The Formation of the Anthocyan Pigments of Plants. Part V.—*The Chromogens of White Flowers.**

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The series of communications of which the present paper forms a part (Part V) deals with the biochemistry and genetics of pigmentation in plants. Parts I and III of the series describe the *rôle* of oxydases in the formation of the anthocyan pigments of flowers; Part IV gives an account of the chromogens which constitute the colourless antecedents of these pigments; and the present paper has for its object the investigation of the chromogens in white flowers.

The subject is of interest from the point of view both of biochemistry and genetics; for, as has been discovered by Mendelian research, the white flowers which occur so commonly in cultivated and wild plants belong to more than one category.

The types of white flowers recognised hitherto are known respectively as dominant and recessive whites. As shown by Keeble and Armstrong, both dominant and recessive white flowers contain oxydase (or peroxydase). In the former the oxydase is inactive owing to the presence of an inhibitor; in the latter it is active.

Inhibition of oxydase suffices to account for the absence of colour from dominant white flowers. In order to account for the absence of colour from recessive whites it is assumed that some part of the colour-forming mechanism—for example, the chromogen—is lacking from the flowers. It is, however, possible that lack of colour may be in some cases the consequence not of absence of an essential constituent of the colour-producing mechanism, but of the failure of these constituents—all of which are present in the flower—to come together and interact with one another.

The results of experiments about to be described show that both kinds of recessive white flowers exist.

As the result of treating the white flowers of *Lychnis coronaria* var. *alba* with alcohol (15–80 per cent.), chloroform, ether, or carbon disulphide, a brown pigment develops.

* Parts I and II, Keeble and Armstrong, 'Roy. Soc. Proc.,' 1912, B, vol. 85, pp. 214 and 460; Part III, Keeble and Armstrong, 'Journal of Genetics,' November, 1912; Part IV, Keeble, Armstrong, and Jones, 'Roy. Soc. Proc.,' 1913.

This pigment is at first limited to the veins, though subsequently the whole petal becomes distinctly coloured. The depth of colour is considerable, and the general aspect of the brown petals resembles closely that produced by the action of benzidine. There seems no doubt, indeed, that the brown coloration obtained by treating petals of *Lychnis coronaria* with an alcoholic solution of benzidine is due to this effect of the alcohol rather than to a reaction between oxydase and benzidine. That this is so is indicated by the fact that addition of hydrogen peroxide to a petal so treated causes a further and immediate darkening.

If, however, petals are immersed in absolute alcohol from which the water has been removed by anhydrous copper sulphate, no browning occurs. This is to be expected if the browning is due to oxydases, for, as shown in Part IV, the oxydases are thrown out of action temporarily by dry alcohol.

Petals transferred to water after soaking an hour or so in dry alcohol rapidly develop the brown colour; but petals that have been left several days in the dry alcohol form no brown pigment on transference to water, nor does the addition of hydrogen peroxide cause it to appear. If now it be assumed that the formation of the brown pigment under the influence of chloroform, alcohol, etc., is due to interaction between a colourless "chromogen" and an oxydase (kept apart in the intact petal but allowed to come together when the alcohol has destroyed the impermeability of the plasmatic membrane); then the failure of the pigment to develop in the case of petals that have been soaking some time in alcohol may be taken to indicate that the chromogen has been removed from the petals and diffused out into the alcohol.

Failure of the brown colour to appear is not due to the destruction of the body that functions as peroxide, since addition of hydrogen peroxide is without effect; nor is it due to the destruction of the peroxydase itself, since the petal, after long immersion in alcohol, gives a good benzidine reaction for peroxydase.

If the above view of what occurs be correct, the absolute alcohol in which the petals have been soaked should contain the chromogen in solution. In order to prove that this is the case, a considerable number (150) of *Lychnis coronaria* flowers were treated with 50-per-cent. alcohol, raised to boiling point in order to destroy the oxydase.

After concentrating the extract to a small bulk, white Lychnis flowers, soaked in dry alcohol as above to remove the chromogen, and known to contain peroxydase, were incubated at 36° C. in the solution. The flowers remained colourless while in the extract, but when they were transferred to water containing hydrogen peroxide a reddish-brown pigment appeared at

once in those parts of the flower which contain peroxydase. Hence it is demonstrated that the petals of Lychnis contain a chromogen, which, when extracted from the flowers, is acted on by the peroxydase contained in the petals and gives rise to a red-brown pigment. The peroxydases of *Primula sinensis, Primula obconica, Dianthus* sp., etc., were shown also to bring about —in the presence of hydrogen peroxide—an oxidation of the chromogen extracted from the petals of Lychnis. A similar chromogen has been extracted from the white-flowered variety of *Anemone japonica.* Like that obtained from the flower of *Lychnis coronaria,* it yields pigments when acted on by the oxydases of petals of various plants.

The white flowers of *e.g. Lychnis coronaria* thus yield an extract which can be used to demonstrate the distribution of oxydases in place of a benzidine solution.

The experiments show, moreover, that these flowers of *Lychnis coronaria,* although they are white, contain both oxydase and chromogen.* It is therefore probable that these constituents are located in different cells or parts of the same cell, and that whiteness is due to the fact that the plant lacks the means of bringing chromogen and oxydase into contact with one another.

As has been mentioned already, the pigment obtained by the action of the peroxydase of the petals of *Lychnis coronaria* on the chromogen extracted from these petals is of a reddish-brown colour. It might, therefore, be urged that the chromogen which gives rise to this pigment is not that which in coloured flowers yields the red anthocyan pigment of the natural petals.

The objection is weighty; but that it may be met is shown by the following considerations and experiments :—

1. It is known that changes in the chemical nature of the chromogen, the degree of oxidation,† the conditions under which the reactions occur, and the presence of traces of other substances,‡ affect the colour of the end product of oxidation. Too much weight, therefore, should not be attached to mere difference in colour as the colour is very susceptible to alteration.

* Since browning of the fresh petal occurs under the influence of alcohol alone, the body that behaves as a peroxydase towards *e.g. a*-naphthol, can behave as an oxydase towards the natural chromogen.

† In this connection it may be noted that if a pink bract of *Bilbergia* sp. be immersed in H_2O_2, the pink pigment becomes changed into brown, presumably as the result of further oxidation. In 'U.S. Dept. of Agric. Bureau of Plant Industry Bulletin,' No. 264, 1913 (received as the present paper goes to press), H. H. Bartlett records a red pigment of Dioscorea as becoming brown on oxidation.

‡ Chodat, R. "Nouvelles Recherches sur les Ferments Oxydants. Les matières protéiques et leurs dérivés en présence du réactif *p*-crésol tyrosinase." 'Arch. Sci. Phys. Nat.,' 1912 (IV), vol. 33, pp. 70, 225.

2. The behaviour of flowers of Brompton stocks, as described in detail in Part IV of this series, provides a convincing proof that it is possible for all the mechanism for colour production to be present in a flower, and yet for the bodies concerned not to interact to produce pigment until the plasmatic impermeability has been destroyed.

The fading and recovery of colour of petals of these plants was observed by the present writer during the preliminary experimental work in connection with the above paper. The facts will be referred to here only in so far as they illustrate the point at issue.

If coloured flowers of Brompton stocks are soaked in absolute alcohol, the contained pigment gradually fades; on transferring the colourless petals to water they quickly become coloured, the "recovered colour" being of exactly the same shade as that of the fresh flower used.

In the paper referred to evidence is presented that the fading of the coloured petal in alcohol is due to the reduction of the pigment to a colourless state, as well as to its diffusion out into the surrounding liquid, and that the formation of pigment when the petal is transferred to water is the result of an oxydase converting into pigment a colourless chromogen substance contained in the petals in addition to a re-oxidation of the reduced pigment remaining.

Thus, in stock, oxydase and chromogen are both present, and the conditions are naturally such as to allow a proportion of these two bodies to come together to produce pigment. In the white-flowered variety of *Lychnis coronaria* the natural conditions are never such as to allow any interaction between oxydase and chromogen. On treatment with alcohol the barrier is removed by the destruction of the plasmatic impermeability, and, as a result, pigment is produced.

The method which serves in the case of Lychnis to bring chromogen and oxydase together, and causes them to interact with one another, serves with Brompton stocks to bring about a large increase in the amount of pigment present—which pigment is of the same colour as that occurring in the flower under natural conditions.

By use of such methods the following types of white flowers have been demonstrated in the course of this investigation:—

1. *White Flowers which contain an Oxydase and a Chromogen, e.g.* Lychnis coronaria, Anemone japonica, Chrysanthemum *sp.*—When petals of these plants are subjected to the action of alcohol, chloroform, etc., a colour change is produced. The colour may be brown, as in *Anemone japonica*, and appear more or less evenly all over the petal; or of a reddish tinge, as in *Lychnis coronaria*, where the colour is located chiefly in the veins. In both these

examples the depth of colour obtained is very considerable; in the case of Chrysanthemum the colour change is only slight.

All the flowers belonging to this class give the characteristic peroxydase reaction with benzidine or α-naphthol solutions and hydrogen peroxide.

2. *White Flowers which contain a Peroxydase and a Chromogen.*—This type of white flower is illustrated by certain varieties of *Dianthus caryophyllus* (*e.g.* var. "Mrs. Sinkins,") and of *Dianthus barbatus* (Sweet William), etc.

These flowers on treatment with dilute alcohol, chloroform, etc., show no development of colour, but the addition of H_2O_2 causes a rapid formation of pigment. In many flowers of this class colour is produced only locally in the petal on the addition of hydrogen peroxide. On testing such a flower with benzidine solution with the subsequent addition of hydrogen peroxide, a peroxydase reaction is obtained only in these same localities. The peroxydase is limited, therefore, to those areas that give a colour reaction when treated with alcohol, etc., and hydrogen peroxide.* Whether the chromogen, which contributes to the reaction occurring in alcohol is present in the regions from which peroxydase is absent is as yet undetermined.

3. *White Flowers which contain a Peroxydase but no Chromogen.*—The white-flowered varieties of *Plumbago capensis* and *Swainsonia Tacsonia* illustrate a third type of white flower.

No colour reaction is given after treatment with chloroform or similar bodies even after the addition of hydrogen peroxide. Such petals, however, give in every case a reaction with benzidine and hydrogen peroxide.

4. *White Flowers which contain no Oxydase or Peroxydase.*—A fourth type may perhaps be inferred from the behaviour of a white variety of Sweet William investigated by Keeble and Armstrong, which was found to give no benzidine reaction, direct or indirect, and was therefore assumed to lack oxydase and peroxydase. The possibility of an inhibitor being present was not overlooked, but was not investigated. Information as to the occurrence of a chromogen in these flowers is also wanting.

The interpretation suggested above of the behaviour of white flowers when treated with alcohol, etc., is the most obvious and simple one, but it is fully recognised that intermediate steps may occur of which no account has been taken.

In *Lychnis coronaria*, it may be that a chromogen, as such, does not occur in the intact petals, but is split off from a body which one may term a pro-chromogen, after the plasmatic impermeability has been destroyed by treatment with alcohol.

* In coloured varieties these same areas are often the only parts of the flower containing pigment.

A modification of the interpretation on these lines, however, does not affect the general hypothesis as to the existence of several types of white flowers, or the inference that pigment is not necessarily produced although all the requisite ingredients for the production of colour may be present.

On the Occurrence of a Ganglion in the Human Temporal Bone not hitherto Described.

By Albert A. Gray.

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(Plate 6.)

The existence of a previously unknown nerve plexus associated with a comparatively large ganglion embedded in the substance of the human temporal bone must be regarded as a somewhat surprising fact at this period in the history of human anatomy. It may be well, therefore, to describe in a few lines the process which led to the discovery.

While making some preparations of the middle ear of animals according to my own method, I discovered the presence of a large plexus of nerves on the posterior surface of the bulla of the sheep. This plexus was found to be composed of bundles derived from the pneumo-gastric and the facial nerves. Since the preparation was only macroscopic, I was unable to ascertain whether nerve ganglion cells were present. Such a plexus has not been described in the human subject, unless the two minute bundles of fibres which pass between Arnold's nerve and the facial nerve be dignified by the name of plexus. It seemed, therefore, highly probable to me that some corresponding structure might exist in the human subject which had not hitherto been described, and a search was accordingly made.

The initial difficulty lay in the fact that this portion of the temporal bone is very different in man from that in the sheep. In the latter there is a large bulla, but no mastoid process; whereas in man there is a large mastoid process and no bulla. In man the only indication of a bulla is the little *cul-de-sac* which runs backwards from the lower, inner, and posterior corner of the tympanum. In the human subject a mass of bone fills the space which in mammals is occupied by the bulla. The plexus,