

Pigment Production in Non-Chlorophyllous Plants

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Introduction

It is well known that many bacteria and fungi are vividly colored but at present the significance of the pigments involved remains unexplained. The present paper is devoted to a review of some features of the general problem of pigmentation in these two groups including the chemical nature of the pigments, possible metabolic functions, and the factors influencing pigmentation.

Pigments of Bacteria, Fungi, and Lichens

In recent years a number of pigments responsible for coloration in the bacteria, fungi, and lichens have been rather well characterized chemically; many of these have been isolated and studied in a highly purified state so that it has been possible to ascertain structural formulas. From the three plant groups mentioned a total of 53 seem distinct, 59 pigments from the fungi, 15 from the bacteria, and 9 from the lichens (1, 3, 4, 9, 14, 23, 29, 30, 31, 37, 39, 40, 42, and 49).

A few colored substances seem to occur in (and be essential for) all organisms: the cytochromes, riboflavin compounds, carotenes, and, perhaps, thiochrome (the oxidized form of thiamin). These substances are metabolic agents or components of substances probably necessary for carbohydrate utilization in all living cells.

Chemically the pigments are quite varied in structure and only the more important classes need be considered. Some believe that acids, antibiotics, and pigments may be compounds related to one another in the organism (42, 50).

Significance of Pigmentation

The role in metabolism of the majority of pigments of bacteria and fungi is not known. The activity of the respiratory pigments, cytochromes and flavins, in the breakdown of carbohydrates now seems well established. There can be no doubt of the essential nature of the bacteriochlorophylls for the normal existence of the photo-synthetic bacteria. However, in comparison, little of a specific nature has been discovered of the activity of the carotenoids, the quinones, anthraquinones, the oxonium compounds, and other classes of biochromes. It seems certain that substances with the potential chemical activity of the above pigments play important roles and that studies in this field should be extremely promising. The dismissal, by some, of pigments as unimportant end-products seems unwarranted; in any case, it would be of great importance to know the reactions leading to the formation of pigments since in most cases they represent syntheses of compounds not easily made in the laboratory at the present time.

Despite the paucity of experimental work on the function of pigments in the organism some roles have been reported for them and others have been suggested on a hypothetical basis.

The photosynthesis by bacteriochlorophyll in the synthesis of carbon compounds (sugars?) from CO_2 is very similar to that of the higher green plants (39). It seems probable that the photochemical roles of both chlorophyll and bacteriochlorophyll are the same although the details of some of the steps in photosynthesis may differ.

With regard to the cytochromes there is an increasing amount of evidence that they are of very general occurrence and are of fundamental importance in biological oxidation as respiratory pigments. They form an oxidation-reduction system which is responsible for the transport of hydrogen and oxygen in the process of respiration leading to the release of energy and the final products, H_2O and CO_2 (49).

A point of great interest is the similar features of the cytochromes of organisms in general, the chlorophylls of plants, and hemoglobin of animals. One cannot help wondering whether the cytochromes may be the parent materials phylogenetically, of both hemoglobin and chlorophyll. From this standpoint the pyrrole pigment prodigiosin is of great interest since it may represent a precursor of the porphyrins or a degradation substance. In any case a search for other pyrrole pigments seems well worth while.

Friedhem (18), reported that phoenicin stimulated the respiration of unpigmented, washed cells of *Bacillus pyocyaneus* by 200-300 percent. A similar function was found for aspergillin which was therefore considered to be a respiratory pigment (42). Pyocyanine, also, in lower concentration stimulates respiration but in higher concentration it inhibits the growth of many bacteria and fungi (27). Pyocyanine is subject to either oxidation or reduction and it appears to act as a respiratory enzyme or coenzyme (49).

Raistrick (42) has expressed the view that several pigments probably function in oxidation-reduction systems. One line of evidence for this view is the occurrence in organisms of both reduced and oxidized forms in the cases of physcion, phoenicin and luteoleersin. The existence of equilibrium mixtures of the reduced and oxidized forms undoubtedly make them a part of the oxidation system but their relative importance in this respect remains to be studied quantitatively. The activity of such pigments can be compared with that of cytochrome and other hydrogen carriers in oxidative metabolism since they readily accept H^+ ions to become reduced and lose them in oxidation.

A study of the effect of xanthenes on metabolism revealed that rubrofusarin gave a 3% inhibition of the rate of dehydrogenation in non-pigmented *Fusarium lini* (36). However, there was a 40% increase in dry weight. Other xanthenes (synthetic) increased the rate of dehydrogenation from 5 to 12%. Mull and Nord conclude that the xanthone derivatives may have a dual but interdependent effect and that synthesis may be enhanced or inhibited while dehydrogenation is depressed or increased according to the structure of the xanthone employed. They

consider that substances which have hitherto been regarded as insignificant "waste products" must be taken into account in enzyme studies.

It is readily seen that there are many pigments whose biological activity remains uninvestigated. From the structures represented these substances seem potentially as active in metabolism as many of the intermediates whose functions are now known. A number of pigments—fumigatin, spinulosin (42), citrinin (34) pyocyanine, iodinin, and litmocidin (19)—have been reported to be antibiotics. This again is an indication of reactivity but does not explain their role in normal schemes. The antibiotic activity of the quinones fumigatin and spinulosin is due to their oxidation of essential sulfhydryl compounds (42)—a process which may be of value under certain conditions. The mechanics of inhibition in other cases has apparently not been reported.

Another function ascribed to pigments is that of some unknown role in reproduction. In many non-green forms the spores and reproductive structures have a distinctly localized pigmentation. Metz (33) expressed the view that in *Aspergillus niger* the pigment was important in sporulation. Thom and Raper (51) discuss the change of color during development of the *Aspergilli* and suggest that pigment studies should be related to this change. They cite evidence relating certain changes in metabolism of the *Aspergillus flavus* group with color variations due to pH. Gray (22) found evidence for a photochemical reaction in the induction of myxomycete plasmodia to form sporangia. Only the pigmented forms were stimulated to fruit, the non-pigmented forms being insensitive to light in this regard. Gould and Railstrick (21) working with auroglaucin and flavoglaucin produced in *Aspergillus novus* noted that auroglaucin almost entirely disappeared during conidia formation. They further believed there was a correlation between perithecia formation and the accumulation of the two pigments. It is reported that γ carotene is the only carotene present in most species and it is restricted to the male reproductive cells in *Allomyces* (16). In *Mucor hiemalis* the plus strains produce greater amounts of β carotene than the minus strains (7).

Thus far no discoveries of pigment activity in the fungi and bacteria compare with the remarkable effect on sexuality in *Chlamydomonas* reported by Moewus and Kuhn (see reviews: 46, 47) of carotenoid pigments and other related compounds which act in a most striking system to control the motility of cells, the sex of gemates, their agglutination preliminary to conjugation, and the actual fusion of male and female gametes. Unfortunately this work has not received the confirmation or condemnation which it deserves. Nevertheless it suggests many possibilities and, for the first time, ascribes definite functions in reproduction to the carotenoids.

There are indications that the absorption of light prevents injury in several forms: *Microcera coccophila* (14), *Polyporaceae* (8), and *Puccinia graminis* spores (15). In the latter case red or gray-walled spores have a higher resistance to injurious effects or ultraviolet radiation than orange or clear-walled spores.

In some forms the accumulation of pigments appears to represent an unhealthy condition. Roberts (44) concludes that the pigmented cells

of *Torulopsis pulcherrima* are no longer able to grow and divide. In the Polyporaceae, Bose (8) found the coloring substances accumulated in the walls or in dead cells but not in living cells. These occurrences, however, do not necessarily signify that the pigments were the cause of the death of the cells since a deficiency or excess of many other substances may have been responsible. In the case of *Aspergillus novus*, which produces flavoglauclin and auroglauclin. Gould and Raistrick (21) obtained a yield of crude pigment equivalent to 43% of the mycelium dry weight. Yields in other cases are as high as 10-30%. Such high accumulations could surely not occur with excessively toxic compounds.

The Effects of Various Factors on Pigment Production

In the fungi the pigments may be variously localized, in some forms occurring in the mycelium and substrate, in others the color may be restricted to the aerial parts, and, in still other forms, only the spores may be colored. The coloration of aerial parts seems to be a relatively constant genetic feature and is universally used in the taxonomy of species. Most—but not all—of the quantitative measurements have been concerned with the biochromes found in both the mycelium and substrate. The latter pigments appear to result from an intimate interaction of the organism and its substrate and thus is an inherited characteristic sensitive to nutritive and environmental factors.

Physiological factors:—There have been many references to the influence of various environmental and nutrient factors on coloration, many conflicting statements have appeared, and very few general conclusions may be made. However, only recently has there been any effort to make quantitative measurements of pigments produced by organisms growing on a synthetic medium. Such quantitative observations seem necessary—in addition to the knowledge of qualitative effects—if an orderly understanding is to be gained. In addition, since the pigments represent a variety of compounds chemically, each fungus and each pigment constitute a different problem.

Among the factors found to influence pigmentation are light, temperature, organic nutrition, and inorganic nutrition.

Light:—The effect of light has been investigated in a number of species with variable results. Pigment formation is apparently induced or stimulated by light in *Microcera coccophila* (41), *Neurospora crassa* (23), and *Coccosporium* sp. (25). Light has no apparent effect on color formation of *Torulopsis pulcherrima* (44), several species of the Polyporaceae (8), and several myxomycetes (22).

Haxo (23, and private communication) found that the formation of carotinoids in *Neurospora* is stimulated by exposure to light. Relatively short exposures followed by incubation in complete darkness were sufficient to stimulate pigment formation.

Unpublished studies of *Coccosporium* show that in this form some pigment is formed in complete darkness but more is produced in the presence of light (25). Light is necessary for production of color in some bacteria also: *Serratia marcescens* (2); two unidentified species (6).

Temperature:—In general it appears that the temperature range optimal for growth also favors greatest pigment formation (12, 21). In *Torulopsis pulcherrima* temperature appeared to have no effect on the amount of pigment produced as estimated visually, but at temperatures above 19° C the pigment diffused out of the cells into the substrate (44). In *Coccosporium* sp. best pigment formation occurs at 12-18° C while greatest mycelium growth requires about 25° C (24). On the other hand with *Fusarium coeruleum* on a sucrose medium no pigment is formed below 30° C, whereas 15-25° C is the optimal growth temperature (35).

In some chromogenic strains or coliform bacteria strong coloration occurs at 19° C but not at 37° C although growth is favored at the latter temperature (20). Bunting (11), in a study of *Serratia marcescens*, concluded that in this form lower temperatures favor a higher proportion of dark red to light-colored variants.

Oxygen:—Aeration plays an important role in pigment formation in many cases (24, 39, 44) and it seems probable that oxygen is usually essential for the process. However, in view of the recent discovery that non-green organisms may assimilate carbon dioxide, the problems of aeration in pigmentation should be more critically investigated. Brown (10) noted that *Sphaeropsis malorum* was stimulated in growth by carbon dioxide but he failed to record an effect on coloration. In the bacteria aeration is usually needed, but McClung (32) has already pointed out that despite a statement to the contrary there do exist a number of pigmented anaerobic bacteria.

According to Roberts (44) when *Torulopsis pulcherrima* cultures were grown in the absence of air no pigment developed but, then, if air was admitted, color appeared in 5½ hours. Roberts concluded that there must develop under anaerobic conditions a precursor substance, a chromogen, which requires only the presence of oxygen to be oxidized to the colored compound.

Osmotic concentration:—Species of the *Aspergillus glaucus* series do well in media of high osmotic concentrations, some tolerating a 50% glucose solution; greater pigmentation occurs under these conditions (21). Most species of bacteria and fungi do best on much weaker solutions and it seems improbable that osmotic concentration has any direct effect.

Hydrogen-ion concentration:—As in the case of most other environmental factors the optimal pH range for both growth and pigmentation are about the same (12, 14a, 21, 26, 44). However, hydrogen-ion concentration is a function of the organism and its substrate so that a marked shift of pH may occur during development, the direction being largely dependent upon the organic carbon and nitrogen sources. Frequently such changes may go beyond the range for best growth in which case less pigment is to be expected.

In *Penicillium carmino-violaceum* (26) and *Torulopsis pulcherrima* (44) changes toward an alkaline range increases the outward diffusion

of pigment. In *Coccosporium* photometric measurement of pigment in the substrate and the mycelium extract showed no significant difference in outward diffusion due to pH according to Higinbotham and Powers (24). However, the later authors found that the initial pH value of the medium had a much greater effect on pigment production than on mycelium dry weight so that at certain values good growth was obtained with little pigment formation.

Mineral nutrition:—There are numerous reports of the effects of minerals on coloration of the bacteria and fungi. It seems obvious that the minerals essential for growth are also necessary if pigmentation is to occur. The need for Mg, P, and S has been affirmed repeatedly but the reports regarding the effect of Fe and other trace elements are in conflict. It seems probable, however, that Fe, Zn, Mn, B, Mo, Ga, and Cu, are essential for the growth of fungi and some of these particularly Fe and Zn, affect pigmentation in a number of species (17). Appropriate iron concentrations stimulate pigment production in *Penicillium citrinum* (?) (5) and *Torulopsis pulcherrima* (44). In *Fusarium oxysporium* the relation of zinc and iron may control the formation of one or both the red and blue pigments normally present in this species (17).

There is good evidence that at least some bacteria require Fe, Mn, B, and Cu (48). Fe has been reported by Bunting (11) to be necessary for pigmentation in *Serratia marcescens* but Dewey and Roe (14a) reported that Fe was not needed. Waring and Werkman (52) in a careful study found that Fe was necessary for six bacteria including *Serratia marcescens*. In the latter form the critical range of Fe concentration was much narrower than that for growth. Waring and Werkman also found that organisms with a complete cytochrome system required greater amounts of Fe than those having only partial cytochrome systems.

Most reports of the effect of mineral elements on chromogenic organisms have been of a qualitative nature. It is to be hoped that new studies will be made with quantitative estimation of pigment production due to the addition of trace elements so that more specific information may be obtained.

Carbon and nitrogen sources:—It appears that the source of organic carbon is of great importance in pigment production since frequently organic compounds may support growth with little or no coloration (12, 19, 21, 26, 45). In some cases the failure of pigmentation appears to be due to pH changes during growth (26) but generally the unfavorable action has not been explained.

Reference to Table 1, which shows suitable carbon and nitrogen sources for growth and pigmentation of several forms, is indicative of the relatively simple compounds on which various organisms may grow and synthesize pigments. Glycerol is an excellent carbon source for several bacteria and fungi and is evidently metabolized by them. An exception to this, perhaps, is *Bacillus pyocyaneus* which is stimulated to produce pigment by glycerol but the latter is not used in the process (28).

TABLE 1. Some sources of carbon and nitrogen suitable for pigmentation as reported in a few recent studies of chromogenic bacteria and fungi.

| Organism | Pigment (if known) | Suitable Carbon Source | Suitable Nitrogen Source | Reference |
|---------------------------------------|--|---|--|-----------|
| BACTERIA | | | | |
| Actinomyces coelicolor | ———— | glycerol glucose NH ⁴ acetate | asparagine NH ₄ acetate | 13 38 |
| A. violaceus ruber | ———— | glycerol glucose | asparagine | 13 |
| Aerobacter sp. | ———— | glycerol | NH ₄ citrate | 20 |
| Bacillus pyo- cyaneus | pyocyanin | glycerol | NH ₄ citrate | 28 |
| Escherichia sp. | ———— | glycerol | NH ₄ citrate | 20 |
| Proactinomyces cyaneus | litmocidin | glucose | tryptone and peptone | 19 |
| Pseudomonas aeruginosa | ———— | glucose | (NH ₄) ₂ SO ₄ | 52 |
| Serratia mar- cescens | prodigiosin | glucose | (NH ₄) ₂ SO ₄ | 52 |
| Serratia mar- cescens | prodigiosin | glycerol asparagine | NH ₄ citrate NH ₄ citrate | 11 14a |
| FUNGI | | | | |
| aspergillus glaucus series | flavoglucain auroglucain rubroglucain | glycerol glucose sucrose maltose glucose | NH ₄ tartrate | 21 |
| Coccosporium sp. | ———— | sucrose mannose glucose | NO ₃ ⁻ | 24 |
| Helminthospo- rium gra- mineum | helminthosporin hydroxy-iso- helminthosporin | glucose fructose sucrose maltose starch glycerol | NO ₃ ⁻ | 12 |
| Penicillium carmino-vio- laceum | carviolacin carviolin | ———— | ———— | 26 |
| P. citrinum? | citrinin | glucose | NH ₄ tartrate | 5 |

Several carbon sources of equal value have been reported for *Aspergillus glaucus* spp. and *Helminthosporium gramineum* (Table 1). This conclusion was apparently based on visual comparison. However, in a similar instance Higinbotham and Powers (24) measured pigment concentration photometrically and found distinct differences between glucose and sucrose as carbon sources for *Coccosporium*. Pigmentation was much greater with glucose than with sucrose although this might well have gone undetected visually. Furthermore, sugar concentration optimal for color formation was lower than that giving maximum dry weight.

The nitrogen source may be inorganic for most forms studied (Table 1). The combination of NH_4 and an organic acid appears to chiefly prevent undue acidification of the medium, although it may also act as an additional source of carbon in some cases. Some of the more complex nitrogen sources may give little or no pigmentation (12, 19, 21).

The carbon/nitrogen ratio is an important factor in pigment production with the *Aspergillus glaucus* series (21), *Fusarium coeruleum* (35), *Penicillium purpurogenum*, *Paecilomyces* sp., and *Coccosporium* sp. (43). In the latter species it was found that excessively high or low C/N ratios may give appreciably less pigment production than a median C/N ratio. Growth, as measured by dry weight, was less affected by the C/N ratio.

Discussion and Summary

At the present stage little of a conclusive nature may be said of the significance of pigmentation in the bacteria and fungi. Interest has grown in this field recently and now the chemical structure of more than 70 of the pigments has been elaborated. The chemical nature of many of the pigments is such that they potentially could have important functions in metabolism such as hydrogen carriers or respiratory pigments. There is evidence that pyocyanine, phoenicin, and rubrofusarin are active in enzyme systems. It has been suggested that pigments may have a role in development, in determination of sex, and in sexual reproduction and there is some evidence of each of these possibilities. Several pigments have an antibiotic action.

The accumulation of pigments in a number of species indicates that they are products which are not deeply involved in metabolism. Yet this is no criterion of their importance since the cells in which they accumulate are older and less active. Furthermore the studies of pigment production under the influence of various factors shows that pigments accumulate in many cases only in a relatively narrow range of conditions. This is suggestive of alternative metabolic systems under which growth, but not pigmentation, may occur. The precise systems leading to pigment synthesis cannot fail to be significant.

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