Acta Tuberculosea Japonica Vol. 6, No. 2, 63-68, 1956

Studies of Metabolism in Tuberculous Lesions

II. Further Studies on the N-acetyltyramine Formation by Mycobacterium Tuberculosis

Hiromi SAKURAI*

From the Pathological Division (Cheif : Hidao TAKAMATSU) of the Tuberculosis Research Institute, Kyoto University and the Osaka Abuyama Red Cross Hospital (Director : Seitaro YANO, Chief of the Chemical Laboratory : Kinzaburo HIRAI).

(Received for publication July 2, 1956)

Introduction

As is described in the first report[1] of this series, the N-acetyltyramine formation was detected in a culture medium which contained tyramine and glucose as a carbon-source and even in the experiment of the low concentration of glucose conducted twenty days after the cultivation of mycobacterium tuberculosis avium.

For the course of the formation of N-acetyltyramine and of other compounds from tyramine the author thought the following: tyrosine → tyramine → N-acetyltyramine → tyrosol → para-hydroxyphenyl-lactic acid? → para-hydroxyphenyl-acetic acid. It was desired to discover whether this course would occur or not.

The author noticed a phenomenon in the experiment described in the first report. The bacillar membrane will appear in a suitable culture media on the fifth day of the period of cultivation. Therefore, the author intended to analyse the media for a shorter period of cultivation for the present problem. In this new experiment, the duration of the cultivation was set at five days and at ten days and the concentration of glucose in the media was 0.5 per cent throughout the experiment, because in this concentration a noticeable formation of N-acetyltyramine could be observed. The author also examined the formation of para-hydroxyphenyl-lactic acid at the same time but no satisfactory conclusions were in these experiments. A resume of the experiments and their results are as follows.

Experiments

Material: The concentration of glucose, which was added as a carbon-

* 桜井宏皆

source for metabolic products of bacillary action in the Sauton's synthetic medium, was fixed at 0.5 per cent. The other components of the culture media were the same as those of the experiments described in the first reports. The bacilli used in these experiments were mycobacterium tuberculosis avium (Takeo).

Duration of the cultivation was divided in 5 days and 10 days. Other procedures were the same as reported in the first report.

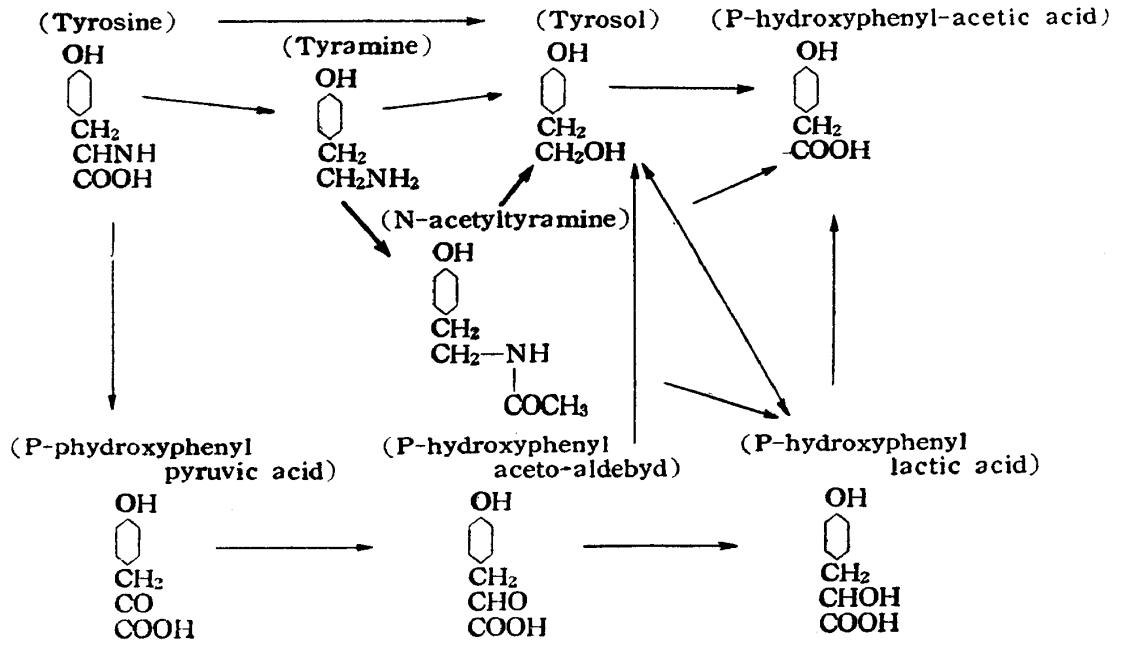
Results

1) Results obtained by 5 day cultivation.

The growth of bacilli was satisfactory and the tendency of the formation of membrane on the surface of culture solution was observed at the end of the cultivation. The colony grew separately. Each fraction was treated as described in the first report. Oily substances were obtained. But no oily substances and white crystals were obtained from the acid fraction. As is shown in the table 1, Millon's reaction was strongly positive in the amine fraction and the tyrosol fraction (2), but the tyrosol fraction (1) reacted faintly and no reaction was observed in the acid fraction.

The results obtained by the examination of paper chromatogram are shown in Table 2. The substances which were contained in each fraction and could be detected as the Rf by paper chromatograph presumably have no relation to each other. The tyrosol fraction (1) was treated

Fig. 1. Hypothetical Scheme of the Metabolism.



(Note) Neubauer[1] said that amino acid decomposition is as follows ; amino acid → keto-acid → aldehyde → acid or alcohol.

Table 1 Results of Millon's test

Fractions \ Duration of Cultivation	5 days	10 days
Amine Fraction	+	+
Tyrosol Fraction (1)	±	+
Tyrosol Fraction (2)	+	+
Acid Fraction	−	−

Table 2 Paper chromatogram.

Sample \ Rf		0.87	0.71	0.14	0.09
5 day culture	Amine Fraction		+		
	Tyrosol Fr. (1)	−			
	Tyrosol Fr. (2)	+			
	Acid Fraction			−	−
10 day culture	Amine Fraction		+		
	Tyrosol Fr. (1)	−			
	Tyrosol Fr. (2)	+			
	Acid Fraction			−	−
Pure Crystal	N-Acetyltyramine	+			
	Tyrosol	+			
	Tyramine-HCl		+		
	P-hydroxyphenyl-acetic acid			+	
	P-hydroxyphenyl-lactic acid				+

Table 3 Rf and Melting Point

	N-acetyltyramine (pure-crystal)	Sample
Paper chromatogram Rf	0.87	0.869~0.87
Melting point	128°C	128°C~128.5°C

with absolute petroleum-ether for crystallization, but no crystal was obtained. The tyrosol fraction (2) was also treated with benzol but no crystal was obtained.

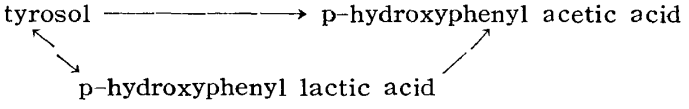
2) Results obtained by 10 day cultvation.

The growth of bacilli was quite adequate and the surface of the culture media was covered with a membrane of bacilli. The Millon's test of each fraction was positive as in the results of the five day cultivation with the exception of that of the acid fraction. The spot tests of each fraction other than the acid fraction by paper chromatograph were positive. Their Rfs agreed with the substances which were presumed to be in each fraction. The tyrosol fraction (1) was extracted with petroleum-ether but no crystal was obtained. After the removal of petroleum-ether, we treated it with benzol but no crystal appeared. The tyrosol fraction (2) was extracted with hot benzol three times and thereafter we obtained a small quantity of white transparent crystal.

In the examination of the liquid solution of the crystal by paper chromatograph, as is shown in Table 3, the substance was confirmed as the Rf coincided with N-acetyltyramine. The melting point of the crystal mixed with N-acetyltyramine was 128°C and this result agreed with the melting point of N-acetyltyramine. After benzol extraction, the residue of the tyrosol fraction (2) was negative for Millon's test.

Comments

At the beginning of this experiment the author anticipated a process on the N-acetyltyramine metabolism as shown in Fig. 1. Even in the experiment of 0.1% glucose concentration, para-hydroxypheny-lacetic acid formation was observed. Because of this para-hydroxyphenyl-lactic acid formation in the step before the para-hydroxypheny-lacetic acid formation was supposed by the author. N-acetyltyramine, it was also assumed, formed in the step before tyrosol or para-hydroxyphenyl-lactic acid formation. The substance which was presumed to be para-hydroxyphenyl-lactic acid by means of paper chromatogram as described in the first report is not confirmed yet, but the process from para-hydroxyphenyl-lactic acid to para-hydroxyphenyl-acetic acid may be assumed. Shirai[2] verified the transition from para-hydroxyphenyl lactic acid to tyrosol in his experiment. Because of this, the following scheme of metabolism may occur.



For this reason, another experiment was performed for short period of the cultivation. In the experiment of the five day cultivation, tyrosol was not

formed even though the formation of N-acetyltyramine could be observed. Although the crystal of tyrosol was not obtained in the ten day cultivation, a substance which took the value of Rf 0.78 was obtained. This value agrees with that of tyrosol. At the same time, the crystal of N-acetyltyramine was obtained. As for this, a course of metabolism from tyramine to N-acetyltyramine and to tyrosol may be constructed. But the other course from tyramine to tyrosol is also possible. As opposed to this, in order to form N-acetyltyramine, tyrosol must be combined with a group of amine. This supposition seems unreasonable, because N-acetyltyramine was detected before the tyrosol formation. The course from tyramine to N-acetyltyramine seemed to be certain. As Shirai's[3] experiment showed, the crystal of tyrosol could not be obtained in the present experiment. For this reason, a rapid process of decomposition after N-acetyltyramine formation may be supposed, but the matter is still remains uncertain.

In conclusion, the following process was conducted: Tyramine → N-acetyltyramine → tyrosol → para-hydroxyphenyl-lactic acid → para-hydroxyphenyl acetic acid. For the study of the metabolism after the N-acetyltyramine formation, some experiments with a particular culture media in which N-acetyltyramine was contained as a carbon-source were performed. The results of these experiments will be reported in another paper by the author.

Summary

Mycobacterium tuberculosis avium was cultivated in a Sauton's synthetic media in which tyramine and glucose were added in the concentration of 0.5 per cent. After five and ten days interval the media were analysed and the results were obtained as follows.

1) Tyrosol was not detected after the five day cultivation but a small amount of tyrosol was detected by Million's reaction after a ten day cultivation.

2) The crystal of N-acetyltyramine was not obtained in the five day cultivation, but its formation could be detected by paper chromatograph. The crystal was obtained from the media of the ten day cultivation.

3) Acid formation was not observed in the both experiments. From these experimental results, the following metabolic processes were supposed.

Tyramine → N-acetyltyramine → para-hydroxyphenyl-acetic acid.

Aknowledgement

The author wishes to express his gratitude to Dr. H. Takamatsu, Chief of the Pathological Division of the Tuberculosis Research Institute, Kyoto

University and to Dr. S. Yano and also to Dr. K. Hirai, Chief of the Chemical Laboratory of the Osaka Abuyama Red Cross Hospital, under whose supervision this work was done.

References

1) Sakurai, H. : Studies of Metabolism in Tuberculous Lesions. Acta Tub. Jap. **6** : no. 1. (in press.).

2) Shirai, Y. : The decomposition of Tyrosine by Tubercle bailli. Kekkaku : **29** (No. 8) : 295, 1954.

3) Shirai, Y. : The decomposition of Tyrosine by Tubercle bacilli. Kekkaku : **29** (No. 11) : 442, 1954.

4) Neubauer and Fromherz : Zschr. f. physiol. Chem. **70** : 326. 1910.