131. *On the Respiratory Pigment, Cytochrome, in Bacteria.*

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The significance of Keilin's cytochrome, which has of late risen in importance as a respiratory pigment, consists in the notable parallelism between its occurrence and respiratory activity of living cells. It is also of almost universal distribution among plants and animals, though the data on this point are yet incomplete. The distribution of this respiratory pigment in lower plants has not been reported except in a few yeasts, (Keilin¹⁾), fungi (Tamiya²⁾) and bacteria (Keilin¹⁾). The observation of Keilin on bacteria included only nonpathogenic species, namely, *B. subtilis*, *B. megatherium*, *B. mycoides*, *B. proteus vulgaris*, *Staphylococcus*, *B. sporogenes*, *Streptococcus acidilactici* and *Vibrio septique*, but he found a very significant fact that anaerobic bacteria show no cytochrome, though aerobic forms contain the pigment abundantly. In the present work we extended the observation to a large number of species of bacteria, especially pathogenic ones, and corroborated Keilin's conclusion, and further noticed a certain peculiarity of the absorption spectrum of bacterial cytochrome which hitherto seems to have been overlooked. Our results will be briefly described in the following lines.

Methods.—For the spectroscopic examination of bacteria we used two microspectroscopes (Abbe), Zeiss and Leitz, with equal facility. A strong source of light, 500 watt "Mazda"-lamp, was used for illumination.

Material.—Bacterial growth cultivated on agar slant and broth at 37°C. for 24–48 hours, was compressed between a slide and coverslip, and examined with the microspectroscope.

The thickness of the layer of bacterial mass was so regulated that in each case the most distinct absorption spectrum can be observed; the thickness of layer was thus varied from 1 to 3 mm. The distribution of cytochrome among the investigated bacteria was as follows:

1) D. Keilin, Proceedings of Royal Society of London, Series B. Vol. **98** (1925) p. 312.

D. Keilin, Comptes Rendus de Séances de la Société de Biologie. Tome **97**, N° 24 (1927) p. 39.

2) H. Tamiya, Acta phytochimica, Vol. IV, No. 1 (1928) p. 215.

Distribution of Cytochrome in Bacteria.

	Species and strains	Absorption bands of reduced cytochrome	The extra absorption band (623-637 $\mu\mu$)	Relative concentration
Aerobic bacteria	<i>B. pyocyaneus</i>	a. b. c! d (2)		5
	<i>B. subtilis</i>	a. b. c! d (2)		5
	<i>V. cholerae</i>	a. b. c! d. a. b.=c! d.		4
	<i>B. diphtheriae</i>	a. b!=c. d. a. b! c. d.		4
	<i>B. tuberculosis</i> (12 days)	a. b! c. d.		4
	<i>B. influenzae</i>	a. b! c. d ?		4-3
Facultative aerobic bacteria	<i>Staphylococcus aureus</i>	a. b! d ?		3-2
	<i>Pneumococcus</i> I.	a. b! d ?		3-2
	<i>B. typhosum</i>	b! d.		2
	<i>B. paratyphi</i> A.	b! d.	?	2
	<i>B. paratyphi</i> B.	b! d.		2
	<i>B. dysenteriae</i> -Shiga	b! d.		2
	<i>B. dysenteriae</i> -Komagome A.	b! d.	very distinct	2
	<i>B. dysenteriae</i> -Komagome B.	b! d.	very distinct	2
	<i>B. dysenteriae</i> -Flexner (original)	b! d.	distinct	2
	<i>B. dysenteriae</i> -Schmitz	b! d.	faint	2
	<i>B. dysenteriae</i> -Nakamura	b! d.	distinct	2
	<i>B. dysenteriae</i> -Kawase	b! d.	faint	2
	<i>Streptococcus erysipclatis</i>	b! d.		2
	<i>B. coli communis</i>	b! d.	faint	2
	<i>B. coli communior</i>	b! d.	faint	2
Anaerobic bacteria	<i>B. sporogenes</i> (Metchnikoff)	0		0
	<i>B. putrificus</i>	0		0
	<i>Vibrio septique</i>	0		0

(N.B.) ! indicates the most distinct absorption band.

= indicates nearly equal intensity.

From the table it can be seen, that the grade of oxygen requirement of bacteria and the number of absorption bands of cytochrome go fairly parallel: The obligate aerobic bacteria show always the four characteristic bands for reduced cytochrome (a, b, c, and d) distinctly, the facultative aerobic bacteria, however, show only 2 or 3 bands, and in obligate anaerobic bacteria no band at all can be observed, showing that these latter are devoid of cytochrome.

The absorption spectrum of cytochrome found in *B. pyocyaneus* and *B. subtilis* differs very little from that of baker's yeast, band c

being the strongest band of all. Band d gives the appearance of being composed of two distinct bands corresponding respectively to 532 and 521 $\mu\mu$.

With the exception of these two strains however, we noticed in almost all other bacteria examined the following peculiarities in their absorption spectrums:

(1). Band c (550 $\mu\mu$), which is in general the strongest absorption band of the usual cytochrome, is much lighter than band b (in *V. cholerae* however, band c is almost as strong as band b) and consequently it disappears in the case of facultative anaerobic bacteria.

(2). Band d lies always on the position of longer wave length, extending between 535–525 $\mu\mu$, the position of its maximum intensity being 532 $\mu\mu$.—While in the case of usual cytochrome the same band lies in 521 $\mu\mu$.

(3). Band b is, as above mentioned, the strongest band in the spectrum and in all the cases of the facultative anaerobic bacteria, in which band c is not visible or possibly be absent, it is found always shifted towards the shorter wave length (563–553 $\mu\mu$). This is a notable fact, because, in this respect the absorption spectrum found in facultative anaerobic bacteria resembles spectroscopically a haemochromogen in alkaline solution showing two absorption bands (I: 567–547, II: 532–510).

As assumed by Keilin, cytochrome is composed of three components (a' , b' , c'). Each of these components (a' , b' , c') gives spectroscopically two absorption bands (α and β).

Diagram showing the three components a' , b' and c' ,
of cytochrome.

	a	b	c	d Z Y X		
Cytochrome	α_1	α_2	α_3	β_1	β_2	β_3
Compound a'	α_1			β_1		
„ b'		α_2			β_2	
„ c'			α_3			β_3
Red				Blue		

α		β	
a' : 614–593 (a)		Z : 532 (d)	
b' : 567–561 (b)		Y : 528 (d)	
c' : 554–546 (c)		X : 521 (d)	

The bands a, b and c of cytochrome would correspond to the α -bands of these three compounds, while the x, y and z of band d would answer the β -bands of the three components.

As band c is obscure in most of the bacteria, we may assume that c'-component in bacterial cytochrome is less concentrated than in the usual cytochrome.

The unusual position of band d can also be elucidated through the same assumption, because the x-component of band d, located as the characteristic band of c'-component on the extreme position of shorter wave length, is absent.

Besides the characteristic absorption spectra of cytochrome we observed a fairly distinct band with the wave length of 623-637 $\mu\mu$ in *B. dysenteriae* (Komagome A and B) and though less distinctly also in Flexner (original), Schmitz, Nakamura, Kawase and in two strains of *B. coli*.

The fact that this extra band can be observed in the para-dysentery but not in the Shiga bacilli, and further in *B. coli*, is of interest.

The chemical and physical meaning of this extra band is worthy of further studies.

Summary.

1. A close parallelism between the cytochrome content and the intensity of aerobic respiration holds in all the bacteria we examined. *B. pyocyaneus*, *B. subtilis* (5) > *V. cholerae*, *B. diphtheriae*, *B. tuberculosis*, *B. influenzae* (4) > *Staphylococcus*, *Pneumococcus* (3-2) > *B. typhosum*, *B. paratyphi* A and B, *B. dysentery*, *Streptococcus erysipclatis*, *B. coli* (2) > *B. sporogenes*, *B. putrificus*, *Vibrion septique* (0).

2. The general aspects of the spectrum, however, varies much with the strains examined:

In almost all the aerobic bacteria examined, cytochrome was always found with its four characteristic bands (a, b, c, and d) and the variation in the intensity of these bands were noted. *B. pyocyaneus*, *B. subtilis*, and *V. cholerae* show most clearly the spectrum of cytochrome, very similar to that of baker's yeast. In the majority of other bacteria however certain notable differences in their spectra from those of usual one was observed. The facts are that in the former the band b is stronger and band c weaker or possibly be absent, and that band d lies always in the position of longer wave length (532 $\mu\mu$).

3. In all strains of *B. dysenteriae*, excluding the Shiga bacilli, and *B. coli* we found a characteristic extra band on 623-637 $\mu\mu$ besides the known bands of cytochrome.