PART I

GENERAL ACCOUNT

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Muriel Wheldale Onslow
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Idea 27. And *first*, their *Colours*; where, with respect to several *Plants* and *Parts*, they are more *Changeable*; as Red, in *Flowers*; or *Constant*, as Green in *Leaves*. Which, with respect to several Ages of one *Part*, are more *fading*, as Green in *Fruits*; or *durable*, as Yellow in *Flowers*. In what *Parts* more *Single*, as always in the *Seed*; or more *Compounded*, as in the *Flower*; and in what *Plants* more especially, as in *Pancy*. Which proper to *Plants* that have such a *Taste* or *Smell*, as both, in *White Flowers*, are usually less strong. To *Plants* that flower in such a *Season*, as a *Yellow Flower*, I think, chiefly, to *Spring Plants*. And to *Plants* that are natural to such a *Soil* or *Seat*, as to *Water-plants*, more usually, a *white Flower*. What, amongst all *Colours*, more Common to *Plants*, as *Green*; or more Rare, as *Black*. And what all these Varieties of *Colours* are upon *Cultivation*, but chiefly, in their natural *Soil*. To observe also with their superficial *Colours*, those within: so the *Roots* of *Docks*, are *Yellow*; of *Bistort*, *Red*; of *Avens*, Purple; but of most, White. Where the Inward, and Superficial *Colours* agree; as in *Leaves*; or vary, as in the other Parts frequently. And in what manner they are *Situated*; some universally spreading, others running only along with the *Vessels*, as in the *Leaves* of Red *Dock*, and the *Flowers* of *Wood-Sorrel*.

From Nehemiah Grew's *Anatomy of Plants, with an Idea of a Philosophical History of Plants*. 1682.

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INTRODUCTORY

By comparison with other products the anthocyanin pigments of plants have now received considerable attention. Such colouring matters have sometimes been spoken of as soluble pigments, since they are in a state of solution in the cell-sap, as contrasted with those which are in some way bound up with the structure of organised protoplasmic bodies known as plastids. The innumerable shades of blue, purple, violet, mauve and magenta, and nearly all the reds, which appear in flowers, fruits, leaves and stems are due to anthocyanin pigments. On the other hand, green, and the large majority of orange and yellow colours, are in the form of plastid pigments. Other colours, again, notably some scarlets and orange-reds, browns and even black, are produced by the presence of both plastid and anthocyanin pigments together in the tissues.

For almost a hundred years botanists have employed one term to denote the soluble pigments—anthocyanin[1] (ἄνθος, κύανος), a word first coined by Marquart (5)[2] for these substances in 1835 and retained in the same sense to the present day; other rival terms, now obsolete, such as erythrophyll, cyanophyll and cyanin have also been used from time to time.

Vegetable pigments served as matter for investigation at a very early date, and the property which first attracted attention was their behaviour towards acids and bases, that is, the reddening by acids and the formation of green coloured products with a base. Thus, in 1664, in the *Experiments and Considerations touching Colours* of Robert Boyle (121), we find the following directions: "Take good Syrrup of Violets, Imprægnated with the Tincture of the flowers, drop a little of it upon a

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[1] In the present volume anthocyanin is largely used in the collective sense, that is as a term which we know to include a number of substances; sometimes however, when the context demands it, the plural form is used.

[2] Numbers in brackets refer to papers, etc., in the Bibliography at the end of the book, and such references are either entirely concerned with anthocyanins, or have some direct bearing upon them. References, on the contrary, in the foot-notes are not directly concerned with anthocyanins.

White Paper...and on this Liquor let fall two or three drops of Spirit either of Salt or Vinegar, or almost any other eminently Acid Liquor, & upon the Mixture of these you shall find the Syrrup immediately turn'd Red,...But to improve the Experiment, let me add what has not...been hitherto observ'd,...namely, that if instead of Spirit of Salt, or that of Vinegar, you drop upon the Syrrup of Violets a little Oyl of Tartar *per Deliquium*, or the like quantity of Solution of Potashes, & rubb them together with your finger, you shall find the Blew Colour of the Syrrup turn'd in a moment into a perfect Green."

And again in *The Anatomy of Plants* of 1682, Nehemiah Grew (1) tells us that "...*Spirit* of *Harts Horn* droped upon a *Tincture* of the *Flower* of *Lark-heel* & *Borage* turn them to a *verdegreese Green*."

"*Spirit* of *Sulphur* on a *Tincture* of *Violets* turns it from *Blew* to a true *Lacke*, or midle *Crimson*."

"*Spirit* of *Sulphur* upon a *Tincture* of *Clove-July-Flowers* makes a bright blood *Red*. Into the like *Colour*, it hightens a *Tincture* of *Red Roses*."

It is not clear from the writings of Boyle and Nehemiah Grew whether these authors considered the great variety of shades to be the expression of one or of many substances, though it is clear that Nehemiah Grew differentiated between red, blue and green pigments as regards their solubilities. For he says, "...The *Liquors* I made use of for this purpose, were three, *sc. Oyl* of *Olives*, *Water*, & *Spirit* of *Wine*. The *Water* I used was from the *Thames*, because I could not procure any clear *Rain Water*, & had not leasure at present to distill any. But next to this, that yields as little *Salt*, as any." The oil he found dissolved the green colour, "...But there is no *Vegetable* yet known which gives a true *Red* to *Oyl*, except *Alkanet Root*." This root "which immediately tinctures *Oyl* with a deeper *Red*, will not colour *Water* in the least. Next it is observable, That *Water* will take all the *Colours* of *Plants* in *Infusion* except a *Green*. So that as no *Plant* will by *Infusion* give a perfect *Blew* to *Oyl*; so their is none, that I know of, which, by *Infusion* will give a perfect *Green* to *Water*[1]." Boyle, on the other hand, was evidently impressed by the uniformity of the acid and alkali reactions: "...it is somewhat surprizing to see,...how Differingly-colour'd Flowers, or Blossoms,...how remote soever their Colours be from Green, would in a moment pass into a deep

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[1] This is entirely in accordance with our present-day knowledge of the pigments. Chlorophyll, like all plastid pigments, is insoluble in water, but soluble in oil. The red, blue and purple anthocyanin pigments are soluble in water, but insoluble in oil. The red pigment of Alkanet root is not an anthocyanin and has quite different solubilities.

Degree of that Colour, upon the Touch of an Alcalizate Liquor." The gradual evolution of the idea of the multiplicity of pigments included under anthocyanin can only be realised in a comprehensive survey of the subject.

After the preliminary and somewhat diffuse observations of the earliest scientists, there follows a period during which certain definite lines of investigation emerge, and it is practically to these lines that the chapters in this book correspond. It may not be out of place, however, to give a general account of the different phases and kinds of investigation showing how they have developed, and how they are related to each other in the complete history.

The above lines of investigation may be enumerated thus: the morphological and histological distribution of the pigments, the factors controlling their formation, their function, their chemical composition, their mode of origin, and lastly, the part they have played in heredity.

First let us consider their distribution. Contributions to this portion of the subject naturally formed a great part, though by no means the whole, of the earlier work on pigments, since it merely involved general observation and microscopical examination of petals and leaves. Various writers published full accounts of the pigments of flowers, fruits, leaves, etc., showing how some colours are due to plastid, others to soluble pigments, and others again are the result of the combination of both in the cells. As would be expected, this form of investigation has tended to diminish in later years for, the histological basis once laid down, the physiological, chemical and biochemical aspects have come to the front. Mention must be made, however, of the publications of Buscalioni & Pollacci (17) in 1903 on the histological distribution of anthocyanin, and of a paper on similar lines read by Parkin (77) at the meeting of the British Association of the same year. Still later work of this kind is that of Gertz (19) which appeared in 1906; this author made a most thorough and systematic investigation of the occurrence of anthocyanin in representative genera of all natural orders, but the publication of his work in Swedish unfortunately restricts the circulation of his results.

The question of factors controlling anthocyanin formation is the next line of enquiry most convenient for consideration. The main factors concerned are light, temperature and nutrition.

The matter of fundamental significance in all questions of factor control, and one which it will be well to understand before turning to the factors in detail, is the dependence of anthocyanin formation upon the supply of an initial product or chromogen, as it is called, from which the pigment arises. This substance, like all others in plant metabolism, ultimately depends for its existence on a supply of carbohydrates (sugars), the first products of synthesis in the plant. The relationship between anthocyanin formation and the presence of sugars in the tissues has provided an important subject for research. Overton (420, 421), in 1899, first drew attention to its significance[1]; this botanist had noticed, while carrying out some experiments on osmosis, that culture of *Hydrocharis Morsus-ranae* in sugar solutions leads to greater production of anthocyanin in the leaves. Further experiments showed the phenomenon to be constant for quite a number of species when isolated leaves and twigs were fed on solutions of cane sugar, dextrose, laevulose and maltose. Repetition of experiments on these lines at later dates by Katić (441), Gertz (473) and others gave full confirmation to Overton's results. This discovery led Overton to the fairly obvious inference that possibly, in the normal plant, reddening of leaves, etc., is correlated with excess of sugar in the tissues, and he states that by tests upon red autumnal leaves he could detect more sugar in red than in green leaves.

More elaborate and conclusive work in this direction was commenced by Combes in 1909 and carried on in the following years. Combes (461, 472) had noted that decortication in some plants brings about a considerable development of anthocyanin in the leaves above the point of decortication. Analyses were made by him, not only of leaves reddened through this cause, but also of autumnal and other red leaves. In all cases he (222) claims to have shown that the red leaves contain greater quantities of sugars and glucosides than green ones from the same plant. From other, more general, phenomena, in addition to Combes's results, it may be safely inferred that an accumulation of such synthetic products as are manufactured in the leaves leads to production of anthocyanin. For example, we frequently find abnormal reddening of a single leaf on a plant otherwise in full vigour, and investigation almost invariably shows the reddening to be accompanied by injury, and the injury, whether it be due to mechanical cutting or breaking, or to the attacks of insects, will be found to affect those tissues which conduct away the synthetic products of the leaf.

There is little doubt that the chromogen of anthocyanin, in the form of a glucoside, is also manufactured in the leaves, and the whole trend of results goes to show that an enforced accumulation of this chromogen,

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[1] The connection between pigmentation and the presence of sugars was also pointed out in 1899 by Mirande (419) as a result of his investigations on the genus *Cuscuta*.

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together with sugars, by stoppage of the translocation current, leads to formation of anthocyanin in the leaf; and a similar result may arise from artificial feeding with sugars. To the more precise relationships between chromogen, anthocyanin and sugars attention will be given in a later chapter.

Thus it will be seen that in all problems connected with effects of temperature, light, etc., on anthocyanin formation, we are confronted with two distinct questions, i.e. the direct effect of light and temperature on the actual production of pigment, and the indirect effect of these factors on the supply of organic compounds from which the chromogen of anthocyanin is synthesised.

The relationship between pigment formation and light constitutes a problem to which there is no very satisfactory solution. Sachs (356, 358), Askenasy (369) and others have tried the obvious methods of growing plants in the dark with controls in the light, of darkening leaves while leaving inflorescences uncovered and so forth. The outcome of these researches, as well as of several others, has been to show that in many cases, for example, in flowers of *Tulipa*, *Hyacinthus*, *Iris* and *Crocus*, anthocyanin develops equally well in the dark; in other cases, such as *Pulmonaria*, *Antirrhinum*, and *Prunella*, the development is feeble or absent. A general survey of anthocyanin distribution leaves us in no doubt that, as far as organs where anthocyanin may be expected to develop are concerned, the greatest production takes place in the most illuminated parts. But we have on the other hand not a few examples, of which the root of *Beta* is a good illustration, of development of pigment in total darkness. In the absence of fuller evidence, the most reasonable point of view is that the actual process of pigment formation may in itself be entirely independent of light, should the tissues contain sufficient reserve materials to supply the chromogen. But if there is a shortage of reserve materials, such as would arise from diminished photosynthesis, the anthocyanin may fail to appear from lack of chromogen.

The problem of the effect of temperature offers similar difficulties. Does the temperature influence the actual formation of pigment, or is it again an indirect cause, making itself felt only through its effects upon the supply of materials from which the pigment is synthesised? That low temperature favours pigment formation would seem to be demonstrated by autumnal coloration, and the winter reddening of leaves of *Hedera*, *Ligustrum*, *Mahonia* and other evergreens. Conversely, Overton (420) found in *Hydrocharis*, the higher the temperature, the less anthocyanin. Klebs (447) also notes that flowers of *Campanula* and *Primula* may be almost white in a hot-house, but the same individuals kept in the cold will bear coloured flowers. The consideration of temperature is perhaps more difficult than that of light; for low temperature, on the one hand, retards photosynthesis by which sugars are formed, but, on the other hand, it also retards growth, starch formation and probably translocation, thereby tending to raise the sugar contents of the tissues. High temperature, on the contrary, accelerates growth and respiration, and consequently tends to prevent the accumulation of any excess of synthetic products.

An interesting application of these views upon light and temperature effects can be made in the case of Alpine flower coloration. The subject has been extensively studied by Gaston Bonnier (375, 376, 381, 394, 415), Flahault (375, 376, 377, 379) and others, and has had a great vogue with the writers on flower coloration in connection with insect pollination. The special features of the case are intensity of flower-colour and the formation of anthocyanin in the vegetative parts. Gaston Bonnier & Flahault have compared individuals grown at heights of 2300 metres with individuals grown in the plains, and have found that the latter produce paler flowers and less anthocyanin in the leaves and stems. It seems most reasonable to suppose that these phenomena form a natural demonstration of some of the relationships which we have just been considering between colour and factors. High Alpine plants are stunted in growth, i.e. little material is expended vegetatively; they are exposed by day to intense insolation while the night temperature is low. One may therefore suppose photosynthesis to be very active, whereas starch formation, translocation and growth are retarded, these being conditions which favour high sugar and chromogen concentrations in the tissues and resultant abundance of pigment.

From considerations of the above nature in greater detail in a later chapter, it will be seen that practically all the conditions which favour anthocyanin production also result in high sugar concentration. The latter leads to increased respiration and oxygen uptake—also a phenomenon accompanying pigment formation. The suggestion will be brought forward, though as yet there is little if any experimental evidence in support, that the acid products of respiration may play a part in augmenting the coloration.

The so-called functions of anthocyanin have provided material for another main line of research. Two essentially different types of function are readily distinguished, the biological and the physiological. The biological function is a subject which, in itself, needs extensive treatment, and does not lie within the scope of this book. It is solely connected with the attractive value of the coloured floral organs for pollination by insects, and the subsidiary question of the attractive value of ripe pigmented fruits for dispersal by birds. The relationship between flower-colour and entomophily has received great attention from botanists, and the whole matter is dealt with most thoroughly in Knuth's *Handbook of Flower Pollination* (527), which includes an excellent bibliography.

The physiological function is a very difficult and far less satisfactory matter. Several different functions of a physiological nature have been attributed to anthocyanin. One of the most famous is the screen theory, the idea of which was first based on work published in 1880 by Pringsheim, who showed that chlorophyll was bleached by intense light, but not if protected artificially by a red screen. Thus the view arose that anthocyanin might be protective in function, but experimental evidence does not altogether favour this hypothesis. For, in 1885, Reinke pointed out that it is those rays absorbed by chlorophyll which have the greatest destructive effect on chlorophyll, and Engelmann (494), in 1887, demonstrated that the absorption spectrum of anthocyanin is on the whole complementary to that of chlorophyll. Hence anthocyanin absorbs those rays which are least harmful to chlorophyll, and cannot therefore be said to provide an effective screen. A second suggestion, brought forward by Stahl (505) in 1896, and largely supported by him, is that anthocyanin absorbs certain of the sun's rays, and by converting them into heat, raises the temperature of the leaf, and this may serve to accelerate transpiration in difficult circumstances, as in damp regions of the tropics, or may protect leaves from low temperature as in Alpine regions. The chief points in favour of this hypothesis are the distribution of anthocyanin in leaves of shade-loving plants, and the fact, observed also by Stahl, and confirmed in 1909 by Smith (520), that the internal temperature of red leaves is greater than that of green.

The next line of investigation, the chemical composition of the pigments, is also difficult, and though spasmodically attacked from time to time, met with no very serious consideration till 1906. So intimately connected with its chemical composition that it can scarcely be considered separately, is the question of the mode of formation of anthocyanin, that is, the chemical reactions involved in the process. Closely connected also, though in a lesser degree, is the part played by anthocyanin pigments in heredity. It is proposed therefore to deal with these three lines of research more or less together.

As already pointed out, the reactions with acids and alkalies are the most obvious and striking chemical properties of anthocyanins, and they have helped to draw the attention of chemists to the subject; for much of the earlier chemical work on anthocyanin, notably of a group of French chemists (1800–1825), Braconnot (124), Payen & Chevallier (127, 128, 129) and Roux (130), centred round these reactions, especially in some cases round their rôle as indicators. But the idea of anthocyanin as an indicator was fully conceived long before 1807 by Robert Boyle (121). "When," he says, "we have a mind to examine, whether or no the Salt predominant in a Liquor or other Body, wherein 'tis Loose and Abundant, belong to the Tribe of *Acid* Salts or not...if such a Body turn the Syrrup of a Red or Reddish Purple Colour, it does for the most part argue the Body (especially if it be a distill'd Liquor) to abound with Acid Salt. But if the Syrrup be made Green, that argues the Predominant Salt to be of a Nature repugnant to that of the Tribe of Acids." After the reactions of anthocyanin with acids and alkalies, other reactions were noticed with iron salts and various reagents, many of which modify the colour as it is modified in nature. These reactions gave rise to views among some chemists, Fremy & Cloëz (140) and Wigand (150), that natural blue, purple and red pigments are modifications of the same substance, brought about by the presence of other compounds in the cell-sap. But as analyses and investigations proceeded, the view of a certain multiplicity of pigments gained the ascendency.

One of the first actual analyses of anthocyanin was carried out in 1849 by Morot (136) who isolated the blue pigment of the Cornflower, and found it to contain carbon, hydrogen and oxygen, with nitrogen as impurity. Ten years later Glénard (143, 144) isolated the pigment of wine, found it also to contain carbon, hydrogen and oxygen, and gave it a percentage formula.

An early suggestion as to the chemical nature of anthocyanin and its mode of formation was that of Wigand (150) in 1862. This author suggested that anthocyanin arises by the oxidation of a colourless tannin-like chromogen, a substance widely distributed in plants and giving a green reaction with iron salts and a yellow reaction with alkalies. The same substance obviously had been noted at an earlier date by Filhol (139, 146) who observed it to be widely distributed, and maintained that the green coloration of anthocyanin with alkalies was due to a mixture of a blue anthocyanin reaction plus the yellow reaction of these accom-