or by the addition of water to the blood, freezing and subsequent thawing several times repeated. Ether, ammonia followed by hydrochloric acid, and solutions of certain salts, are also means of separating hæmoglobin from blood.

Hæmoglobin is capable of existing in two states of oxidation, as oxyhæmoglobin and as hæmoglobin or reduced hæmoglobin. These two are distinguished by their colour and their absorption spectra (see Fig. 2). By means of oxidising and reducing agents the one compound can readily be converted into the other. Hæmoglobin is soluble in cold water, but not in hot. It is insoluble in

Vertebrata.	Mollusca.	Crustacea,	Insecta.	Annelida.	Echinodermata.
All except— Amphioxus and Leptocephalus	Arca Planorbis Solen Cardita	Daphnia Apus Cyprus Cheirocephalus Lernanthropus Clavella	Musca Chironomus	Lumbricus Hirudo Lumbriculus Limnodrilus Eunice Cirrhatulus Nais Terebella Glycera Nereis Capitella Chætogaster Tubifex Arenicola Enchytrachus Aphrodite Phoronis Hamingia Polia Nephelis Hæmophsis	Ophiuridea (*some*) Holothuridea (*some*)

absolute alcohol, chloroform, and benzene. Its solutions do not diffuse through vegetable parchment—in fact, molecules of hæmoglobin, like others mentioned in this work, are too big to get through the pores of a membrane.*

Hæmoglobin plays the part of a weak acid, its aqueous solutions having a feeble acid reaction, and accordingly its very dilute alkaline solutions possess considerable stability. It readily absorbs oxygen; one gramme of hæmoglobin absorbs 1·27 cc. of oxygen at the normal temperature and pressure. Carbon monoxide and nitric and nitrous oxides displace the oxygen in oxyhæmoglobin, the solutions acquiring a bluish tint. Hæmoglobin solutions are precipitated by alcohol, acetic and mineral acids, mercuric nitrate, silver nitrate, mercuric chloride, and potassium ferrocyanide. It likewise gives the protein reactions. Acids, as well as alcohol, while precipitating also decompose it. Although hæmoglobin is a colloidal substance it crystallises, but its readiness of crystallisation differs considerably in different animals.† These crystals belong mostly to the rhombic system, forming long four-sided prisms

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* Traube, *Centralblatt für d. med. Wissenschaften*, 1866, p. 97.

† See *Dictionnaire de Wurtz*, and Gautier's *Chimie Biologique*, p. 384.

in dogs, tetrahedra in guinea-pigs, rhombic tables and prisms in man and the horse.

Derivatives of Hæmoglobin.

Oxyhæmoglobin is decomposed by dilute acids or alkalies, or by the action of heat, into a proteid or *globulin*, and a ferruginous pigment, *hæmatin*, of which about 4 per cent. is obtained for about 96 per cent. of the globulin.

Hæmatin ($C_{68}H_{70}N_8Fe_2O_{10}$) is best prepared by mixing defibrinated blood with a strong solution of potassium carbonate, until the liquid adhering to the separated coagulum becomes colourless. The coagulum is then dried at 50° C., and digested for some days in absolute alcohol; the alcoholic solution after concentration, deposits hæmatin in the form of rhombic crystals. It is of a bluish-black colour with a metallic lustre; and when burnt leaves pure ferric oxide behind. It is insoluble in water, alcohol, ether, and chloroform, but easily soluble in the alkalies and alkaline carbonates. It is with difficulty soluble in acetic and mineral acids, and hydrochloric acid appears to be the only acid which dissolves it without its iron separating. When treated with strong sulphuric acid hæmatin undergoes decomposition according to the following equation:

$$C_{68}H_{70}N_8Fe_2O_{10} + 2H_2SO_4 + O_2 = C_{68}H_{74}N_8O_{12} + 2FeSO_4,$$

in other words, hæmatoporphyrin and iron sulphate are formed.

Hæmin ($C_{68}H_{70}N_8Fe_2O_{10}2HCl$) is hæmatin hydrochloride, and may be prepared by rubbing up a small quantity of blood with sodium chloride, then boiling for a few minutes with glacial acetic acid, and the mixture evaporated to dryness. Among the colourless crystals of sodium chloride and sodium acetate, are rhombic plates and prisms of hæmin, which are of a bluish-red colour by reflected, and brownish-red by transmitted, light. Hæmin is insoluble in water, alcohol, and ether, but is soluble in alkaline solutions.

Hæmochromogen ($C_{34}H_{37}N_4FeO_5$) is a substance obtained by reducing a solution of hæmoglobin by hydrogen and then treating with sulphuric acid. On oxidation hæmochromogen yields hæmatin :

$$2C_{34}H_{37}N_4FeO_5 + O_2 = C_{68}H_{70}N_8Fe_2O_{10} + 2H_2O.$$

Hoppe-Seyler, who first discovered hæmochromogen, has obtained it in the form of crystals.

Hæmatoporphyrin ($C_{68}H_{74}N_8O_{12}$) is prepared by heating hæmatin with strong hydrochloric acid or sulphuric acid. The spectra of hæmatoporphyrin are given in Figs. 2 and 3. Hæmatoporphyrin is not only a derivative of hæmoglobin, as it occurs in the integument of several invertebrates, viz., starfishes, slugs, *Lumbricus*, and certain sponges.[*]

Methæmoglobin.—When a solution of hæmoglobin is exposed for long to the air, or to the action of oxidising agents (*e.g.*, potassium permanganate, sodium nitrite, amyl nitrite, &c.) in neutral or faintly alkaline solutions, it forms a yellowish-brown solution, and is converted into methæmoglobin. Methæmoglobin is soluble in water, but insoluble in alcohol and ether. It is decomposed into hæmatin and an albuminous substance by acids and alkalies.

Parahæmoglobin, of Nencki, is said to be a coagulation product of oxyhæmoglobin by Hoppe-Seyler. It is obtained by the action of alcohol on oxyhæmoglobin. Gautier believes that metahæmoglobin and parahæmoglobin are polymers of hæmoglobin.[†]

Copper-hæmoglobin ($C_{648}H_{1030}N_{178}FeCu_5S_2O_{177}$), or "copper hæmol," is a compound of copper and hæmoglobin, and was discovered by Merck.[‡] Copper appears to be a metal which readily

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[*] MacMunn, *Quart. Journ. Micros. Science*, 1877; *Journ. Physiol.*, vols. vii. and viii.

[†] Concerning the spectra of the derivatives of hæmoglobin, see Graebe's paper in *Zeitschrift für Anal. Chemie*, vol. xxxiii. p. 771

[‡] Merck, *Apoth. Zeit.*, April 17, 1895.

combines with albuminous molecules. Filehne[*] has recently obtained a true compound of copper and albumin.

Histohæmatins.

Allied to hæmoglobin is an important group of pigments which are termed the histohæmatins by MacMunn.[†] Myohæmatin is the histohæmatin characteristic of invertebrate muscle, and is the true intrinsic colouring matter of muscle, whereas the histohæmatins proper are the intrinsic colouring matters of the tissues and organs; both may be reinforced or replaced at times by hæmoglobin when extra activity of internal respiration is required; probably the same radicle may be made use of for building up all these pigments, for they seem to be related, since the same decomposition product —hæmatoporphyrin—is probably yielded by all of them. The histohæmatins are respiratory pigments, holding the oxygen brought to the tissues by the blood until it is required by the tissues.

Concluding Remarks.

Several formulæ have been given for hæmoglobin, among these are the following:

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[*] Filehne, *Deutsch. Med. Wochensch.*, 1896.

[†] MacMunn, *Philosophical Transactions* 1886.

$C_{600}H_{960}N_{154}FeS_3O_{179}$ (*Preyer*),
$C_{544}H_{823}N_{147}FeS_2O_{147}$ (*Kossel*),
$C_{636}H_{1025}N_{164}FeS_3O_{189}$ (*Hüfner*),
and
$C_{712}H_{1130}N_{214}FeS_2O_{245}$ (*Zinoffsky*),

and as readiness of crystallisation, solubilities, &c., differ, it has been suggested there is more than one hæmoglobin. Preyer has found that the absorption spectra of the hæmoglobin of *Chironomus*, *Cardita sulcata*, and *Lumbricus*, are identical with that of the vertebrata.* Notwithstanding this agreement in a fundamental property, the hæmoglobins differ from each other physically and chemically. Their solubility, crystallisability, and their proportion of crystalline water differ. Their quantitative composition, their coagulability, their liability to decompose differ according to the species. If species have been modified in the course of long eras, the hæmoglobins—though fulfilling all the conditions of a true chemical compound—must have become modified step by step with the species (in a morphological sense).† Hence

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* The spectroscopic properties, decomposition products, &c., of hæmoglobin are always the same.

† The researches of Landois (*Die Transfusion des Blutes*, 1875), on transfusion also support the theory that there is more than one hæmoglobin. He has carried out transfusion of blood between the remotest species. When the blood is made to flow directly from

we see that the conception of evolution must necessarily find a place in chemistry, not merely as regards the elements, but as regards the formation of highly complicated compounds.

* * * * *

In conclusion, it appears that there are several respiratory proteids in the blood of animals—four of which contain iron in their molecules, viz., echinochrome, hæmerythrin, chlorocruorin, and hæmoglobin; and chloroglobin or chlorophyll may also contain the same metal in its molecule. Hæmocyanin contains copper, pinnaglobin manganese; and there are four, and probably more, achroglobins whose molocules are devoid of metals. The metals in combination with the globulin molecules have fairly low atomic weights; in fact, all the elements of the living body have low atomic weights. None of them exceeds 63 (copper). The figures are (omitting decimals):—

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the vessels of one animal to the vessels of an animal of a different species, it is stated that in a few minutes the red corpuscles degenerate, and the *hæmoglobin*, becoming dissolved in the blood plasma, soon appears in the urine; but in the case of transfusion of similar blood between individuals of the same or closely-related species, the hæmoglobin does not appear in the urine. Ponfick (*Virchow's Archiv*, vol. lxii.) records similar investigations on the transfusion of blood.

Hydrogen	1	Phosphorus	31
Carbon	12	Sulphur	32
Nitrogen	14	Chlorine	35
Oxygen	16	Potassium	39
Fluorine	19	Calcium	40
Sodium	23	Manganese	55
Magnesium	24	Iron	56
Silicon	28	Copper	63

The specific gravities of these elements (in the solid state) are also remarkably low. If water is taken as 1, H=0·62; C=1·3; N=1·2; O=1·1; F=1; Na=0·97; Mg=1·7; Si=2; P=1·82; S=1·9; Cl=1·8; K=0·86; Ca=1·5; Mn=7·15; Fe=7·8; Cu=8·96. Hence only the last three named elements have higher specific gravities than 2. The elements of the living body have all a high specific heat, which taking that of water as 1, is included within the limits 0·10 and 0·46. That of hydrogen reaches 5·88, whilst all other elements have a specific heat of at most 0·10, and generally much lower down to 0·028. From the high specific heat, as Preyer has remarked, of all the elements of the living body, especially of hydrogen, it follows that all the essential constituents of the tissues of living organisms which are built up of such compounds must have a relatively high specific heat. This inference follows at once from their abundant proportion of water. The biological significance of this fact, as already remarked by Errare, must be sought herein, that living tissues when heat is conveyed to them easily indeed undergo an increase of intermolecular movements, but much less easily a rise of temperature than inorganic structures under similar conditions.

Besides the physical properties so important for life, the elements of the living body have further the chemical peculiarity of forming with each other *most numerous combinations and very large molecules;* and these combinations or compounds are, as a rule, easily soluble and very readily decomposable. The molecules of albumin (protoplasm) without which life is not conceivable, are very large and are easily decomposed. They are modified by the slightest influences.

Protoplasm is continually decomposing as long as it is alive. The supply of the material used up in its self-consumption is yielded by the elements in the assimilable compounds of the food. But we must not imagine that the dissimilation of the entire series of the katаplastic chemical process ensue in the same manner as the imitations of the metabolic processes attempted in the laboratory. However many components of living animals and plants we produce by artificial synthesis, we have not proved in a single case that the living body proceeds in the same manner. With decompositions the case is identical. How the organism forms the carbon dioxide which it evolves is unknown, and yet there is absolutely no life without the formation of carbon dioxide! The reason why this problem has not yet been found capable of solution lies doubtless in our insufficient knowledge of the nature of the place where the formation of carbon dioxide takes place. That the foci of oxidation lie exclusively in the protoplasm is certain, but what is their aspect?

As protoplasm has an extremely complicated structure, recognised only of late since the improvement of the microscope, in which the minute interstices and meshes, often at the very limit of visibility and not even permanent, but altering under the eyes of the observer, the question arises whether in so peculiar a locality chemical reactions can altogether take place in the ordinary manner. An examination of the conditions necessary for the completion of any chemical reaction has shown that one of the most important of these conditions, *the action of mass*, cannot be realised in living protoplasm on account of the smallness of the open spaces. The chemical equilibrium can here be reached only to a very restricted extent. But then the chemism of the living cell-contents, the chemism of protoplasm, *i.e.*, the inter-reaction of the very unstable compounds of the elements of the living body, must be another, and lead to other results than in test-glasses, retorts and combustion-furnaces. Even the extremely fine division of every minutest particle of nutriment which is burnt in millions and millions again of different places in the organism, and then especially the strikingly externally measurable average temperature of the focus of combustion, render it probable that in the narrow interstices of living protoplasm, *it is no longer the large molecules but the atoms which rush together in the moment of their liberation.* It is not the ordinary chemical reactions in which enormous numbers of molecules forming masses enter into action at the same place, but atomistic reactions which here come into play, single combats of atoms in the nascent state with strong unsatisfied affinities, and nowhere in an exactly identical manner, since the protoplasms, hæmoglobins, achroglobins, &c., like organisms, are individually different.

Thus the more accurate determination of the properties of the compounds of the living body, in connection with the scrutiny of the minutest structure of animal and vegetable protoplasm, promises to throw a clear light on the foundation of all life, the biochemical processes (Preyer).

APPENDIX.

I. PELAGINE.

Pelagine is the violet pigment of *Pelagia* (one of the Medusæ), and its chemical composition has been established* in the following way: By treatment with hot alcohol and ether the pigment and fats are dissolved and the solution, after filtration, is evaporated carefully to dryness. The residue is treated with a solution of sodium hydroxide and the pigment then rapidly extracted by means of carbon disulphide. Upon the spontaneous evaporation of the solvent, the violet pigment is obtained as an amorphous residue.

Analysis of this pigment gave the following results:

I.	Substance used	0·2058 gramme
	Carbon dioxide	0·47325 ,,
	Water	0·0810 ,,
II.	Substance used	0·4605 ,,
	Nitrogen	15·15 cc.
	Barometric pressure	742 mm.
	Temperature	15° C

Reduced to percentages, and calculating the formula the following results are obtained:

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* Griffiths and Platt, *Comptes Rendus de l'Académie des Sciences*, Paris, t. cxxi. p. 451 ; *Journ. American Chem. Soc.*, vol. xvii. p. 877.

	I.	II.	Calculated for $C_{20}H_{17}NO_7$.
Carbon	62·71	—	62·66
Hydrogen	4·38	—	4·43
Nitrogen	—	3·75	3·66
Oxygen	—	—	29·24

Analysis, then, would indicate the formula

$$C_{20}H_{17}NO_7.$$

Pelagine is soluble in alcohol, ether and acetic acid ; it is insoluble in water, but it is very soluble in carbon disulphide. Solutions of the pigment are decolorised by exposure to light and, on spectroscopic examination, do not yield characteristic absorption bands. It has, apparently, no respiratory function and probably belongs to the same order of pigments as tetronerythrin, rhodopsin, &c., in other words, pelagine is probably a lipochrome or a nitrogenous derivative of that class. In certain respects it may be said to resemble melaine, the pigment of *Sepia*, investigated by Girod, Variot, and Desfosses.*

II. PUPINE.

This substance has been extracted† from the skin of the pupæ or chrysalides of the following lepidoptera :

Pieris brassicæ, Latreille.
Pieris napi, Latreille.
Pieris rapæ, Latreille.
Plusia gamma, Linnæus.
Mamestra brassicæ, Linnæus.
Noctua pronuba, Linnæus.

by the following process : The skins are boiled for a long time in a solution of sodium hydroxide, and then cut into small pieces—the pieces being exhausted successively with acidulated and distilled water, alcohol and ether. The residue is dissolved in hydrochloric acid and precipitated by

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* Girod, Variot and Desfosses, *Comptes Rendus*, t. xciii. p. 97.

† Griffiths, *Comptes Rendus*, t. cxv. p. 320 ; *Bulletins de l'Académie Royale de Belgique*, 3ᵉ sér. t. xxiv. p. 592.

means of an excess of water, the operation being repeated several times.

Analysis of this animal substance gave results which assign to it the formula

$$C_{14}H_{20}N_2O_5.$$

Pupine is an amorphous and a colourless substance. It is soluble in mineral acids, insoluble in neutral solvents; but prolonged boiling in strong mineral acids decomposes it into leucine and carbon dioxide:

$$C_{14}H_{20}N_2O_5 + 3H_2O = 2C_6H_{13}NO_2 + 2CO_2.$$

It appears that pupine is secreted by the pores of the larvæ, after the change of the skin for the last time; and it is the principal substance in the skin of the pupæ of certain lepidoptera.

III. CUPREINE.

Cupreine is the brown pigment in the elytra of *Curculio cupreus*—the copper-coloured weevil, and its chemical composition has been established* in the following way: By treating the elytra with hot alcohol and ether the pigment is dissolved and the solution after filtration is evaporated to dryness. The residue is dissolved in alcohol, and the solution is again evaporated to dryness—this operation being repeated several times. The brown pigment is an amorphous substance with a tendency to form pseudo-crystals. Analysis of it gave the following results:

I.	Substance used	0·32505	gramme
	Carbon dioxide	0·96705	,,
	Water	0·16875	,,
II.	Substance used	0·1452	,,
	Nitrogen	8·4 cc.	
	Barometric pressure	756 mm.	
	Temperature	18° C	,,

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* Griffiths, *Comptes Rendus*, t. cxx.; *Chemical News*, vol. lxxi. p. 270.

Reduced to percentages and calculating the formula the following results are obtained :

	I.	II.	Calculated for $C_{15}H_{13}NO$.
Carbon	81·13	—	80·72
Hydrogen	5·77	—	5·83
Nitrogen	—	6·24	6·27
Oxygen	—	—	7·18

These results assign to cupreine the formula

$$C_{15}H_{13}NO.$$

Cupreine is soluble in alcohol, ether and acetic acid. Solutions of the pigment are decolorised by exposure to light, and on spectroscopic examination do not yield characteristic absorption bands.

The ash of the whole body of *Curculio* yielded the following results on analysis :

Weight of ash taken.				
6·84 grms.	Weight of $CaCO_3$ obtained	0·102 grm.	= CaO .	0·830
6·84 „	„ MgO „	0·08 „	= MgO .	1·160
6·84 „	„ K_2PtCl_6 „	0·044 „	= K_2O .	0·123
6·36 „	„ $BaSO_4$ „	0·03 „	= SO_3 .	0·150
6·36 „	„ $Mg_2P_2O_7$ „	0·072 „	= P_2O_5 .	0·720
6·84 „	„ $\left\{\begin{matrix} Fe_2O_3 + P_2O_5 \\ + Al_2O_3 \end{matrix}\right\}$	0·385 „	= Al_2O_3 .	2·450
6·84 „	7 cc. $\frac{N}{20}$ $K_2Cr_2O_7$ used		= Fe_2O_3 .	2·450
5·55 „	2 cc. $\frac{N}{10}$ $AgNO_3$ used		= Cl .	0·120
Silica (by difference)	. . . . .		= SiO_2 .	91·997
				100·000

The body of *Curculio* yielded 2·16 per cent. of ash or mineral matter, and the ash contains traces of sodium and lithium—the presence of lithium being proved by the spectroscope.

IV. DIEMYCTYLINE.

Diemyctyline is a red pigment which occurs in the integument of *Diemyctylus viridescens* (*i.e.*, in its red stage), and its chemical composition has been established* in the following way: By treating the skins with hot alcohol and ether the pigment and fats are dissolved and the solution, after filtration, is evaporated to dryness. The residue is treated with a solution of sodium hydroxide and the pigment then rapidly extracted by means of carbon disulphide or benzene. Upon the spontaneous evaporation of the solvent the red pigment is obtained as an amorphous residue.

Analysis of this pigment gave the following results:

I.	Substance used	0·24570 gramme
	Carbon dioxide	0·54315 ,,
	Water	0·10185 ,,
II.	Substance used	0·10280 ,,
	Nitrogen	6·25 c.c.
	Barometric pressure	760 mm.
	Temperature	15° C.

Reduced to percentages, and calculating the formula, the following results are obtained:

	I.	II.	Calculated for $C_{20}H_{18}N_2O_7$.
Carbon	60·27	—	60·30
Hydrogen	4·52	—	4·52
Nitrogen	—	7·16	7·04
Oxygen	—	—	28·14

These results assign to diemyctyline the formula

$$C_{20}H_{18}N_2O_7.$$

The pigment is soluble in alcohol, ether, benzene and

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* Griffiths, *Comptes Rendus*, t. cxix. p. 912; *Journ. Chem. Soc.*, vol. lxviii. p. 174. Living specimens of this small American lizard were sent to the author by Prof. S. H. Gage of Cornell University.

carbon disulphide, but insoluble in water, the acids and alkalies. By the prolonged action of boiling hydrochloric acid it is converted into uric acid. Hence it is probably a derivative of uric acid, and is of an excretory nature. Solutions of the pigment are rapidly decolorised by exposure to light, and on spectroscopic examination do not show characteristic absorption bands.*

V. TURACIN.

This red pigment was discovered by Church† and first described by him in 1869. It occurs in the wing-feathers of the plantain-eaters; in fact all the known species of the three genera, *Turacus*, *Gallirex*, and *Musophaga* contain turacin.

Turacin is soluble in water, more soluble in alkaline liquids, and it can be precipitated from its solutions by the addition of an acid. It is soluble in ammonia. Turacin, in the dry state, has a dark crimson hue, is non-crystalline, and has a purple semi-metallic lustre. The spectrum of turacin in alkaline solutions shows two absorption bands between D and E and a faint broad band on either side of line F, and extending from λ496 to λ475. The spectrum of isolated turacin in ammonia shows in addition to the three bands already mentioned a fourth band lying on the less-refrangible side of line D, and extending from λ605 to λ589. This extra band is said to be due to a decomposition product of turacin, which may be identical with Krukenberg's turacoverdin.‡

Analysis of this pigment gave the following results:

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* See also Gage in the *American Naturalist*, 1891, p. 1095.

† Church, *Philos. Trans.*, vol. clix. p. 627; *Chemical News*, vol. lxv. p. 218.

‡ Krukenberg, *Vergleich Physiol. Studien.*

Carbon	53·69
Hydrogen	4·60
Copper	7·01
Nitrogen	6·96
Oxygen	27·74
	100·00

These results assign to turacin the formula

$$C_{82}H_{81}Cu_2N_9O_{32}.$$

Turacin presents some analogies with hæmatin, and yields, by solution in sulphuric acid, a coloured derivative, turacoporphyrin. The spectra of this derivative, both in acid and alkaline solutions, present striking resemblances to those of hæmatoporphyrin, the corresponding derivative of hæmatin. But copper is present in the derivative of turacin, while iron is absent from its supposed analogue, the derivative of hæmatin. The beautiful researches of Church have proved that the copper is in true combination with the other elements in the molecule—in fact, strong hydrochloric acid removes no copper from turacin, even on boiling.

The feathers of a single bird contain on an average two grains of turacin (= 0·14 grain of Cu). Turacin is merely a decorative pigment, and has no respiratory function.

VI. GORGONINE.

This substance has been isolated* from the horny skeleton of *Gorgonia cavolinii* (one of the Actinozoa) and contains iodine in organic combination. By the action of barium hydroxide on gorgonine, it yields an amido-acid containing iodine (iodogorgonic acid $C_4H_8NIO_2$), which is a crystalline substance, and is said to have the composition of

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* Drechsel, *Zeit. Biol.*, vol. xxxii. p. 85. See also Halliburton in *Science Progress*, August 1896.

an iodoamidobutyric acid. Gorgonine is a proteid, and by the action of hydrochloric acid it yields leucine, tryosine, lysine, lysatine, iodogorgonic acid and ammonia.

The living substance of the Gorgonias or sea-fans contains no iodine or only mere traces. It is also a proteid, and by the action of hydrochloric acid yields lysine and probably lysatine.

VII. CARMINIC ACID.

This substance, to which varying formulæ have been assigned by different chemists, is the colouring principle of the *Coccus cacti*. Liebermann has thrown doubt on its glucosidal character, and states that it yields no sugar on boiling with dilute acids, whilst it was described by Hlasiwetz as a glucoside having the formula $C_{17}H_{18}O_{10}$, which by boiling with dilute acids is split up into carmine red and a non-crystallisable inactive sugar, according to the equation

$$C_{17}H_{18}O_{10} + 2H_2O = C_{11}H_{12}O_7 + C_6H_{10}O_5.$$

It is a weak dibasic acid, forming purplish-violet compounds with the alkalies and purple with the alkaline earths.

VIII. LEPIDOTIC ACID.

Lepidotic acid, discovered by Hopkins,* is the yellow pigment contained in the wings of the Pieridæ (yellow). It is a derivative of uric acid, which Hopkins has obtained synthetically by heating uric acid with water in sealed tubes at high temperatures. Its constitution is still under investigation. The same pigment occurs in the wings of a large number of day-flying Lepidoptera. It may be obtained from the wings by treatment with hot water, in

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* Hopkins, *Proc. Chem. Soc.* 1889, p. 117 ; *Proc. Roy. Soc.*, vol. lvii. p. 5.

which it is freely soluble. It yields murexide when evaporated with nitric acid and subsequent treatment with ammonia.

The wings should be first treated with hot alcohol and ether, in which the pigment is insoluble, then boiled up with water, and the yellow aqueous solution filtered while hot. If the aqueous solution be concentrated, and allowed to cool, the substance separates out as a yellow amorphous precipitate. It may also be separated from the wings by the action of dilute alkalies in the cold, and afterwards precipitated by the addition of acetic acid. The common brimstone butterfly yields somewhat less than a milligramme of pigment from each insect, larger species belonging to the genus *Callidryas* yield as much as five milligrammes. The yellow pigment is an amorphous substance, insoluble in alcohol, ether, chloroform, and other organic solvents. It is very sparingly soluble in cold, but freely so in hot water. Its aqueous solutions are strongly acid to litmus. It is a derivative of uric acid, and probably allied to mycomelic acid; and it has been suggested that it is a condensation product of the two acids uric and mycomelic, though the hydrogen is too high for the formula suggested in Hopkins's original paper. It is supposed that the two acids unite, with elimination of two molecules of water, to form a new dibasic acid:

$$C_5H_4N_4O_3 + C_4H_4N_4O_2 = 2H_2O + C_9H_4N_8O_3.$$

Hopkins has also found that the wing scales of the group of butterflies known as the white Pieridæconta in uric acid.

IX. LEPIDOPTERIC ACID.

Lepidopteric acid* is the green pigment which occurs in the wings of the following Lepidoptera:—Rhophalocera—

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* Griffiths, *Comptes Rendus de l'Académie des Sciences*, Paris, t. cxv. p. 958; *Chem. News*, vol. lxvi. p. 305; *Journ. Chem. Soc.*, vol. lxiv. p. 236.

Papilio (several species), *Parthenos gambrisus*, *Hesperia*, *Limenitis procris*. Noctuæ—*Halias prasinana*. Geometræ—*Larentia* (several species), *Cidaria miata*. Sphingidæ—*Ino statices*. The same pigment is always yielded by each of these species. The wings are first treated with hot alcohol and ether, in which liquids the pigment is insoluble. The wings are then boiled in acidulated water and the filtrate is concentrated by evaporation—the green pigment being deposited as an amorphous substance. The mean of six analyses of this green pigment gave results answering to the formula

$$C_{11}H_{12}N_8O_{10}.$$

The pigment is a dibasic acid, soluble in acids and precipitable by silver nitrate. The mean of three analyses of the silver salt leads to the formula $C_{11}H_{10}Ag_2N_8O_{11}$. The silver salt appears in silky, colourless needles very soluble in alcohol, but insoluble in ether. The alcoholic solution deflects the plane of polarisation to the right. Its specific rotatory power for the ray D is $[\alpha]_D = +29^\circ$. By prolonged boiling with water the acid pigment yields urea, alloxane, and carbon dioxide:

$$C_{11}H_{12}N_8O_{10} + O_2 = 2CH_4N_2O + 2C_4H_2N_2O_4 + CO_2.$$

By the prolonged action of boiling hydrochloric acid the pigment is converted into uric acid:

$$C_{11}H_{12}N_8O_{10} = 2C_5H_4N_4O_3 + CO_2 + 2H_2O.$$

It is very probable that the green pigment is a derivative of uric acid, and that it is deposited in the wings by the wandering cells, *i.e.*, it is of an excretory character. The solutions of lepidopteric acid give no characteristic absorption bands when examined by means of the ordinary spectroscope or the microspectroscope. Lepidopteric acid is merely a decorative pigment.

X. NERVOUS TISSUES OF CERTAIN INVERTEBRATES.

In 1812 Vauquelin made the first chemical analysis of nervous tissues; and since that date Chevreul, Fremy, Müller, Gobley, Liebreich, Kühne, Gautier, Hoppe-Seyler and many other chemists have added to our knowledge of the compositions of these tissues of the vertebrates. But our knowledge concerning the chemical composition of the nervous tissues of the invertebrates is very meagre and requires extending. The nervous tissues of the invertebrates on p. 119 have been submitted to analysis with the following results.*

The nerves were separated from the body, and the analyses made on the fresh tissues—except in the case of *Homarus*, the figures of which represent the composition of the nervous in the dry state. The nervous tissues of *Homarus* contain in the fresh state 70·21 per cent. of water.†

In the nervous tissues of some invertebrates (*e.g.*, insects and crustaceans) the neurokeratine is replaced by neurochitine—the composition of the latter being $C = 50·21$; $H = 7·64$; $N = 4·06$; and in the fresh state the nervous tissues of the invertebrates are slightly alkaline, but after death become acid.

XI. MISCELLANEOUS SUBSTANCES.

Melanins are the black pigments occurring in the body. Most of them are derivatives of hæmoglobin, and are present in the skin, hair, retina, shells of molluscs, stems of *Gorgonidæ*, &c. Some melanins contain iron, while others are devoid of that metal. The melanins are amorphous

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* Griffiths, *Comptes Rendus de l'Académie des Sciences*, Paris, t. cxv. p. 562; *Chemical News*, vol. lxvi. p. 230.

† See Griffiths, *Physiology of the Invertebrata*, pp. 336, 337.

	Albuminoids.	Lecithine.	Cholesterin and Fats.	Neuro-keratine.	Neuro-chitine.	Cerebrine.	Salts.	Water.
Lucanus cervus .	8·76	2·48	13·99	—	1·20	1·52	0·19	71·86
Blatte orientale .	8·54	2·50	12·97	—	1·14	1·32	0·17	73·36
Carcinus mœnas .	7·20	3·05	14·00	—	1·06	1·21	0·23	73·25
Astacus fluviatilis .	7·58	2·99	13·98	—	1·08	1·19	0·25	72·93
Anodonta cygnea .	7·92	2·86	13·82	1·12	—	1·16	0·20	72·91
Mya arenaria . .	7·96	2·85	13·86	1·20	—	1·14	0·25	72·74
Helix pomatia .	8·25	2·36	12·98	1·18	—	1·20	0·19	73·84
Helix aspersa .	8·28	2·38	13·10	1·16	—	1·18	0·17	73·73
Sepia officinalis .	7·99	2·76	13·00	1·21	—	1·16	0·24	73·64
Loligo vulgaris .	8·02	2·80	13·11	1·20	—	1·19	0·23	73·45
Homarus vulgaris .	20·61	7·79	58·34	—	—	8·26	0·90	—

pigments, soluble in alcohol, ether, acids, and alkalies. According to Schérer the melanin present in the retina contains $C = 58{\cdot}08$; $H = 5{\cdot}91$; $N = 13{\cdot}76$; $O = 22{\cdot}23$.

Punicin is the purple pigment obtained from certain molluscs (*Purpura* and *Murex*). It is secreted by the epithelium situated between the branchiæ and the hind-gut in the mantle cavity. It is colourless when fresh, but becomes purple on exposure to air. When dissolved in aniline and allowed to crystallise it assumes star-shaped groups of acicular crystals.

Aphidein, discovered by Sorby, is a pigment obtained from the *Aphis*, and it is said to possess a respiratory function. It contains carbon, hydrogen, oxygen, nitrogen, and probably sulphur.

Stentorin, discovered by Ray Lankester, is a blue pigment obtained from *Stentor*, and it has probably a respiratory function.

Pentacrinin, antedonin, aphysiopurpurin, janthinin, are animal pigments discovered by Moseley. They are lipochromes.

Tetronerythrin is a red pigment or lipochrome found in a large number of invertebrates and vertebrates. It is soluble in alcohol and ether, and has been investigated by Halliburton, MacMunn, and others.

Zoonerythrin is a bright red pigment found in the integument of certain sponges, tunicates, crustaceans, and fishes. Gautier says that it aids cutaneous respiration. It is insoluble in water, soluble in alcohol, ether, turpentine, carbon disulphide, and acetic acid. According to Blanchard, this pigment resembles carrotin (the lipochrome of the carrot and tomato).

Melaine is the black pigment secreted by the ink-bag of *Sepia*. Schwartzenbach and Hosacus found that it contains

80 per cent. of solids; and Nencki and Sieber have recently separated from the pigment a definite compound termed sepiaic acid, which contains carbon, hydrogen, nitrogen, oxygen, and sulphur. Girod, Variot, and Desfosses state that the pigment has the formula $C_{22}H_{20}N_3O_{10}$. It is insoluble in water, alcohol, and acids, but is soluble in a strong solution of potassium hydroxide.

Chitin occurs in various parts of the body of the invertebrates. It is readily prepared by treating the shells of crabs and lobsters with hydrochloric acid, so as to dissolve out the salts of lime. It is also obtained by digesting the wings of beetles and other insects in a solution of NaOH. In both cases the chitin remains undissolved. The residue is then dissolved in strong HCl, and re-precipitated from this solution by the addition of water. This operation is repeated several times, when the chitin is obtained in a pure state. It is a colourless substance, and is soluble in strong mineral acids. When heated with strong acids it is decomposed into acetic acid and glucosamine:—

$$2\ C_{15}H_{26}N_2O_{10} + 2\ H_2O = 3\ HC_2H_3O_2 + 4\ C_6H_{13}\,NO_5.$$

Conchiolin ($C_{30}H_{48}N_9O_{11}$) is the skeletin or basis of the shells of the gasteropoda.

Cornein ($C_{30}H_{44}N_9O_{13}$) is the skeletin of *Gorgonia* and other corals.

Spongin is the skeletin of the Porifera. Its composition is unknown.

Fibrorin is the substance of which the webs of spiders are composed.

These four substances all yield leucin and glycocine on decomposition.

Cantharidin ($C_{10}H_{12}O_4$) is the active principle of the cantharides beetle or so-called Spanish fly. It is insoluble in water, carbon disulphide, soluble in chloroform, ether, acetone, and acetic acid; and forms sublimable plates.

Hyalin is allied to chitin, and is found in the echinoderms and other invertebrates. It contains C = 45·3 to 44·1; H = 6·5 to 6·7; N = 5·2 to 4·5; O = 43 to 44·7 per cent.

Tunicin is the carbohydrate found in the tunicates, *Ophrydium*, &c. Its simplest expression is represented by the formula $(C_6H_{10}O_5)_n$. The value of n is unknown.

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