XXXIV. MERCURIALIS.

II. THE OCCURRENCE OF A CHROMOGEN SHOWING A REMARKABLE AVIDITY FOR FREE OXYGEN.

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WITH the object of extracting from *Mercurialis* the precursor of the permanent blue compound whose isolation is described in the previous paper [Haas and Hill, 1925], fresh tissues were closely packed into a glass cylinder provided with a glass stopper; the tissues were then completely covered with 10 % alcohol, the cylinder being filled to the top and the stopper inserted. On removing the stopper after 48 hours and withdrawing the tissues, the aqueous extract was found to be colourless, but after a few minutes the superficial layer assumed a blue colour which slowly disappeared on further exposure to air. These facts indicate that *Mercurialis* contains a chromogen which by oxidation yields a transient blue compound distinct from the permanent blue compound mentioned above. In view of the sensitivity of this chromogen to atmospheric oxidation, the following method of preparation was adopted.

Exp. 1. Cleaned white rhizomes or apical regions of young aerial shoots are cut into short lengths, the severed portions being allowed to fall into a wide-mouthed glass bottle containing just enough cold freshly-boiled water to cover the fragments of tissue used. Roughly 100 g. of rhizome cut into quarter-inch lengths require about 100 cc. of liquid, whilst the same weight of aerial shoot will need about 175 cc. of water. About 2.5 cc. of chloroform for each 100 cc. of water are then poured in and a rubber cork, fitted with inlet and outlet tubes, is rapidly placed in position, and a rapid stream of nitrogen is passed through in order to remove the air. After a few minutes the nitrogen stream is moderated and the bottle is placed in a thermostat at 45° for one hour.

If the experiment is carefully conducted the liquid in the bottle will be colourless, but on removal and filtration a striking change takes place; the extract rapidly alters in colour to a deep azure blue which may be preserved for some time if the solution be kept in an atmosphere of nitrogen. If, however, the extract be fully exposed to air, the blue increases to a maximum and then changes through green to yellow, the latter being permanent; the green phase is due to a mixture of the yellow with the blue. The depth of colour developed

varies with the strength of the solution; thus from vigorous young shoots the blue pigment may be so deep as to appear almost black and this, on complete oxidation, will give a brown colour. The residual tissues remaining after extraction are no longer able to form the permanent blue on drying.

Another way of demonstrating the colour changes resulting from atmospheric oxidation is to fill a glass cylinder with the blue solution and to leave it open to the air. In time the surface layer will change to yellow, so that a sharp line of demarcation is formed between the yellow and the blue. This line moves slowly downwards as the oxidation of the blue compound proceeds. The sharp separation between the yellow column above and the blue column below is easily broken, wherefore the greatest care must be taken to avoid shaking and the formation of convection currents. This also serves to demonstrate the rate of solution and diffusion of oxygen into the solution and it is possible that this pigment may find application in the investigation of gaseous diffusion, not only in liquids but also in gels.

A similar cycle of changes in colour can also be produced in the tissues by cutting, bruising or otherwise injuring them. Thus if a fresh piece of white rhizome is cut across with a sharp knife, the cortical layer will in a short time assume a blue colour which gradually fades away. Or again, if a small quantity of rhizome is crushed in a white porcelain dish the material quickly assumes a blue colour which changes to dull green and finally to yellow. If the tissue has been well crushed and frequently turned over to ensure thorough exposure to air the pulp will no longer give rise to the permanent blue on desiccation. Similarly it is found that exposure to chloroform vapour induces in a colourless rhizome the formation of the transient blue, the oxygen in this case being presumably supplied by the air contained in the intercellular space system of the tissues. Here again it is found that a portion of the tissue which has been exposed to chloroform vapour for a sufficient length of time to ensure the maximum formation of the transient blue and its subsequent oxidation, will no longer yield the permanent blue on drying. This may be conclusively shown by passing a long rhizome through a cork into a test-tube containing a little chloroform, in such a way that part of the rhizome is within the tube and the part on the other side of the cork is exposed to the air. The enclosed portion will soon turn blue and next day it will be yellowish white, whilst the part exposed to the air will be blue, owing to the formation of the permanent blue.

Exp. 2. That the changes here described are due solely to direct atmospheric oxidation without the intervention of an enzyme was shown as follows. Young white rhizomes were extracted with chloroform water in an atmosphere of nitrogen as described above. The vessel was connected by a rubber tube to a distillation flask through which nitrogen also circulated. After warming for an hour at 45° the extract was transferred through the rubber tube from the extracting bottle to the distillation flask without exposure to air. The extract was then boiled vigorously over a naked flame for one minute in a current of nitrogen and, after cooling, exposed to air. The characteristic colour changes

from colourless through blue and green to yellow were obtained, showing that the phenomenon is able to take place under circumstances which preclude the possibility of enzyme action.

It has been shown that a permanent blue compound is formed by *Mercurialis* on drying [Haas and Hill, 1925]; it is now seen that the plant also yields a transient blue compound by atmospheric oxidation. The question arises whether the same chromogen is involved in each phenomenon—a precursor which by loss of water yields the permanent blue and by oxidation gives the fugitive blue. To test this the following experiment was carried out to see whether the chromogen extract, when evaporated to dryness in an atmosphere of nitrogen, could be made to yield the permanent blue.

Exp. 3. A chloroform water extract was transferred to a distillation flask as described in Exp. 2 and there evaporated to dryness under reduced pressure in a current of nitrogen. The flask contained a small quantity of a deep blue solid which, when taken up with water, and exposed to the air retained its colour unimpaired, from which it may be concluded that the conversion of the chromogen into the permanent blue had been effected by desiccation, as had been anticipated.

It will be remembered that tissues which have produced the transient blue to their fullest extent never yield the permanent blue on desiccation (p. 237). For these reasons we incline to the view that it is the same chromogen which yields either the one or the other blue compound according to the conditions, but a sufficient answer to this question cannot be given until the chemistry of the substances involved has been fully investigated.

The possible physiological significance of such an extremely labile substance as the chromogen described is of the greatest interest, but speculation at this early stage is unprofitable; much more information is requisite. Thus, before considering this substance as a respiratory chromogen in Palladin's sense, but differing from his in that enzyme action is not involved, it is necessary to ascertain whether the oxidative changes producing the blue and yellow compounds are reversible or not. In answer to this question it may be said that so far only the oxidation of the chromogen to blue has been reversed; thus, if to a solution of the fugitive blue a little sodium hydrosulphite is added the blue colour is discharged, but is restored by careful addition of hydrogen peroxide, only to be further and irreversibly oxidised to the yellow compound on the addition of excess of the reagent. Attempts to reduce the yellow compound back either to the blue or to the chromogen by means of the same or other reagents have so far not met with any success and solutions of the yellow compound treated with excess of hydrosulphite give no indication of production of the blue on carefully regulated addition of hydrogen peroxide.

These and other aspects of the subject are under investigation and our observations, together with some account of the chemical properties of the substances involved, will form the subject of a future communication.

SUMMARY.

1. In *Mercurialis* there is a chromogen which undergoes oxidation by gaseous oxygen in two stages, the first stage results in the production of fugitive blue compound which is further oxidised to a permanent yellow substance.

2. The oxidation of the chromogen is independent of enzyme action.

3. The fugitive blue is distinct from the blue produced on desiccation.

4. There are reasons for considering that there is only one chromogen concerned, which forms either the fugitive blue or the permanent blue, according to conditions.

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REFERENCE.

Haas and Hill (1925). *Biochem. J.* **19**, 233.