MICROBIOLOGICAL ASPECTS OF RIBOFLAVIN

I. INTRODUCTION. II. BACTERIAL OXIDATION OF RIBOFLAVIN TO LUMICHROME

JACKSON W. FOSTER

Research Laboratory, Merck & Co., Inc., Rahway, N. J.

Received for publication September 2, 1943

I. INTRODUCTION

In view of the vast amount of study devoted to the chemical, physiological, biochemical, nutritional and clinical aspects of riboflavin, it is surprising that a direct approach has not been made to the biosynthesis and biological degradation of this vital substance. The unique and complex nature of riboflavin raises intriguing possibilities in regard to the mechanism of its synthesis and degradation in biological systems. One can envisage from its distinctive structure that a sequence of a large number of individual enzymatically-catalyzed synthetic reactions, all integrated by the orderly workings of the living cell, are involved.

The ultimate object of this and succeeding papers is to study these steps in so far as they can be identified and separated. Although it is hardly to be believed that all the specific answers can be obtained as to how cells put together the structure of riboflavin from metabolic fragments derived from simple substances such as carbon dioxide, carbohydrates, amino acids and minerals, it is hoped that some further understanding of biological synthetic reactions will eventuate from such studies.

A direct approach to the problem of the mechanism of natural synthesis of riboflavin is not immediately available, or at least is not immediately obvious. The first requirement would be to employ a system which lends itself to this type of study by its ability to synthesize appreciable amounts of riboflavin. This system should be capable of being handled under controlled conditions. At present the indications are that microorganisms provide the only feasible approach.

However, despite having an organism which under certain conditions can synthesize riboflavin in amounts greater than its own dry cell weight, an attack on the mechanism has not been as fruitful as expected, due primarily to an inability to separate the numerous reactions involved in the biological synthesis of the riboflavin molecule. It has long been the feeling of the writer that the study of the biosynthesis of substances possessing relatively complex structures might be more expeditiously approached in an indirect manner, namely, by elucidating the intermediary steps in the biological decomposition of the particular compounds in question. There is ample evidence in the biochemical literature that the mechanisms or steps involved in the degradation of biological molecules are not unrelated to those which accomplish the reverse process. Probably only because our knowledge of the dissimilation of other biologically important compounds is so fragmentary, is the reversibility of other reactions still a matter for speculation.
Biological degradation of riboflavin in nature must proceed at all times and doubtless at a marked rate, when one considers the widespread occurrence of this compound and the fact that it never accumulates. Little positive information is available concerning the ability of the animal or plant organism to metabolize riboflavin. Almost certainly, however, microorganisms actively decompose riboflavin in nature (Starkey, 1942) and possibly in the intestines of animals. There is indirect evidence that rat intestinal tissue itself destroys riboflavin (Selye, 1943). The next and following papers afford abundant proof for the microbial decomposition of riboflavin. Hitherto, the microbiological aspects of riboflavin have been limited largely to a study of the riboflavin requirements of certain bacteria, such as lactic acid bacteria, streptococci, etc., and to microbiological methods of assay for this vitamin. These aspects are touched upon in the following program only in so far as they occasionally supplement the approaches employed.

II. BACTERIAL OXIDATION OF RIBOFLAVIN TO LUMICHROME

Recently an unusual opportunity for the isolation of microorganisms capable of attacking riboflavin was made available through the discovery by the writer of a localized area of soil in Rahway with an uncommonly high content of riboflavin. Although it is, without doubt, possible to reproduce such conditions by microbiological enrichment culture techniques, the present instance of finding a soil rich in riboflavin without deliberately enriching the soil is believed to be without precedent in the microbiological literature.

EXPERIMENTAL

Small portions of the riboflavin-rich soil were added to two flasks containing a solution of 0.1 per cent riboflavin and a small amount of phosphate and magnesium sulfate. To one flask 0.10 per cent glucose was added. After a few days’ incubation at room temperature, the riboflavin had disappeared in both cultures, as indicated by the loss of the typical orange color of the riboflavin. The disappearance was shown not to be due to simple reduction of the riboflavin to the leuco form. The cultures were plated out on the same solutions incorporated into agar. A variety of bacteria, yeasts and fungi were isolated, not all of which necessarily attacked the riboflavin. Undoubtedly, impurities carried over from the soil provided sufficient nutrients for their development. The majority of them are being reserved for future study.

One bacterial culture was singled out for detailed study. Riboflavin agar cultures did not reveal any apparent disappearance of the vitamin, and by superficial examination the growth had a dry, bright yellow appearance. Interestingly enough, this type of growth was obtained only when the organism was cultivated on riboflavin-containing agar, and, even then, only when the concentration of riboflavin was greater than about 0.02 per cent. The organism develops abundantly on yeast extract and yeast-extract glucose agar but without the yellow color obtained on riboflavin. Further inquiry on this point revealed that the yellow appearance of the growth on the riboflavin was due to a local
concentration of a yellow crystalline substance within the colonies or streaks of the bacterial growth. Figure 1 shows the appearance of such cultures. This substance was later isolated and identified as 6,7-dimethyl alloxazine (lumichrome). Lumichrome is obtained chemically through the action of Na$_2$CO$_3$ on lumiflavin (Kuhn and Rudy, 1934a, b) and by irradiation of solutions of riboflavin or lumiflavin with sunlight (Karrer et al., 1934; Karrer and Schöpp, 1934; Kuhn and Rudy, 1934c). It has been synthesized chemically (Stern and Holiday, 1934). The only record available of the occurrence of lumichrome in nature is that of its isolation from hog retinas (Brunner and Baroni, 1936). From its occurrence there along with riboflavin and lumiflavin, a physiological relation between these compounds might be inferred, but the present example is the first demonstrating a direct physiological or biochemical sequence between riboflavin and lumichrome.

Fig. 1. Crystallization of Lumichrome Formed from Riboflavin by P. riboflavinus

The light areas are crystalline lumichrome on the surface of yeast (0.1%) agar containing 0.1% riboflavin. Under these conditions the riboflavin is entirely soluble. The arrangement of the crystalline material resulted from streaking the organism on the surface of the agar. The isolated white spots are where single isolated colonies of the bacteria developed. The bacterial growth made is so scant as to be invisible on the photograph, due to the very limited amount of energy liberated by the conversion of riboflavin to lumichrome, 7 days old. Approx. $\times \frac{1}{2}$.  

1934; Kuhn and Rudy, 1934c). It has been synthesized chemically (Stern and Holiday, 1934). The only record available of the occurrence of lumichrome in nature is that of its isolation from hog retinas (Brunner and Baroni, 1936). From its occurrence there along with riboflavin and lumiflavin, a physiological relation between these compounds might be inferred, but the present example is the first demonstrating a direct physiological or biochemical sequence between riboflavin and lumichrome.
The relation between riboflavin and lumichrome is shown by their structural formulae; loss of the ribityl side chain converts riboflavin into lumichrome.

\[
\begin{align*}
\text{Riboflavin} & \quad \text{Lumichrome} \\
\text{6,7-dimethyl-9-(1'-ribityl)-isoalloxazine} & \quad \text{6,7-dimethyl alloxazine}
\end{align*}
\]

Microscopic examination of the crystals showed them to be long, needle-shaped prisms and microcrystalline in dimensions. Figure 2 is a photomicrograph of a preparation made directly from a culture and shows the crystals of lumichrome mixed with cells of the bacteria which formed them. On the surface of agar cultures the crystals tend to be arranged in peculiar horseshoe-shaped aggregates. Figure 3 is a photomicrograph (high, dry magnification) of a streak on riboflavin agar and shows the nature of these aggregates. Each aggregate consists of hundreds or thousands of individual crystals piled in match-like fashion and arranged like a horseshoe. The detailed structure of these horseshoe aggregates of lumichrome crystals is brought out clearly in figure 4.

*Pseudomonas riboflavinus* nov. sp.

The ability of this particular organism to oxidize riboflavin to lumichrome is believed to be a distinctive enough physiological property to warrant designating the organism as a new species. It will be shown subsequently that other representative bacteria tested do not have the ability to attack riboflavin and oxidize it to lumichrome.

The name *Pseudomonas riboflavinus* nov. sp. is proposed for the organism. It is a thin, variable length, gram-negative, actively motile rod. It is non-sporulating and obligately aerobic. Small amounts of complex organic nitrogenous substances apparently supplying amino acids or an unknown factor or factors are required for growth. A mixture of 20 of the known water-soluble accessory substances could not replace yeast extract in a synthetic mineral glucose medium. Urea, glycine, \( \text{NH}_4\text{Cl} \) and \( \text{NaNO}_3 \) cannot substitute as nitrogen sources for or-
Fig. 2. LUMICHROME CRYSTALS IN A RIBOFLAVIN CULTURE SHOWING THE BACTERIAL CELLS WHICH PRODUCED THE LUMICHROME. 1800 X

Fig. 3. ARRANGEMENT OF LUMICHROME CRYSTALS IN HORSESHOE-LIKE FASHION
Photograph taken directly on undisturbed surface of a streak culture on riboflavin agar. The lateral piling up construction from long prism-shaped crystal units is visible in some of the aggregates. The bacterial cells are in the background. 95 X.
ganic material in media containing riboflavin as the sole energy source. No acid or gas is formed in 1 per cent peptone broth containing the following sugars although the sugars are attacked: levulose, mannitol, sucrose, maltose, lactose, xylose and galactose. Abundant growth can be obtained in the absence of riboflavin. Acetymethyl carbinol, gelatin liquefaction, and methyl-red tests are all negative. Test for nitrate reduction is slightly positive. No pigment is formed on any medium. Colonies on yeast extract agar are smooth, convex, small (0.1–0.2 mm.) and transparent with a slightly dentate circular edge. In yeast-extract glucose agar growth is considerably more abundant and milky in appearance, with formation of copious quantities of a polysaccharide so that the large colonies appear drop-like, confluent, glistening and very gelatinous. Polysaccharide formation also occurs on levulose, mannitol, sucrose, maltose, lactose, xylose and galactose plates. Cultivation in liquid yeast-extract 1% glucose, particularly with good aeration, causes polysaccharide formation sufficient to make the culture so viscid that it barely flows when the vessel is inverted. Op-

**Fig. 4.** Enlarged Horseshoe Aggregate Showing Its Construction Out of Numerous Laterally Piled Micro Prism-Shaped Crystals of Lumichrome

The mottled background is made up of bacterial cells. 450 X
timum temperature is 30–33°C. Starch is not attacked and milk is slowly peptonized with soft curd formation.

In organic media containing a small amount of organic matter, such as yeast extract or peptone, and 0.05–0.2 per cent riboflavin, the riboflavin is attacked and converted into lumichrome which accumulates in the culture as a lemon-yellow crystalline material. On the surface of riboflavin agar small aggregates of lumichrome crystals form (as described above). These vary between 0.01 and 0.2 mm. in size. In liquid culture the crystals appear as a flocculent sediment in stationary cultures and after shaking will settle out slowly, giving the culture a lemon-yellow appearance. The disappearance of riboflavin in the presence of lumichrome can be measured fluorometrically since lumichrome, unlike riboflavin, does not fluoresce in the ultraviolet.

Isolation and identification of lumichrome

Liquid cultures containing a trace of K$_2$HPO$_4$ and MgSO$_4$·7H$_2$O, 0.1 per cent yeast extract and 0.1 per cent riboflavin show virtually complete disappearance of the riboflavin in 5 to 10 days in 80 ml. amounts in a 250 ml. Erlenmeyer flask. This time is reduced to 1 to 2 days if the cultures are continuously aerated, and all experiments in liquid media described below were done in this manner. Riboflavin analyses were done fluorometrically. Riboflavin media were always adjusted to pH 6.0 before heat sterilization. Although by far the major portion of the riboflavin always disappears, invariably a few micrograms remain undecomposed. The bright orange riboflavin color of the liquid changes to a lemon-yellow color with appreciable quantities of crystalline lumichrome occurring free in the liquid, provided the initial concentration of riboflavin is above 0.05 per cent. The organism does not further attack the lumichrome.

Lumichrome is practically insoluble in water at neutral to acid pH values but it is readily soluble in weak alkalis, forming the corresponding salt. Alkaline solutions of lumichrome have a deep yellow-orange color, which turns to light lemon-yellow upon neutralization. Free lumichrome is very slightly soluble in organic solvents. A culture with some free lumichrome was acidified to pH 2.0 with H$_2$SO$_4$ whereupon a flocculent mass of lumichrome precipitated from solution. The solution was extracted with ether in a continuous extractor, the lumichrome depositing in the receiver as a flaky coating of lemon-yellow material contaminated with some dark brown matter. After evaporation of the ether, the lumichrome was purified by dissolving in a minimum amount of N/100 NaOH and extracting out with ether some of the impurities. The liquid was then made slightly acid again with H$_2$SO$_4$ and re-extracted with ether. The ether-soluble material was again dissolved by warming in weak NH$_4$OH and reprecipitated by acidification with dilute acetic acid. The amorphous precipitate was centrifuged, washed with 1 per cent acetic acid, redisolved in a minimum amount of N/100 NaOH and 5 per cent acetic acid added slowly until incipient precipitation of the lumichrome. Then an equal volume of acetone was added, and the mixture placed on a steam bath. As the acetone slowly evaporated, the lumichrome separated out from solution as a flocculent mass
of microcrystals. These were centrifuged, washed twice with water and dried at 105°C.

Conditions for the crystallization of lumichrome are apparently extremely limited since with few exceptions, and those unrepeatable, the lumichrome is obtained as an amorphous flocculent mass. pH is a critical factor in the precipitation of lumichrome, and as soon as the pH is lowered to about the neutral point, precipitation of amorphous lumichrome begins with suddenness and, depending on the concentration, may cause the solution to gel due to the hydrophilic nature of the precipitate.

![Absorption Spectrum of Bacterial Lumichrome](image)

**Fig. 5. Absorption Spectrum of Bacterial Lumichrome**

The substance analyzed as follows:

<table>
<thead>
<tr>
<th>Element</th>
<th>Found</th>
<th>Calculated</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>59.72</td>
<td>59.92</td>
</tr>
<tr>
<td>H</td>
<td>4.36</td>
<td>4.13</td>
</tr>
<tr>
<td>N</td>
<td>23.52</td>
<td>23.14</td>
</tr>
</tbody>
</table>

The substance does not melt up to 370°C. Its absorption spectrum in 1.0 N Na₂CO₃ shows three maxima (fig. 5), a sharp band at 2600 Angstrom units, a lower broad band between 3400 and 3500 and a faint inflection at 3900-4100 Å.

I wish to thank Mr. D. F. Hayman for these analyses.

I am grateful to Dr. N. R. Trenner for these measurements.
The absorption spectrum data are summarized and compared with literature values as follows:

Maxima

Bacterial product ........................................... 2600, 3450, 3900-4100 (inflection)
Rudy and Kuhn (1934a) ........................................... 2600, 3400, 3900, 4100 (inflection)
Stern and Holiday (1934) ...................................... 2550, 3490, 3980 (inflection)
Karrer, et al. (1934) ...............................................

The color in alkaline solution of bacterial lumichrome is not discharged by hydro-sulfite.

Conversion of riboflavin to lumichrome by Pseudomonas riboflavinus

Effect of pH. Twenty-four hour Blake bottle cultures of P. riboflavinus on 0.1 YE-0.1 B₂ agar were suspended in sterile tap water, centrifuged and washed once. The centrifugate consisted of a homogenous mass of bacterial cells and lumichrome crystals. The latter did not interfere in any way with the handling or activity of the cells. The centrifugate was suspended in distilled water, divided into aliquots in 50 ml. Erlenmeyer flasks and adjusted to different pH values. Phosphate buffer of the appropriate pH was added to a final concentration of M/50. Each treatment received 168 micrograms of riboflavin in aqueous solution. The final volume was 3 ml. One flask (pH 7.2) was boiled before riboflavin was added and served as the control. The flasks were shaken at 30°C. on a rotary shaking machine for 75 minutes and analyzed for residual riboflavin. The disappearance of riboflavin as a function of pH is shown in figure 6. The optimum pH is approximately 7.7, but the action takes place over a wide pH range.

3 The elementary analysis given for the compound by Rudy and Kuhn (1934a) shows it was impure. [Cf. Rudy and Kuhn (1934b).]
Effect of glucose and yeast extract. Supplementary energy sources, such as 1 per cent glucose and 0.1 per cent yeast extract, accelerate appreciably the rate of riboflavin oxidation by washed cell suspensions of \textit{P. riboflavinus}. Results of a typical experiment are shown in figure 7. While it may be argued that the increase caused by yeast extract is due to cell proliferation in the presence of this nitrogen and energy source, this idea is not supported by the fact that glucose also enhances the process, though to a lesser degree. In this instance, a source of nitrogen is not available, and presumably it would be necessary for cell multiplication since the nitrogen in the riboflavin and lumichrome molecules cannot be utilized. However, the possibility that cell proliferation did occur to some extent is not entirely eliminated.

\begin{figure}
\centering
\includegraphics[width=0.5\textwidth]{figure7.png}
\caption{Effect of Yeast Extract and Glucose}
\end{figure}

Riboflavin synthesis by \textit{Pseudomonas riboflavinus}

This organism develops abundantly in ordinary bacteriological media in the absence of riboflavin as a source of energy so long as small amounts of complex nitrogenous supplements are present. The exceedingly luxuriant growth made in the presence of glucose in the absence of added riboflavin raised the interesting question of riboflavin synthesis in such cultures. The media listed in Table 1 were inoculated with \textit{P. riboflavinus}, incubated with aeration for three days and assayed for riboflavin content by the \textit{Lactobacillus casei} method. The yeast extract used in this experiment was selected for its exceptionally low content of riboflavin. The data show that \textit{P. riboflavinus} synthesizes appreciable quantities of riboflavin when developing in the absence of this substance. The synthesized riboflavin is probably required for the respiratory mechanisms of the cells.

Quantitative conversion of riboflavin to lumichrome

Recovery experiment. One hundred ml. of a mineral-0.1 per cent yeast extract medium received exactly 100 mg. of riboflavin and was inoculated with \textit{P. riboflavinus}. A control containing no riboflavin was also inoculated. Both cultures were shaken four days at 30°C. The lumichrome-containing culture was acidi-
Microbiological Aspects of Riboflavin

Fed to pH 2.0 with H₂SO₄, transferred quantitatively to a liquid-liquid extractor fitted with a small weighed receiving flask. The culture was continuously extracted with ether for 48 hours, after which the ether was evaporated, the receiver flask dried at 100°C. for 1 hour and weighed. The culture without riboflavin was treated in exactly the same manner, extracted in the same extractor for 48 hours and the receiver dried and weighed. The data are contained in the following summary:

<table>
<thead>
<tr>
<th>MEDIUM</th>
<th>RIBOFLAVIN CONTENT micrograms/ml.</th>
<th>RIBOFLAVIN SYNTHESIZED micrograms/ml.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1% YE uninoculated</td>
<td>0.008</td>
<td></td>
</tr>
<tr>
<td>0.1% YE inoculated</td>
<td>0.010</td>
<td>0.002</td>
</tr>
<tr>
<td>0.1% YE + 1% dextrose inoculated</td>
<td>0.078</td>
<td>0.070</td>
</tr>
<tr>
<td>1.0% YE uninoculated</td>
<td>0.021</td>
<td></td>
</tr>
<tr>
<td>1.0% YE inoculated</td>
<td>0.060</td>
<td>0.029</td>
</tr>
<tr>
<td>1.0% YE + 1% dextrose inoculated</td>
<td>0.18</td>
<td>0.16</td>
</tr>
</tbody>
</table>

TABLE 1

Riboflavin synthesis by P. riboflavinus

This is in good agreement with the theoretical recovery of 64.5 mg. lumichrome from 100 mg. riboflavin.

Manometric experiment. The complete reaction for the oxidation of riboflavin to lumichrome is represented by the following:

\[
\text{Riboflavin} \quad \text{H} \quad \text{HNO}_3 + \frac{5}{2} \text{O}_2 \rightarrow \text{Lumichrome} \quad \text{C}_{12} \text{H}_{10} \text{O}_4 \text{N}_4 + 5 \text{CO}_2 + 5 \text{H}_2\text{O}
\]
Essentially, the physiological reaction is the oxidation of the ribityl side chain:

\[
\begin{align*}
\text{CH}_2\text{OH} & \\
(\text{CHOH})_3 & \\
\text{CH} & \\
\end{align*}
\]

\[
\text{C}_8\text{H}_{10}\text{O}_4 + 5\frac{1}{2} \text{O}_2 \rightarrow 5 \text{CO}_2 + 5 \text{H}_2\text{O}
\]

A cell suspension was prepared as described above from YE-B2 agar and 0.4 ml. placed in the side arm of a Warburg vessel. Because of the limited solubility of riboflavin, 2.0 ml. of the substrate containing by analysis 174 micrograms was placed in the main chamber. The experiment was set up for the measurement of rate of oxygen uptake and total CO₂ production according to the customary Warburg manometric technique. Since the pH of the vessel contents was 7.2 initially, the final total (gaseous + bound) CO₂ was determined by dumping 0.2 ml. 5 per cent H₂SO₄. A typical course of oxygen uptake is plotted in figure 8 (curves marked No DNP), which, although for a different experiment, shows the rate of attack of riboflavin. Data on the gas exchange in a typical experiment are summarized as follows:

**Fig. 8. Inhibition of Oxidative Assimilation by Dinitrophenol**
Theoretical O₂ uptake and CO₂ production for complete oxidation of 174 micrograms of riboflavin if the molecule is broken down to CO₂, H₂O and NH₃ would be 166 and 176 cmm., respectively. Theoretical gas change for the conversion to lumichrome would be 56 and 51 cmm., respectively. It is obvious that the data come more nearly fulfilling the requirements of the second reaction than the first, especially since lumichrome was formed during the process and the riboflavin disappeared entirely. The observed values for O₂ and CO₂ are 64.5 and 43.2 per cent of the theoretical for the lumichrome reaction.

Table

<table>
<thead>
<tr>
<th></th>
<th>AUTORESPIRATION</th>
<th>RIBOFLAVIN</th>
<th>GAS EXCHANGE DUE TO OXIDATION OF 174γ RIBOFLAVIN</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cmm.</td>
<td>cmm.</td>
<td>cmm.</td>
</tr>
<tr>
<td>O₂ uptake</td>
<td>15</td>
<td>51</td>
<td>36</td>
</tr>
<tr>
<td>Initial bound CO₂</td>
<td>13</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Final total CO₂</td>
<td>28</td>
<td>51</td>
<td></td>
</tr>
<tr>
<td>CO₂ produced by cells</td>
<td>15</td>
<td>37</td>
<td>22</td>
</tr>
</tbody>
</table>

Undoubtedly, oxidative assimilation was inhibited and the oxygen uptake approached more nearly the theoretical 55 cmm. required for the conversion of 170 micrograms of riboflavin to lumichrome. The data are tabulated in the following summary:

<table>
<thead>
<tr>
<th></th>
<th>No DNP</th>
<th>With M/1300 DNP</th>
</tr>
</thead>
<tbody>
<tr>
<td>O₂ consumed</td>
<td>39 cmm.</td>
<td>51 cmm.</td>
</tr>
<tr>
<td>Autorespiration</td>
<td>5 cmm.</td>
<td>8 cmm.</td>
</tr>
<tr>
<td>O₂ due to riboflavin oxidation</td>
<td>34 cmm.</td>
<td>43 cmm.</td>
</tr>
</tbody>
</table>

61.8% of theory 78.2% of theory

Apparently the concentration of DNP used did not stop entirely the assimilation process but its effect was clear-cut enough to warrant the conclusion that the discrepancy between observed and theoretical O₂ consumption can be ascribed to oxidative assimilation.
Adaptive enzyme

Washed cells of *P. riboflavinus* which have been grown in the presence of added riboflavin oxidize riboflavin immediately and at a rapid rate. Cells grown in the absence of riboflavin do not attack riboflavin immediately but attack it after exposure to this substrate for a period of several hours, after which the rate may be quite rapid. This behavior can probably be explained as adaptive enzyme formation according to the ideas of Karström (1930).

In one experiment, washed cells grown in the presence and absence of riboflavin, respectively, were incubated with 100 \( \gamma \) riboflavin on a shaking machine and riboflavin analyses were made periodically. The cells previously exposed to riboflavin destroyed 100 \( \gamma \) in less than 2 hours, whereas those previously unexposed showed very little destruction up to 48 hours. However, during the following overnight period, all the riboflavin had disappeared. A manometric experiment demonstrating the adaptive enzyme formation is presented in figure 9. Adapted cells (solid line curves, fig. 9) began to oxidize the riboflavin immediately and at a high rate so that all the riboflavin (170 \( \gamma \)) was oxidized in 1 hour. The unadapted cells (dotted line curves, fig. 9) showed no sign of attacking the riboflavin for 2 hours, after which the rate of attack was very slow and greatly prolonged so that even after 12 hours less than one-half the available riboflavin had been oxidized. The true adaptation rate is the difference between autorespiration and the substrate respiration and is shown in figure 9. The adaptive nature of the reaction is indicated by the small and slow but nevertheless clear-cut increasing rate of oxidation over the 12 hour period. Doubtless the rate would have become much greater had the experiment been continued.

**SUMMARY**

*Pseudomonas riboflavinus* nov. sp. is described. This organism oxidizes riboflavin to lumichrome stoichiometrically according to the reaction:

\[
C_{17}H_{20}O_6N_4 + 5\frac{1}{2}O_2 \rightarrow C_{12}H_{19}O_2N_4 + 5CO_2 + 5H_2O
\]

riboflavin \hspace{1cm} lumichrome

Some characteristics of the reaction are described.
REFERENCES


SELYE, H. 1943 The role played by the gastrointestinal tract in the absorption and excretion of riboflavin. J. Nutrition, 25, 137-142.
