that the next American Pilgrim Trust lecturer to address you may not feel obliged to discuss the war, but will be able to treat of some interesting aspect of the progress of science in accord with the original conception of Sir William Bragg and as a happy feature in the post-war forward march of science.

THE BIOCHEMISTRY OF ANTHOCYANINS

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A GREAT deal of work has been done all over the world on the chemistry of plant pigments. Very little work has been done anywhere on the stages through which the pigment develops in the living plant. This should be called the biochemistry of the plant pigments and is temporarily a neglected field of science. My attention has been called by Professor Bruce of Cornell to a book entitled "The Chemistry of Natural Coloring Matters" by Mayer and Cook, published in 1943. I can not find in it any information as to the probable precursors of the carotenes, to take one instance. If somebody would rewrite this book, making an exhaustive presentation of the biochemical side, he would have a masterpiece.

The red pigments in leaves, fruits and flowers and the blue pigments in flowers and fruits are mostly due to anthocyanins, though the red in the tomato, the watermelon, the berry of the mountain ash, the grape haws and the pink-fleshed Texas grapefruit is due to a lycopene. It is now known that anthocyanins are formed in plants in at least two different ways, through reduction of flavones or flavonols by ultraviolet light,[1] or through hydrolysis of what are called leuco-anthocyanins.[2] The latter reaction is often, but apparently not necessarily, accompanied by oxidation. There may be other ways of developing anthocyanins in plants; but this has not yet been proved definitely for any anthocyanin in any leaf, flower or fruit.

Light will not reduce flavones in a test-tube; but it will in some plants at some times. Therefore there must be found in some plants, under as yet unknown conditions, some substance or substances which will make flavones photosensitive. We do not yet know what this sensitizer is in any case. Flavones can be reduced electrolytically to anthocyanins,[3] but it is necessary to have a cathodic over-voltage. Consequently the reduction in the plant is undoubtedly an enzyme reaction.[4]

One important biochemical question is to determine the substance from which the anthocyanin is formed, and this has not been done in a great many cases.

With leaves which turn red in the autumn it is a relatively simple matter to test the green and then the red one so as to determine the probable precursors of the anthocyanin. This was done by Rutzler[5] in some cases. Leuco-anthocyanin appears to be the precursor in 14 per cent. of the cases. The red autumn pigments of the leaves of the sumach, the dogwood and the barberry come from a flavone and those of the sugar maple, the Virginia creeper and the Seckel pear from a leuco-anthocyanin. The leaf of the Japanese creeper, when green, contains both flavone and leuco-anthocyanin. We do not yet know which gives rise to the anthocyanin or whether both do.

When the leaves come red before they turn green, another technique becomes necessary and one was apparently devised by Abbott[6] over thirty years ago. A small copper beech was kept partially covered in the spring, and the leaves under the sacking came green. When these green leaves were exposed to the sun, red could be detected inside of two days. Abbott of course did not test the green leaves for flavones or leuco-anthocyanins. Since light turns the leaves red quickly it seems probable that flavones were present. The development of enough acid to hydrolyze a leuco-anthocyanin would probably take longer.

We do not know whether the red leaves which appear as new leaves in the tropics could come green if the leaves were shaded, as Abbott did his. The botanists and the chemists have not yet got together on this point.

With red flowers one can not usually apply the leaf technique, because it is only in a few cases that we can examine the flower before and after it has turned red. Kuyper[7] reports that the flowers of *Hibiscus mutabilis* come out white at dawn and turn red during the day. Temperature is important in producing the color change. At temperatures under 16° C. there is practically no development of pink. This makes it probable that Kuyper was dealing with a leuco-anthocyanin.

Shibada, Nagai and Kishida[8] found that the flowers of *Diervilla grandiflora* S. and Z. bloom white; but turn rose color during the day. They did not test for

[1] Bancroft and Rutzler, *Jour. Am. Chem. Soc.*, 60: 2738, 1938.
[2] Robinson and Robinson, *Jour. Chem. Soc.*, 744, 1935.
[3] Chapman, Cornell University Ph.D. thesis, 1938.
[4] Wheldale, *Jour. Genetics*, 1: 113, 1911.
[5] *Jour. Am. Chem. Soc.*, 61: 1160, 1939.
[6] *Nature*, 80: 429, 1909.
[7] Kuyper, *Rec. Trav. bot. néerl.*, 28: 1, 1901.
[8] *Jour. Biol. Chem.*, 28: 93, 1916.

a flavone; but they report that the colorless alcoholic extract produces a purple-red anthocyanin solution on reduction with mercury, magnesium and hydrochloric acid. This indicates that a flavone was present.

Sorby[9] found that by diminishing the exposure to light of a dark variety of the common wallflower, *Cheiranthus cheiri,* no anthocyanin developed, though this pigment was abundant in the flowers exposed to the sun. Apparently Sorby made no attempt to find out whether the phenomenon is general or is confined to this one flower.

We know now that the phenomenon is not general because von Sachs[10] says that "bulbs of hyacinths, tulips, etc., when germinating in profound darkness, nevertheless produce magnificent normal flowers, while the leaves at the same time become etiolated, though not strongly."

Askenasy[11] confirmed some of the results of von Sachs, but obtained a different result with *Hyacinthus orientalis.* The darkened flowers were not colorless, but they were distinctly a paler blue.

Hugo Fischer[12] says that the first appearance of color in the flower buds is generally two weeks before the time of blooming in the case of the red and blue flowers, and three to four weeks on the average with the yellow flowers. In order to minimize the disturbances of assimilation in the plants due to the cutting off of the light Fischer used small sacks or hoods of dark cloth which were not fully light-tight, but which let so little light through that one could ignore the physiological effect of the transmitted light.

When grown under a black hood the flowers of *Cydonia Japonica* [Japanese quince] were nearly pure white with just a touch of pink in the middle of each petal. Two weeks after the black hoods were taken off, the bleached flowers could not be distinguished from the normal ones.

When hooded, *Iberis umbellata* came almost completely white. *Campanula rapuncaloides, Phacelia Campanularia, Agapenthus umbellatus* and *Digitalis purpurea* were bleached somewhat, while *Althala rosea* (dark red) and *Dahlia variabilis* (double and pink) were distinctly paler but not much. On the other hand, no change could be detected with *Tradescantia virginia, Geranium pratense, Pelargonium zonale* (brilliant red) and a number of other flowers.

Although one of the yellow flowers, *Calendula officinalis,* which is a marigold, probably contains no anthocyanin, some of the petals, though not all, bleached to a pale yellow while the buttercup was unchanged.

Fischer says that there is absolutely no regularity to be found among the anthocyanins and that there seems to be no reason why one flower should bleach when grown in the dark and the other not. Fischer is ignoring possible chemical differences in the development of the anthocyanins. He could not avoid this in 1908; but we can do better now.

I read this paper by Fischer some years ago; but I read it uncritically, as apparently the other people did. I should have seen that Fischer had proved that there are at least two ways in which the plant can synthesize anthocyanin. In one case, the Japanese quince, no anthocyanin is formed when the flower is shaded; in the other case, typified by the geranium, anthocyanin is formed when the plant is shaded.

Since we know that there are two ways in which anthocyanin can be formed—[from flavones and from leuco-anthocyanins]—it seems natural to ask whether this fact will help us account for the work of Fischer and of others. If the anthocyanin is formed in the flower from a flavone it will not develop if ultra-violet light is excluded. If the anthocyanin is formed from a leuco-anthocyanin there is at present no reason to ascribe any appreciable effect to light, and the anthocyanin should form in the shaded flower. If the anthocyanin is formed in both ways in the flower, as we know it is in some leaves, we might easily get any intensity of color in the shaded flower.

It has not yet been proved that the shaded white flower of the Japanese quince contains flavone; but we know that nearly all normally white flowers do. Consequently, the white Japanese quince either contains flavones or there has been devised a method for preparing white flowers containing no flavone. While this is not theoretically impossible, the burden of proof is on the man maintaining it.

It is not clear how ultra-violet light gets into the flower bud three weeks or so before the flower blooms, and yet it must if keeping out the ultra-violet light prevents the formation.

If we water a geranium with a suitably buffered solution, perhaps a urea solution, we might be able to prevent the hydrolysis of the leuco-anthocyanin and should get white flowers which would turn red when treated with acid. I suggest urea because it is taken up readily by plant cells.

Fischer did not grow a white-flowered geranium, but he did not have any theory to guide him. Being professor emeritus I have no graduate students. Being crippled by a motor accident I can not do experimental research myself. All I can do is to point out what I think is important. I have the hope that some day it will interest some more competent person.

Somebody should extend Fischer's tests to cover all the known anthocyanins. We ought to have data on the ordinary lilac, pink carnations, peonies, oxalis,

[9] Sorby, *Proc. Roy. Soc.*, 21: 479, 1873.
[10] von Sachs, "Lectures on the Physiology of Plants," 534, 1882. Translated by H. Marshall Ward.
[11] Askenasy, *Bot. Zeitung*, 34: 1, 27, 1876.
[12] Fischer, *Flora*, 98: 380, 1908.

Christmas cactus, the red-bud or Judas tree, pink dogwood, phlox, cardinal flower, salvia and no end of other flowers. In Southern California one would include the bougainvillea.

Some red flowers, like the wild geranium, turn blue when exposed to ammonia and some do not. We do not yet know what substance should be added to the cell sap to stabilize the blue; but it should not be difficult to learn this. When we have done so, and when we have learned how to make a flavone photosensitive, we should be able to start with a plant which normally has red, white or blue flowers and make it flower either of the other two colors. We can make a hydrangea bloom red, white or blue; but this is not really a case of a patriotic posy, for we do not get the white by cutting off the ultra-violet so we are undoubtedly dealing with a different variety, as in the case of the white lilac.

It should be possible to ripen a strawberry without permitting any red color to develop. That would have no scientific value; but it would have news value.

When I was a boy we used to be told that a blackberry is red when it is green, but that is not necessarily true for a biochemist.

After the biochemistry of the anthocyanins shall have been straightened out people ought to start on the biochemistry of the carotenes and of lycopene. We know that the green tomato will turn red in the dark and of course there can be no ultra-violet light reaching the inside of the watermelon or of the pink-fleshed Texas grapefruit.

Willstätter and his successors have cleared up the chemistry of chlorophyll pretty well; but we still know very little about the biochemistry of chlorophyll.

Coming back to the anthocyanins, it is possible that zymin or reductase[13] is the enzyme or one of the enzymes that makes a flavone photosensitive. There is no proof of this as yet, but it may be true and will serve as a starting point.

SUMMARY

1. An explanation has been given for the effect of subdued light on the development of anthocyanins. This effect was observed by von Sachs and by Sorby seventy years ago. Nobody has made an exhaustive study of the subject, although Askenasy, Hugo Fischer and others have done work along these lines.

2. When a flavone is reduced to an anthocyanin, as with the Japanese quince, cutting off ultra-violet light prevents the formation of the anthocyanin.

3. When the anthocyanin is formed by the hydrolysis of a leuco-anthocyanin, cutting off of ultra-violet light will not necessarily prevent the formation of the anthocyanin. This occurs with the geranium.

4. We do not know at all approximately how many or which flowers belong to what I call the flavone type and how many or which to the leuco-anthocyanin type.

5. After the biochemistry of the anthocyanins shall have been worked out the botanists and chemists should concentrate on the biochemistry of the carotenes, the lycopenes and chlorophyll.

OBITUARY

JOSEPH SWEETMAN AMES

THE death of Dr. Joseph Sweetman Ames on June 24, 1943, brings to a close a long and eventful chapter in the history of Johns Hopkins University.

Dr. Ames' career is a striking example of a life devoted to one institution. Born on July 3, 1864, in Manchester, Vt., he went to Baltimore at the age of eighteeen to enter the university. He won his baccalaureate degree in 1886, spent a short time in study in Berlin, held a fellowship in physics at his alma mater in 1887 and 1888, was assistant in physics the two following years, and received his Ph.D. in 1890. After graduation he continued his connection with the university and rose rapidly through the positions of associate and associate professor to a full professorship in physics in 1899. Following Professor Rowland's death in 1901 he was made director of the physical laboratory. He filled this post for a quarter of a century, when he was made provost of the university. The culmination of his university career came with his appointment as president of the university in 1929. In 1934 he announced that he would retire the following year, at which time he was made president emeritus. Unhappily the period of his career as president coincided with the worst years of the depression with an increased burden of financial problems.

In only one important instance did Dr. Ames share his university allegiance with another institution. He became deeply interested in the development of aeronautics through his appointment by President Wilson as a member of the National Advisory Committee for Aeronautics in 1917. For twenty years he was chairman of the executive committee of that agency, and through his guidance the committee's facilities for aeronautical research were expanded until they now comprise three great laboratories—at Langley Field, Va.; Moffett Field, Calif.; and Cleveland, Ohio. The committee's laboratory on the West Coast is officially

13 Paladin, *Z. physiol. Chem.*, 26: 81, 1908; *Biochem. Jour.*, 18: 15, 1909.