RIBOFLAVIN SYNTHESIS IN CULTURES OF *PHYSARUM POLYCEPHALUM*[1]

WILLIAM D. GRAY

Department of Botany and Plant Pathology, The Ohio State University, Columbus 10

The Myxomycetes, of which there are probably no more than 400 species, are rather unique among the fungi in that the vegetative phase is not mycelial in the usual sense but consists of a typically fan-shaped, coenocytic, reticulated structure, the filaments of which have no rigid cell walls. This structure, the plasmodium, is capable of moving slowly about over the substratum and obtains its food by engulfing bacteria, fungus spores and other small bits of organic matter. Viewed by some as merely a large mass of naked protoplasm, the plasmodium is on the contrary a well-developed, well-coordinated organism. Myxomycete plasmodia may be pigmented or colorless, depending upon the species, and a variety of different plasmodial colors are known. Of the pigmented forms, however, the majority are some shade of yellow.

The culture of some 20 or more species of Myxomycetes upon artificial media has been reported by various workers, but by far the greatest success in so culturing has been experienced with the yellow-pigmented form, *Physarum polycephalum* Schw. Howard (1931) first reported the successful cultivation of this species, and Camp (1936) devised a moist chamber culture method which makes it possible to easily obtain relatively large quantities of plasmodium. In view of the success experienced with the cultivation of *P. polycephalum*, it is not surprising that many of the physiological studies conducted with Myxomycete plasmodia during the past two decades have been performed with this species. Seifriz and Zetzmann (1935) found that a natural pH indicator was present in this species and that the plasmodial color would change from green to reddish-range through various intermediate colors over the pH range of >8 to <1. These workers suggested that the plasmodial pigment belongs to the group of respiratory "ferments" known as flavones, lyochromes or flavins. In another study Gray (1938), working with both pigmented and non-pigmented plasmodial types, found that in pigmented plasmodia the pigment seems to be intimately associated with the fruiting process, and that in general yellow-pigmented plasmodia would form fruiting bodies (sporangia, aethalia, plasmodiocarps) only if exposed to visible light, whereas the non-pigmented species studied formed their fruiting bodies equally well in light or darkness. These general findings were subsequently substantiated by Sobels and van der Brugge (1950). It was later found (Gray, 1939) that if light intensity remained constant, time required for initiation and completion of the fruiting process varied directly with pH value over the range of pH 3.0 to 7.0; no fruiting was obtained when the liquid in moist chamber cultures was adjusted to values as high as pH 8.0.

In a more recent study (Gray, 1953) it was reported that the effect of pH on the fruiting process was not a direct one but that pH value was effective only in that it affects light absorption by the plasmodial pigment, and that the factor primarily responsible for initiation of the fruiting process is light as was initially postulated. Thus, at a pH of 3.0, the pigment absorbs far more visible light than it does at pH 6.0 and hence fruiting would be expected to occur in a shorter period of time at the lower pH value; this latter expectation was verified experimentally.

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On the basis of these observations, the important role which the yellow plasmodial pigment plays in the fruiting process seems apparent, and hence an attempt to make a more specific characterization of the pigment seemed to be indicated. For that reason the present study was initiated.

EXPERIMENTAL METHODS AND RESULTS

The Myxomycete used in this work was the L–2 strain of *Physarum polycephalum* (Gray, 1944). The organism was cultured by the moist chamber method devised by Camp (1936) at room temperature (ca. 28° C) in strong natural light.

Quantitative riboflavin analyses were made by the microbiological method of Snell and Strong (1939). For analysis of a Myxomycete culture, the entire culture (i. e., plasmodium, partially digested rolled oats, filter paper and water) was extracted with 0.1 N HCl by autoclaving for 15 min. at 15 lb. gauge pressure; the volume of the extract was then measured and aliquots of different volume were used for the determination of riboflavin content.

Since Seifriz and Zetzmann had suggested the possibility of the plasmodial pigment being a flavin and since there exists a good microbiological method for the estimation of this vitamin, the first experiment was purely qualitative and was conducted primarily to determine if the plasmodium contains this compound. Aliquots of extract from a small amount of wet, freshly cultured plasmodium were added to the basal medium of Snell and Strong in lieu of riboflavin and it was found that this extract supported growth of *Lactobacillus casei* and hence contained riboflavin. The possibility existed, however, that the riboflavin present in the plasmodium may have had its origin in the rolled oats which were fed to the organism and so a more carefully controlled experiment was then conducted.

TABLE 1

The synthesis of riboflavin in moist chamber cultures of Physarum polycephalum

Expt. *No.*	Age of culture (*days*)	Rolled oats fed (*grams*)	Riboflavin in rolled oats (*micrograms*)	Total riboflavin in culture (*micrograms*)	Riboflavin *increase* (*micrograms*)	(*percent*)
1	4	6.0	46.5	470	423.5	910
2	4	8.6	66.7	264	197.3	296
3	3	4.3	33.3	228	194.7	584

A moist chamber culture of *P. polycephalum* was started using a small piece of fresh plasmodium as inoculum. The plasmodium was fed with small portions of rolled oats at 24-hour intervals, each portion of rolled oats being carefully weighed. At the end of 4 days the entire culture was extracted and the extract used for riboflavin analysis. A similar extraction and analysis was made with finely-ground, dry rolled oats from the same source as those used as plasmodial food. The extract from the 4-day old culture was found to contain 470 μg. of riboflavin. The average riboflavin content of the dry rolled oats was found to be 7.75 μg./gm., and since a total of only 6.0 gm. of rolled oats had been fed to the plasmodium during the 4-day culture period, only 46.5 μg. of riboflavin had been introduced into the culture in rolled oats. Thus, during the culture period there was an increase in riboflavin in the culture of 423.5 μg.—an increase of approximately 910 percent. The results of this experiment as well as those obtained in two similar experiments are summarized in table 1.

DISCUSSION

On the basis of the results presented in table 1 it is obvious that relatively large amounts of riboflavin are synthesized in moist chamber cultures of *P. polycephalum* during a growth period of 3 to 4 days. The largest increase both in terms of actual amount as well as percentage was obtained in the first experiment in which 423.5 μg. representing an increase of 910 percent over the quantity supplied to the culture in rolled oats, were synthesized. The total riboflavin values reported in each experiment are very probably low and do not truly reflect the amounts actually synthesized, since the growing cultures were exposed to natural visible light for about 12 hr. out of each 24, and some of the riboflavin synthesized may have been destroyed by light, since this compound is known to be photolabile.

Thus far in the discussion it has been stated only that riboflavin was synthesized in Myxomycete cultures, and this is the only statement that can be made with certainty at this time; however, it seems highly probable that riboflavin was in fact synthesized by the plasmodium. Plasmodia cultured in moist chambers as described above are not pure cultures, and the possibility exists that compounds found in such cultures may have been synthesized by bacterial contaminants; however, when plasmodia are not overfed bacterial contamination is not great. In the experiments here reported, small quantities of rolled oats were fed and bacterial contamination was slight.

SUMMARY

Analyses of whole cultures of plasmodia of *Physarum polycephalum* have shown that relatively large quantities of riboflavin may be synthesized in such cultures. In view of the fact that rolled oats were fed to the plasmodia in small quantities with resultant low level of bacterial contamination, it seems probable that most if not all of the riboflavin was synthesized by the Myxomycete plasmodia. While it is not suggested that the yellow pigment of the plasmodium consists only of riboflavin, it seems evident that part of the yellow coloration may be due to this vitamin.

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