LIGHT AS A FACTOR IN THE PRODUCTION OF PIGMENT BY CERTAIN BACTERIA

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Pigment formation is a phenomenon common in bacteria. Some workers have attempted to show that pigmentation serves a useful purpose, but, except for the purple bacteria, there is little evidence to indicate that pigments are really of value to the organisms that possess them. The bacteriologist makes use of them, it is true, for purposes of identification, but this can hardly be said to serve the organism.

Pigments are often variable and not infrequently the ability to form pigment is permanently lost without altering other metabolic activities in any noticeable way. That the presence of molecular oxygen is necessary for pigment formation has long been known. Obligatory anaerobes, therefore, are never pigmented. It is also well known that high incubation temperatures are unfavorable for pigment formation, and that continuous incubation at body temperature will often permanently deprive many of the saprophytic chromogens of their color-forming habits. The pH of the culture medium has an influence in many cases and also the presence of certain chemical substances in the culture medium.

Recently, while studying a group of acid-fast bacteria, an organism was observed which developed a rich pigment when grown in the presence of light but which was wholly unpigmented when incubated in darkness. The examination of a rather large collection of acid-fast organisms, most of which were pigment-forming, revealed an additional strain exhibiting this peculiar action toward light.

This experience was wholly new to every one in our laboratory, and a search of textbooks and manuals failed to unearth any reference to such a phenomenon. Finally, however, a reference (Prove, 1887) was found to an early observation of this sort.[1] Since this observation apparently has been overlooked by modern bacteriologists, we considered it desirable to call attention to it, and to describe the phenomenon as we have seen it in our cultures.

EXPERIMENTAL

The phenomenon was first observed in an acid-fast organism pathogenic for the small tropical killifish (*Platypoecilus maculatus*) with which we are working. This organism grows quite slowly. Its optimum temperature is about 30°C.; it does not grow at 37°C. From ten days to two weeks' incubation is required for fairly luxuriant growth, but the maximum growth is not attained until considerably later. Cultures which were incubated in the dark were essentially colorless, whereas those that were allowed to stand on the laboratory table in the presence of light developed a deep orange color. The work that is described below was done with this strain. The only other strain in our collection which exhibited the property was one which had been isolated from cow's milk by Plum.[2] This culture is quite unlike the killifish strain in all respects except the behavior toward light. It grows at 37°C. and develops quite rapidly. When grown in the dark it is unpigmented. In the light it takes on a rosy pink shade.

EXPOSURE TO DIFFERENT LIGHT RAYS

In these brief studies, no attempt was made to measure the quality or the intensity of the light sources used. Three kinds

[1] *Micrococcus ochroleucus* found in the urine of man.

Morphology—micrococcus from 0.5–0.8 microns in diameter, solitary, in pairs, or in short chains.

Biological characters—aerobic, non-liquefying, chromogenic micrococcus. Develops in usual culture media at room temperature. Colonies appear on gelatin plates at the end of twenty-four hours. From the description this organism apparently does not belong to the acid-fast group of bacilli.

[2] Culture received in 1932 from N. Plum, Serum Laboratory, Royal Veterinary and Agricultural College, Copenhagen, Denmark.

of light were tested for their efficiency in producing coloration in fully developed cultures which had been grown in the dark—sunlight, light from a 100-watt Mazda lamp, and ultra-violet light from a small, water-cooled, mercury-vapor lamp equipped with a Wood filter.

The sunlight exposures were made in the middle of the day during August and September. The exposures to the incandescent lamp were made at a distance of approximately two feet. Exposures to the mercury vapor lamp were at a distance of about three inches.

TABLE 1

The effects of light on pigmentation

LIGHT SOURCE	TIME EXPOSED	RESULT
Sunlight	1 minute	Partially colored
Sunlight	15 minutes	Full coloration
Sunlight	60 minutes	Partially colored
Sunlight	100 minutes	No pigmentation
Incandescent lamp	5 minutes	No pigmentation
Incandescent lamp	15 minutes	Partially colored
Incandescent lamp	30 minutes	Full coloration
Ultra violet	10 seconds	Partially colored
Ultra violet	1 minute	Full coloration
Ultra violet	20 minutes	No pigmentation

Exposures to the incandescent lamp and to sunlight were made in tubes (glycerol-agar slants). Cultures exposed to the ultra-violet lamp were growing on agar plates, from which the lids were removed during the exposure period.

The appearance of pigment does not occur immediately upon exposure to light. Usually coloration can be seen within twenty-four hours after exposure is begun and virtually the full intensity is reached within forty-eight hours. The results which are shown in the following table were recorded after forty-eight hours' incubation in darkness following the exposure period.

These data suggest that it is the shorter light rays that affect pigmentation. Although direct comparisons can not be made

because of the differing intensities of the light sources, it is significant that the ultra-violet operated very quickly, and that sunlight was much more effective than light from a Mazda lamp. It will be noticed that exposure to ultra-violet for 20 minutes and to sunlight for 100 minutes produced no pigmentation. Evidently these exposures were lethal; at any rate subcultures could not be obtained.

TABLE 2

The temperature influence on pigmentation

EXPOSURE TEMPERATURE	STORAGE TEMPERATURE	RESULT
°C.	°C.	
4	4	No pigment
	24	Partial (+)
	37	Partial (++)
24	4	No pigment
	24	Partial (++)
	37	Partial (+++)
37	4	No pigment
	24	Full color (++++)
	37	Full color (++++)
Unexposed controls	4	No pigment
	24	No pigment
	37	No pigment

EFFECTS OF TEMPERATURE ON PIGMENT FORMATION

Experiments were conducted to test the influence of temperature on the formation of pigment. Three temperatures, 4°, 24°, and 37°C. were employed. The cultures were on agar slants, and were fully-grown and non-pigmented in the beginning. The culture tubes were placed in glass beakers filled with water which was maintained at the desired temperature. All beakers were freely exposed to bright, midday sunshine for thirty minutes. After exposure the cultures were grouped into three lots which were then held in the dark at 4°, 24°, and 37°C. respectively. The degree of pigmentation in each case is indicated in table 2.

Thirty-seven degrees Centigrade, a temperature at which the

organism will not grow, favored chromogenesis more than 24°C., a temperature at which growth will develop. Color formation did not take place when the cultures were stored at 4°C. Further observations have shown that when the cultures stored at 4°C. were subsequently placed at 37°C. for 48 hours, the same degree of pigmentation developed as in those stored at 37°C. immediately after exposure to sunlight. Pigmentation did not occur in the control cultures.

OXYGEN RELATIONSHIP TO PIGMENT FORMATION

Wright's method of chemical absorption of oxygen with alkaline pyrogallol was used to remove the free oxygen from fully developed, non-pigmented cultures. These cultures, as well as aerobic cultures of the same age and grown on the same medium, were then exposed to sunlight for 30 minutes. Pigment did not develop in the anaeorobic tubes. The anaerobic environment apparently did not harm the bacteria and subsequent exposure under aerobic conditions showed the usual pigmentation. The control tubes, having access to atmospheric oxygen, showed color formation. Molecular oxygen was, therefore, apparently necessary for pigment formation.

THE LIVING ORGANISM AND PIGMENT FORMATION

We have not attempted an exhaustive study of the mechanism of pigment formation by this organism under the influence of light. We wondered whether light acted simply to activate a chemical change which conferred color on some substance which had already been elaborated in an uncolored state, or whether the color-containing substance was formed only under the influence of light.

Three observations, however, tend to indicate that light so alters the metabolic activities as to cause the organism to form pigment, and that a precursor of this pigment is not present in cultures incubated in darkness. These observations are:

1. Unpigmented cultures killed by heating at 60°C. for thirty minutes will not produce pigment.
2. Unpigmented cultures exposed to sunlight or ultra-violet

light long enough to destroy their vitality do not thereafter produce pigment.

3. The pigment of this organism is readily soluble in alcohol. When unpigmented cultures are extracted with alcohol, however, and the extract is exposed to light while being constantly aerated, no pigment appears in the solution.

The fact that pigment forms rather rapidly after light exposure in fully grown cultures, which are colorless as a result of having been grown in darkness, is an indication that the coloring substance appears in old cells. This is also indicated by its formation at 37°C., a temperature which is too high for multiplication but not high enough to destroy the vitality of the culture.

SUMMARY

1. Two cultures of acid-fast bacteria have been found which form pigment when cultivated in the presence of light and are wholly devoid of it when cultivated in darkness.

2. Brief exposure to ultra-violet and sunlight, and longer exposures to electric light, confers on fully developed unpigmented cultures the ability to form pigment during a subsequent period in darkness.

3. The mechanism of pigment formation under the influence of light is not known; however, it is evident that it is a vital phenomenon and is not merely the result of a chemical reaction induced by light in substances preformed by the organism. The evidence for this statement is as follows: (*a*) Unpigmented organisms killed by heat or by ultra-violet light do not develop pigment. (*b*) Pigment is not formed in living cultures incubated at 4°C. after exposure. (*c*) Whereas, alcohol will extract the pigment from cultures grown in the presence of light, alcoholic extracts of non-pigmented cultures do not become colored when so exposed.

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ADDENDUM

Since this paper was submitted for publication it has been discovered that the phenomenon described is not a rare one.

Of a lot of 185 strains of acid-fast bacilli, 24 strains have been found in which light affects pigment formation. Of these, 22 produced slight pigment when grown in the dark and brilliant orange when grown in diffuse daylight. Two other strains were wholly colorless when cultivated in darkness and pigmented when exposed to light. One of the latter was a strain of the avian type of tubercle bacillus. Although cultures of the mammalian tubercle bacillus have not been tested for this specific reaction, it has been noticed that strains handled by students in class work often exhibit considerable pigmentation, whereas the same strains kept in our stock collection do not exhibit color. It is thought that exposure to light may account for this difference.

REFERENCE

PROVE, O. 1887 *Micrococcus ochroleucus*, eine neue chromogene Spaltpilzform. Beit. z. biol. Pfl., **4**, 409-439.