The Distribution of Oxydases in Plants and their Rôle in the Formation of Pigments.

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Whereas our knowledge of the mode of inheritance of flower colour has made rapid and sure advance of recent years, our understanding of the chemistry of the process of pigment formation in flowers is still uncertain and incomplete.

The researches of Miss Wheldale (1911) on flower-pigments and of Gortner and others on animal-pigments have confirmed and extended the conclusions of earlier workers, and it may now be regarded as an established fact that the formation of pigment in plants and in animals is due to the action of an oxydase on a more or less colourless chromogen.

According to the experiments of Gortner (1911) the superficial pigmentation of various insects is the outcome of the action of tyrosinase on the chromogen tyrosin. As a result of that action a black pigment, melanin, is produced. Gortner has shown moreover that this reaction may be inhibited by certain phenolic compounds such as phloroglucinol, orcinol and resorcinol. These dihydroxyphenols Gortner describes as anti-enzymes and he attributes the phenomenon of dominant whiteness, that is the suppression of pigment-formation in tissues, believed, by reason of their mode of colour-inheritance, to contain both chromogen and oxydase, to the presence—local or general—of these anti-bodies.

Miss Wheldale has obtained evidence that pigment formation in flowers is consequent upon the interaction of an oxydase and a chromogen and has suggested that the latter body is a product of hydrolysis of a glucoside.

Pending the introduction of more precise and convenient methods for determining the distribution of oxydases in plant tissues our knowledge has remained in this interesting but incomplete state. It is true that a number of oxydase reagents is known but the method of their application, that of adding the reagents to macerated tissues, cannot but lead to inconclusive results. It is possible, however, as Clarke (1911) has demonstrated, to use certain oxydase reagents for micro-chemical purposes and, as we show, appropriate methods admit of both macroscopic and microscopic demon-

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stration not only of the presence but also of the distribution of oxydases in such delicate tissues as the petals of flowers.

Further, as will be apparent to all students of Genetics, the application of precise chemical methods to the investigation of the distribution of oxydases in plant- and animal-tissues is of special importance at the present time. For, thanks to the work of Bateson, Baur, Gregory and many others, not only do we possess a detailed knowledge of the modes of inheritance of flower-colour in many different species of animals and plants, but we have also at our disposal many pure-bred strains of animals and plants, the genetical relationships of which are known. Therefore we may hope that precise tests applied to plants or animals of known genetical constitution may throw new light on the physiological *rôles* of oxydases in the organism and may contribute also to an understanding of the nature of Mendelian characters, the sum total of which appears to determine specific character.

The method which we employ consists in the treatment of a tissue with the colour-indicator constituent of the oxydase reagent together with a "hormone" (see H. E. and E. F. Armstrong, 1910, 1911), that is a substance which causes the plasmatic membrane to become permeable to the reagent and also renders active the oxydases and other enzymes present in the cell. When this component of the oxydase reagent is added to the intact petals of a flower the first visible effect is the decolorisation of the flower. As soon as the colourless state has been induced, the second component of the reagent, hydrogen peroxide, is added. Owing to the previous treatment of the tissues the hydrogen peroxide penetrates rapidly into the cells and the colour-reaction indicative of oxydase is produced, generally in the course of a few minutes. By the use of various oxydase-indicators, α-naphthol, benzidine and others, we have been enabled to establish the following facts with respect to (1) the distribution of oxydases in the Chinese primrose (*Primula sinensis*) and (2) the relation between oxydases and pigment formation in that plant:—

1. The distribution of pigment in the flower coincides exactly with that of a peroxydase.

2. The alcoholic solution of the oxydase-indicator brings about decolorisation of the flower. In most cases the decolorisation is rapid and complete; but in certain colour-varieties the flower-colour is more resistant. Surface sections of such flowers may however be readily decolorised. The colourless state is maintained till hydrogen peroxide is added. Hence the oxydising agents present in the flower in *Primula sinensis* are to be regarded as peroxydases.

3. The use of different oxydasic reagents reveals the presence of two

peroxydases in *P. sinensis*. One, the "epidermal" peroxydase, occurs in the epidermis and in some cases in the layers subjacent to the epidermis. Another, the "bundle" peroxydase, is localised in layers of cells neighbouring the woody tissues of the vascular bundles.

Both epidermal and bundle peroxydases occur in the vegetative parts of the plant as well as in the flower. In the stem the epidermal peroxydase is separated widely from the bundle peroxydase by many layers of cortical cells, which in many varieties at all events contain no peroxydase; but in the tenuous petals of the flower the bundle peroxydase occurs of necessity in close proximity with the epidermal peroxydase.

4. Certain varieties of *P. sinensis* such as Crimson King, Coral Pink, and Sirdar give, under certain circumstances, a *direct* oxydase reaction: that is a characteristic coloration is produced when they are treated with the oxydase reagent alone.

5. The bundle peroxydase of the petals of the flower of *P. sinensis* is located in the cells of the bundle sheath which surrounds the veins. The peroxydase accompanies that sheath throughout the repeated ramification of the veins, and may be seen in microscopic preparations to extend to the tips of their finest branches; the epidermal peroxydase occurs in the superficial papillate cells of the petals.

6. Where, as is the case with many varieties of *P. sinensis*, the flower has a yellow eye, no epidermal peroxydase is demonstrable over the eye except in the hairs which are produced as outgrowths from the epidermis.

7. The epidermal and bundle peroxydases differ from one another both in their distribution and in their colour reactions. Thus bundle peroxydase reacts with α-naphthol and hydrogen peroxide to yield a lavender-blue colour which picks out the veins in exquisite detail. With this reagent the epidermal peroxydase yields generally no colour reaction.

Selective coloration in the opposite sense though less precise is produced by the addition of benzidine and hydrogen peroxide to the flower. Treatment with an alcoholic solution of benzidine brings about first decolorisation of the sap (anthocyan) pigments of shoot and flower. The subsequent addition of hydrogen peroxide activates both epidermal and bundle peroxydases and results in a rich brown uniform coloration of the surface layer of the petals, which coloration extends also to the veins.

8. White flowers which by breeding tests are known to be dominant whites fail to give the epidermal peroxydase reaction; but in such flowers a faint bundle peroxydase reaction may occur.

Both epidermal and bundle peroxydases are, however, present in dominant white flowers; for if such flowers are treated with hydrogen cyanide and

subsequently with benzidine or α-naphthol and hydrogen peroxide the characteristic peroxydase reactions are produced. The whole surface of the petals becomes deeply coloured and the veins also stand out prominently. Hence the flowers of dominant white primulas contain a substance which inhibits but does not destroy the pigment-producing peroxydase.

Where dominant white patches occur on otherwise self (uniformly) coloured flowers, as is the case with certain strains of blue *P. sinensis* with which we have worked, the benzidine reagent picks out the coloured areas in brown and leaves the dominant white areas uncoloured. The α-naphthol reagent picks out the veins with the utmost sharpness and leaves the dominant white patches unstained except for an occasional fine line of colour along the course of a vein which traverses the white patch. Beyond each dominant white area as well as before reaching it, the bundle sheath surrounding each vein is more deeply stained. Recessive whites possess both epidermal and bundle peroxydases.

9. The observations on the epidermal and bundle peroxydases throw light on the significance of the phenomenon presented by many cultivated flowers which are known to florists as "ever sporting." Such flowers are characterised by the sporadic appearance of splashes of colour on a white or self-coloured ground. Ever sporting strains are familiar to everybody in carnations and azaleas. In *P. sinensis* ever sporting strains are also common. Thus the variety Mont Blanc Star bears white flowers with magenta flakes. We are inclined to regard flaking as the effect of the bundle peroxydase on the chromogen-containing cells neighbouring on the bundle sheath. The white ground colour is to be attributed to an inhibitor associated with and nullifying the epidermal peroxydase.

The marked localisation of the pigmentation effected by the bundle peroxydase, which localisation expresses itself in splashes, flakes, or lines of colour, appears to be due to anatomical causes, such as the degree of development of the cells of the bundle sheath and the nearness of the veins to one another.

10. The existence of two localised peroxydases which may induce pigmentation and may reinforce one another along certain tracts of tissue provides material facts for the explanation of colour-range and colour pattern in flowers.

The investigations which are the subject of this communication have been carried out jointly by the authors, who wish to share equally in the responsibility for the results which they have obtained.

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On the Nature of Pancreatic Diabetes. (Preliminary Communication.)

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Many explanations have been proposed for the fact, discovered by von Mering and Minkowski (1), that extirpation of the pancreas is followed immediately by severe and fatal diabetes. It has been suggested on the one hand that the normal function of the pancreas is to diminish excessive production of sugar, and that, in the absence of its restraining influence, excessive sugar production and mobilisation are the results. On the other hand, the fact that carbohydrates are not utilised by the body when administered to animals in this condition has been interpreted as showing that the tissues have lost their normal power of assimilating and utilising glucose. It has also been suggested, though without much experimental support, that the sugar of the blood has to be built up into some other form before it can be utilised by the tissues.

We have recently, in a research on the influence of mechanical conditions and of temperature on the heart beat, modified the procedure described by Jerusalem and Starling (2) for working with a heart-lung preparation, so that we are able to keep a heart, connected with the lungs but isolated from the rest of the body, beating for many hours in approximately normal