which resembled torula cells, and occasionally penicillium acrospores.

From this result we do not, however, conclude that it is by these forms that the cosmopolitan fungus (as Hallier calls it) is usually propagated; it frequently happens that liquids which have been once exposed, although they contain no visible cells whatever, rapidly germinate without further exposure. We are also certain that although air is the main source of what we may venture to call fungus impregnation, as distinguished from impregnation with microzymes, yet the two acts may take place at the same moment—germs of torula being often contained in the same liquid media as the germ particles of microzymes. That this is so is proved by instances already referred to, in which liquids protected from air filled with torula cells. Here we relinquish this question, although in a biological point of view it is of the greatest interest and importance.

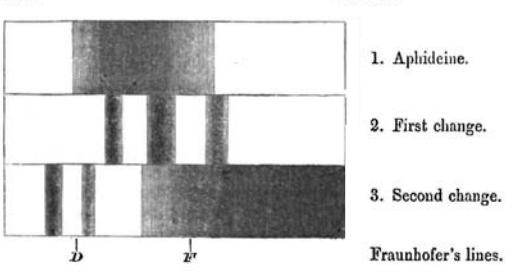
On the Colouring Matter *of some* Aphides.

By H. C. Sorby, F.R.S., &c.

Those who have orchards are no doubt often only too familiar with the red Aphides found in downy patches on the bark of the apple tree. These are coloured by a substance possessing somewhat remarkable properties, connecting it on the one hand with cochineal, and on the other with the hæmoglobin of the blood of vertebrate animals. It rapidly changes into a series of new products, which have remarkable optical characters, and are in some respects analogous to the colouring matters of oils and fats.

In order to obtain this red colouring matter in a state suitable for examination, the insects, fresh taken from the tree, should be crushed up in a small quantity of boiling water, and the solution filtered. It is then of a fine crimson colour, giving a spectrum with a broad general absorption, extending from the yellow over the whole of the green to the centre of the blue, without any well-marked narrow band, as shown in No. 1 of the accompanying fig. 1.

Fig. 1.—*Spectra of the light transmitted by aqueous solutions.*



The addition of a small quantity of citric acid immediately alters the colour to yellow, and then the spectrum merely shows an absorption of the blue end, extending to about the centre of the green, without any definite absorption-bands. A little ammonia restores the colour to its original state, and therefore the crimson colour is characteristic of a neutral or slightly alkaline solution. When a small quantity of the double sulphate of protoxide of iron and ammonia is added to the solution in its natural state (as in all similar cases, using along with it some of the double tartrate of potash and soda, to prevent the precipitation of oxide of iron), it is changed at once to a pale flesh-colour; and, if a little ammonia had been previously added, the solution becomes quite colourless. On exposure to the air, it changes back again to the original tint, from the surface downwards. No such alteration is produced by adding the ferrous salt to an acid solution. This red substance, therefore, like hæmoglobin and hæmatin, exists in an oxidised and in a deoxidised condition, and, like them, can be deoxidised by the above-named process only when the solution is somewhat alkaline. It thus seems reasonable to suppose that it may perform the same functions in the economy of those insects which contain it that hæmoglobin does in the case of the vertebrata. For convenience, it may be well to call this red colouring matter of Aphides *Aphideine*. It is entirely different from any substance on which they feed, and is the same in several species living on entirely different plants.

One of the remarkable peculiarities of hæmoglobin is that it can be changed into a number of substances, each giving a

well-marked spectrum, and in this respect Aphideine is little, if at all, less remarkable. On very gradually adding a small quantity of hypochlorite of soda to a recently prepared solution, the original spectrum No. 1 is changed to that shown in No. 3; but the compound then formed changes quickly into another, the spectrum of which shows two similar narrow absorption-bands, somewhat nearer the red end, not removed by the addition of ammonia or citric acid, disappearing at once when the ferrous salt is added to an alkaline solution, and partially restored by reoxidisation, if not kept long in a deoxidised state. The same results may be obtained by using the Aphideine extracted cold by crushing the insects in a small quantity of water, but this solution, which is often turbid, changes so rapidly on exposure to the air, that it is difficult to examine it before it has been considerably altered. On crushing the living insects in a watch-glass with a little water, the solution is at first pink, but rapidly becomes orange. On pouring this off into another watch-glass, leaving it for a short time, and then pouring the comparatively clear solution into an experiment cell, it will be found that the original Aphideine has been completely altered. On adding a little ammonia, instead of the spectrum showing a broad, continuous band like No. 1, three well-marked narrow bands are seen, as shown by No. 2. For the actual position of these and those in other spectra, I refer to the table given at the end of this paper.

The relative intensity of these three bands varies considerably, and this led me to conclude that two different substances were present, as was subsequently proved in the manner described in the sequel. A weak acid entirely removes the narrower band nearest the red end, raises the others somewhat, and develops a new band still nearer the extreme blue, which can only be seen with excellent sunlight. On adding the ferrous salt to the alkaline solution, the absorption-bands gradually vanish, and, if kept deoxidised for some time, a new compound is formed with an absorption-band between the orange and yellow, and another in the green, disappearing when reoxidised. On the contrary, if the solution which gives the spectrum No. 2 be kept for a while exposed to the air, it is gradually changed into another compound, giving the two absorption-bands shown in No. 3. On keeping still longer these disappear, and the spectrum shows only a general absorption extending over the blue and green without any narrow bands. I am therefore inclined to believe that the compounds which give spectrum No. 2 are gradually altered into two other substances, which when mixed give

spectrum No. 3, the narrow bands being due to one and the greater part of the broad absorption of the blue end to the other. These two narrow bands are at once removed by citric acid. The addition of the ferrous salt to an alkaline solution also removes the bands, and they are restored if re-oxidised in a short time. When the solution is kept for a day or two deoxidised, and then rapidly reoxidised, no bands make their appearance; but if, after having been thus kept deoxidised, the cell be exposed uncovered to the air, so as to reoxidise slowly, another compound is formed, which gives a spectrum with an absorption-band nearer the red end than that shown in No. 3, made much more faint by citric acid, removed at once by deoxidising the alkaline solution, and reappearing when reoxidised. Since some of these solutions are often turbid, it is requisite to use strong concentrated sunlight to penetrate through them.

It will thus be seen that by exposing the solution to the air Aphideine passes successively into four different coloured products, and by deoxidisation and by subsequent exposure two others are formed. These complicated changes do not thus rapidly occur in the comparatively pure solution obtained by boiling the insects in water. It seems requisite that it should contain some of the (perhaps albuminous) substances present when the insects are crushed up in cold water, which by their rapid decomposition seem to induce the above-named changes in the Aphideine itself.

In my paper on some compounds derived from the colouring matter of blood,[1] I briefly described some of the products of the oxidisation of hæmoglobin. Of these there are at least four, three of which are characterised by the presence of absorption-bands at the red end of their spectra when the solutions are deoxidised. The products of the change of Aphideine are in some respects analogous to these, only that except in one the bands are characteristic of the oxidised state. The physical and optical properties of Aphideine and its products differ completely from those of the colouring matter of the cochineal insects of commerce. Whether this is a normal constituent of the living insects or a product can only be decided by examining them when alive, which hitherto I have not been able to do. I have met with Aphideine only in several dark-coloured species of Aphides, but at the same time I must confess that my acquaintance with the colouring matters of insects is very limited.

When carefully selected living Aphides of the apple tree are quickly crushed up in ether, and the clear solution agi-

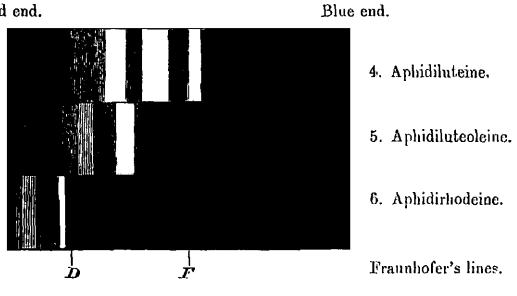
[1] 'Quart. Journ. of Micros. Science,' x, 1870, pp. 400—402.

tated with about an equal quantity of water, it sinks to the bottom coloured pink-red by the Aphideine, whilst the supernatant ether is of pale yellow colour. On evaporating this to dryness, and dissolving in bisulphide of carbon, the yellow solution gives a spectrum without any decided absorption-bands, and seems to be coloured by a substance like that occurring in the fat or wax of other insects. If, however, similar living Aphides are crushed up in a test tube, kept in that state for a few minutes, and then treated with ether, on agitating with water it subsides almost colourless, whilst the ether is coloured deep yellow, and its spectrum shows two well-marked absorption-bands in the blue. When this solution is agitated with water, no colour is dissolved from it, but on adding a little ammonia the greater part of the colouring matter passes to the water in the alkaline modification, of orange colour, giving two well-marked absorption-bands between the blue and the green part of the spectrum, corresponding exactly to the two bands in No. 2, fig. 1, which are nearest to the blue end. On adding a little citric acid that on the green side is removed, and another developed still nearer to the blue end than the one which remains nearly in the original position. If the crushed Aphides are kept longer and treated in the same manner, we obtain a spectrum with three bands, analogous to No. 2, fig. 1; and after they have been kept crushed and damp for half a day, the spectrum shows only two bands, which lie so much farther from the blue end than in the former that the band nearest to it in this case almost coincides with that farthest from it in the other. On agitating this solution with water and a little ammonia, the colouring matter is deposited as a pink layer between the ether and the water, the alkaline modification of this substance thus differing from that of the former in being insoluble in water as well as in ether. Separating it and mixing in alcohol it gives a spectrum with two well-marked absorption-bands in the green and green-blue, corresponding exactly with the two bands in No. 2, fig. 1, which lie towards the red end; and on adding a little citric acid the band in the green disappears, and another is developed in the blue. There is thus good evidence to show that the variation in the relative intensity of the bands in spectrum No. 2 of fig. 1 is really due to a variable mixture of these two substances. Both are of yellow colour when the solution is neutral, and when dry are of waxy consistence. They are manifestly formed by an alteration of the original Aphideine, and therefore it may perhaps be well to call the former *Aphidiluteine*, and the latter *Aphi-*.

diluteoleine. On still further exposure to the air a red colouring matter is formed, which may be distinguished by the name of *Aphidirhodeine;* but this may be more conveniently obtained pure in the manner described in the sequel.

As in the case of all such substances, their spectra are best seen when they are dissolved in bisulphide of carbon, for then the absorption-bands lie farther from the blue end, and there is no chance of there being any variation in their position, owing to any difference in the amount of water that may be present in alcohol or ether. When carefully picked out living Aphides are crushed up in a test tube with the bisulphide, the colour is at first red, but almost immediately changes to yellow; and on stirring them up so as to expose to the air and to the bisulphide, the original Aphideine is rapidly altered into Aphidiluteine, which dissolves in the liquid, giving a bright yellow solution. This should be filtered and examined at once. The spectrum of transmitted light shows two well-marked absorption-bands in the blue, situated much nearer to the extreme blue than those of any other analogous substance which has come under my notice. It is also very fluorescent, of a fine green colour, and this light of fluorescence gives the spectrum shown in No. 4 of the following woodcut, fig. 2:

Fig. 2.—*Spectra of the Light of Fluorescence.*



The whole of the green part of the spectrum is seen, with the exception of two somewhat faint bands, which I believe are due to the Aphidiluteine itself, but am not quite certain, since it rapidly changes into other compounds which have absorption-bands nearly in the same situation. On keeping the above-named solution for some hours it is completely

changed. The spectrum of transmitted light shows two absorption-bands situated very considerably further from the blue end than before, and the light of fluorescence is yellow-green, giving the spectrum No. 5 with a bright band nearly in the centre of the green and a fainter between the green and yellow. This change takes place much more slowly in the case of the solution in ether, but much more rapidly when crushed insects are exposed to the air, and a third compound is formed, which may be obtained in a very satisfactory manner by digesting dead insects, kept dry for some weeks, in a solution of bisulphide of carbon in alcohol, and after it has remained for a few days agitating the clear solution with excess of the bisulphide. This sinks to the bottom with the greater part of the required substance, and leaves various impurities dissolved in the alcohol. After washing with more alcohol, the solution in bisulphide when evaporated leaves an oily or waxy substance coloured brown orange. When dissolved in bisulphide of carbon this gives most remarkable spectra. The transmitted light is of an orange-red colour, giving five well-marked absorption-bands, one in the orange, dark, narrow, and well defined; one at the yellow end of the green, very dark and well defined, with some general shading on the green side; a third and a fourth, less dark than the above two, one nearly in the centre of the green and the other at the green end of the blue, whilst the fifth is nearly in its centre. This spectrum is not only remarkable for the number of bands thus spread over so large a space, but also for the manner in which they are related to one another. This is much like what might be due to a mixture of two substances, and yet there is no further evidence of its being so.[1] The solution is strongly fluorescent, the light of fluorescence is orange-coloured, and its spectrum is as shown by No. 6. The yellow, green, and blue are entirely absent; there is a red band, but it is comparatively so faint that the light may be said to be nearly monochromatic, being almost entirely due to the well-defined orange band shown by the figure, which is so narrow that it is only about $\frac{1}{50}$th part of the whole visible spectrum of daylight. As will be seen, it is quite on the red side of the sodium line D, but when the substance is dissolved in ether instead of bisulphide of carbon, the centre of the bright band almost exactly coincides with D, and all the various bands in the other spectra already described are raised to about the same

[1] See my late paper, "On the Examination of Mixed Colouring Matters," 'Monthly Micros. Journal,' vol. vi, pp. 124—134.

extent towards the blue end, when ether is employed as the solvent.

On agitating the solution of this Aphidirhodeine in ether with water containing a little ammonia, the greater part of the colour is deposited as a green layer between the water and the ether, as though the alkaline modification were insoluble in both water and ether. Separating this and mixing it up in dilute alcohol it gives the spectrum No. 3 of fig. 1, and this fact led me to think it probable that the substance which gives these bands, formed on exposing a solution of aphidieine to the air, is really Aphidirhodeine remaining in a state of very unstable solution. I therefore added to such a preparation two or three times its bulk of alcohol, and on agitating with excess of bisulphide of carbon obtained a red solution of Aphidirhodeine with some Aphidiluteoleine. It therefore appears that though the products derived from Aphideine are not dissolved by water, they may in some cases remain in solution for a time, so as to give a more or less clear liquid. I specially mention this because as an almost universal rule colouring matters soluble in water are insoluble in bisulphide of carbon, or in fats and oils; and misled by the apparent solubility in water, it was some time before I discovered that this brown, dirty-looking solution was in great measure coloured by the clear red and highly fluorescent substance obtained as already described by the use of bisulphide of carbon, for on superficial examination they seem to have so very little in common.

As already named, when the living insects are crushed up in ether, a small quantity of a yellow colour is obtained analogous to that in the fat or wax of other insects, but no Aphidiluteine, which, therefore, appears not to be a normal constituent. If the insects be killed by exposure for a short time to the vapour of bisulphide of carbon, and the colouring matter dissolved out by ether in the course of a few minutes, the amount of Aphidiluteine obtained is very small; but, if the insects have been kept dead for a quarter of an hour, there is no difficulty whatever in proving that a considerable part of the Aphideine has changed into Aphidiluteine even in so short a period of time. After having been kept dead for about a day very little unaltered Aphideine remains. On keeping them much longer they turn darker and transmit red light, showing the absorption bands of Aphidirhodeine. These facts clearly prove that in such inquiries it is most important to decide whether the colouring matters are or are not present in the living insects. The change from Aphideine to Aphidiluteine is so rapid that I was for a consider-

able time led to conclude inaccurately that Aphides contained a waxy substance coloured yellow by that compound. Such an instance of rapid and remarkable changes may be rare, but at the same time it serves to show the importance of our taking into consideration the possibility of its occurrence, even when circumstances are not so favorable for deciding the question. When exposed to the vapour of ether, though apparently killed, the insects sometimes revive, and, even if they do not, the Aphideine changes far more slowly, which may explain why bisulphide of carbon has a so much more poisonous action.

Since it may, perhaps, be convenient for reference, I here subjoin a table of the character and position of the more important absorption-bands seen in some of the spectra roughly described in this paper, making use of the notation explained in a previous communication.[1]

TABLE OF SPECTRA.

Fraunhofer's lines, D *is at* $3\frac{1}{2}$ *and* F. *at* $7\frac{1}{2}$.

1. As dissolved in water:			
Aphideine, alkaline	$3\frac{1}{2}$		$8\frac{1}{2}$
„ acid	6 . .	7 . .	8—
The first mixed product:			
When alkaline varying as thus shown	$5\frac{1}{8}$	7	$8\frac{1}{2}$
	$5\frac{1}{8}$	$6\frac{7}{8}$	$8\frac{1}{2}$
When acid	$7\frac{3}{8}$	$8\frac{7}{8}$	$10\frac{1}{2}$
The second product		$2\frac{2}{4}$	$4\frac{1}{8}$
2. As dissolved in ether, &c.:			
Aphidiluteine in ether		9	$10\frac{5}{8}$
„ in ammoniacal solution of ether in water		$6\frac{3}{4}$	$8\frac{3}{8}$
„ in acid solution of ether in water		$8\frac{3}{4}$	$10\frac{1}{2}$
Aphidiluteoleine in ether		$7\frac{1}{2}$	$9\frac{1}{8}$
„ suspended in dilute alcohol with ammonia		5	$6\frac{1}{2}$
„ „ „ „ citric acid		$7\frac{3}{8}$	9

[1] "On Some Technical Applications of the Spectrum-microscope," 'Quarterly Journ. of Micros. Science' (N.S.), Vol. IX, pp. 358 and 359.

Aphidirhodeine in ether . . .	$\frac{3\frac{1}{2}}{\cdot\cdot}$	$\frac{4\frac{3}{5}}{-}$ ... 5	6	$\frac{7\frac{2}{5}}{\cdot\cdot}$	$\frac{9\frac{1}{4}}{-}$
,, suspended in dilute alcohol with ammonia				$\frac{2\frac{3}{4}}{\cdot\cdot}$	$\frac{4\frac{1}{5}}{\cdot\cdot}$

3. As dissolved in bisulphide of carbon :

Aphidiluteine						$\frac{8\frac{1}{2}}{\cdot\cdot}$	$\frac{10\frac{1}{4}}{\cdot\cdot}$
Aphidiluteoleine						$\frac{7\frac{1}{5}}{\cdot\cdot}$	$\frac{8\frac{3}{4}}{\cdot\cdot}$
Aphidirhodeine	$\frac{3\frac{1}{4}}{\cdot\cdot}$	$\frac{4\frac{1}{5}}{-}$ ... $4\frac{3}{5}$	$5\frac{3}{4}$	$\frac{7\frac{3}{8}}{\cdot\cdot}$	9

Observations *and* Experiments *on the* Red Blood-corpuscle, chiefly *with* regard *to the* action *of* Gases *and* Vapours. By E. Ray Lankester, Radcliff Travelling Fellow, University of Oxford.

Preliminary.—1. The uses of gases and vapours as a means of micro-chemical research.—2. Opinions and doubts concerning the red blood-corpuscle (bibliography).—3. The normal appearance of the frog's red blood-corpuscle.—4. The normal appearance of the human red blood-corpuscle.—5. Means of studying the changes of the blood-corpuscles in disease.—6. Effect of pressure on the red blood-corpuscle.—7. Effect of isolation: *a*, by adhesion; *b*, by oil.—8. Effect of water in minute quantities gradually added.—9. Effect of CO_2 gas.—10. Effect of osmic acid (vapour).—11. Effect of acetic acid (vapour and liquid). 12. Effect of alcohol.—13. Effect of ammonia gas.—14. Effect of chloroform (vapour and liquid).—15. Effect of bisulphide of carbon.—16. Effect of benzine.—17. Effect of turpentine oil.—18. Effect of solution of acetate of rosanilin and of tannin (Robert's experiments).—19. Effect of carbonic oxide.—20. Effect of cyanogen gas.—21. Effect of sulphuretted hydrogen.—General conclusions and summary.

The object of the disconnected observations which are here recorded was threefold: firstly, to ascertain whether certain vapours and gases having marked physiological influence on animals exert any direct action on the red blood-corpuscles, and to determine whether those known, by investigation with the spectroscope, to affect the hæmoglobin produce visible changes in the corpuscle; secondly, to examine into the chemical and formal structure of the red corpuscle; thirdly, to obtain, by a detailed examination of the influence of reagents, and especially gaseous reagents, on a typical histological element, a starting-point for further micro-chemical studies. I cannot consider, as far as relates to the chemical