

Studies of Metabolism in Tuberculous Lesions

III. Further Studies on the Decomposition of Tyrosine-Derivatives by Mycobacterium Tuberculosis.*

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The author carried out those experiments to investigate the decomposition of tyrosine and its derivatives by tubercle bacilli and found that the formed substances were dependent on their C-source both qualitatively and quantitatively.

By reason of the above, I tried to see how the formative substance are converted into other substances by using another kinds of C-source.

The author investigated these various substances that were obtained by decomposing N-acetyltyramine formed by acetylation in the media containing Glucose or Glycerin as the C-source.

The author had already reported on the previous papers^{13,14)} as follows :

Tyrosol was formed from the decomposition of tyrosine by using Glycerin as the C-source. N-acetyltyramine was formed by the decomposition of tyramine in Glucose-Media, and also tyramine was formed from the decomposition of tyrosine.

Considering that the results of the above mentioned experiments, perhaps the composition of C-source has influence not only on the quantity of the N-acetyltyramine and tyrosol formation, but on the quality a little.

The separation of N-acetyltyramine and tyrosol was very difficult by routine methods using the paper-chromatography, paper-electrophoresis and etc..

The melting-point of the produced substance was measured. by using the reaction of 20 per cent sodium hydroxide and benzene chloride¹²⁾ for the analysis of N-acetyltyramine and tyrosol

EXPERIMENTS AND RESULTS

Experiment (1) The decomposition of Tyramine in Lactose-media.

Methods and Materials : Lactose (20 gm in a liter) was used in the media

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instead of glucose as was used in the previous reports.^{8,13)} *Mycobacterium tuberculosis avium* (Takeo) had been cultivated for 20 days. Tyramine (0.5 gm) was used in this experiment. The extraction method was the same as described in the previous report.¹³⁾

Results :

Table 1.

| | Diazo R. | Millon R. | Ninhydrin R. | Crystallization | Chromatography |
|--|----------|-----------|--------------|-----------------|----------------|
| Fraction 1 Amine Fraction (Tyramine) | ## | ## | ++ | ## (0.2) | ## |
| Fraction 2 Tyrosol Fraction (Tyrosol) | ++ | ++ | -- | + | ++ |
| Fraction 3 N-acetyltyramine Fraction (N-acetyltyramine) | ## | ## | -- | ## (0.05) | ## |
| Fraction 4 Acid Fraction (p-hydroxyphenyl acetic acid) | + | + | -- | -- | + |

About 0.2mg of unaffected tyramine still remained in Fraction 1, and a small quantity of oily substances appeared in Fraction 2. (Fraction 1. is used for Amine fraction¹³⁾ and Fraction 2. is used for Tyrosol-Fraction (1) from this point on).

After exclusion of water by clay-plate and vacuum desiccation, the above substance was dissolved in ether and diluted with petroleum ether in complete absence of water. It was then placed in an ice box for crystallization.

The crystals were analyzed by paper-, column-chromatography, and paper electrophoresis etc. (Chromato will be used for them from now on).

At the same time, other portions of it were dissolved in 5 ml of sodium hydroxyd (20 per cent). Then 0.5 ml benzene chloride was added to the solution stirring to obtain crystals.

This precipitated crystal was analyzed through chromato. It was confirmed to be tyrosol-dibenzoate, and therefore, I presumed that the original substance was tyrosol.

The oily substance which appeared in Fraction 2 was extracted repeatedly with hot benzene and then the filtrate was kept in an ice box.

After 24 hours, about 0.05 gms white needle crystals appeared and it was confirmed to be N-acetyltyramine by using chromato, melting-point determination and another methods.

From Fraction 4, the amounts of the isolated substances were very minute.

It had the same Rf value of P-hydroxyphenyl-acetic acid in paper-chromatography.

Experiment (2) The decomposition of Tyramine in Pyruvic acid-Media.

Methods and Materials: Pyruvic acid was used as the C-source instead of glucose in Glucose-Media. The rest of the experiment followed the previous mentioned methods and materials.

Though a large numbers of bacilli were inoculated, the condition for the bacillary growth was poor in the pyruvic acid-media and only thin bacillary membrane formed on the surface of media in 30 days' cultivation.

Results :

Table 2.

| | Diazo R. | Millon R. | Ninhydrin R. | Cryst. | Chromato |
|-------------|----------|-----------|--------------|--------|----------|
| Fraction 1. | ‡‡ | ‡‡ | + | ‡‡ | ‡‡ |
| Fraction 2. | ‡ | + | — | — | + |
| Fraction 3. | ‡‡ | ‡‡ | — | + | ‡‡ |
| Fraction 4. | + | + | — | — | + |

As shown in the above figure, N-acetyltyramine was recognized as crystalline. The large amounts of remained tyramine resulted from the poor growth of the bacilli. According to the above results, it was a very interesting fact that N-acetyltyramine crystals were formed in such bad condition.

Experiment (3) (a) The decomposition of N-acetyltyramine in Glucose-Media.

The bacilli were cultivated at 37°C for 20 days in the Glucose-Media added N-acetyltyramine, and the substance decomposed from N-acetyltyramine by the bacilli was investigated as noted above.

The methods of extraction and the identification of the formed substance,

Results :

Table 3.

| | Diazo R. | Millon R. | Cryst. | Chromato. |
|-------------|----------|-----------|--------|-----------|
| Fraction 1. | ± | ± | — | ± |
| Fraction 2. | ‡‡ | ‡‡ | ± | + |
| Fraction 3. | ‡‡ | ‡‡ | ‡‡ | ‡‡ |
| Fraction 4. | + | + | — | + |

etc., were the same as the previous experiments.

As for N-acetyltyramine, I refined the substance obtained from tyramine on the culture of mycobacterium tuberculosis, and used 0.2 gm of it in this experiment.

As shown in Table 3 in Fraction 1, it was doubtful whether the reactions with every reagent were positive or not. The methods of crystallization and chromato etc., were useless for their identifications.

In Fraction 2 the substance, such as tyrosol, was obtained in such minute amounts that it could be identified only by the methods of chromato. On the chromato, the revealed spot of the substance coincided with tyrosol.

In Fraction 3, 0.1 gm of the remaining N-acetyltyramine was recognized.

Fraction 4, two spots appeared in paper chromatogram, one was the same Rf value of p-hydroxyphenyl-acetic acid and the another was an unknown substance.

Experiment (3) (b) The decomposition of N-acetyltyramine in Glycerin-Media.

Following after the experiment (3) (a), I used glycerin instead of glucose as C-source.

The growth of bacilli in glycerin-media was better than glucose-media, and a large amounts of dried bacilli were obtained. N-acetyltyramine, 0.2 gm, was used for this experiment.

Results :

Table 4.

| | Diazo R. | Millon R. | Cryst. | Chromato. |
|-------------|----------|-----------|--------|-----------|
| Fraction 1. | — | — | — | — |
| Fraction 2. | ## | ## | + | + |
| Fraction 3. | ## | ## | ## | ## |
| Fraction 4. | ++ | ++ | ± | + |

As shown in the Table 4, I could find no special substance in Fraction 1. The substance in Fraction 2 was too small quantitatively but its reactions with Diazo R. and Millon R. were strongly positive.

The amount of the remained N-acetyltyramine in this media was less than in glucose-media, (0.08 mg.).

In Fraction 4, p-hydroxyphenyl-acetic acid was identified by the chromato.

COMMENTS

The author had reported in the previous paper that the decomposition of tyra-

mine was possible in glucose-media and N-acetyltyramine would be obtained by using only 0.1% glucose as C-source and by cultivating the bacilli for only 5 days.

From these results, I tried to find the possibility of N-acetyltyramine formation in the glycerin media and pyruvic acid as C-source.

Moreover, I examined on what process N-acetyltyramine proceeded in the next decomposition step.

The results of these experiments were almost the same in other papers,^{7,18)} explaining that the possibility of tyrosine decomposition was reasonable, and these results would give great aid to the study of the mechanism of tyrosine and its derivatives.

Lactose containing glucose group was used as C-source in this experiment, and tyramine may be decomposed by the action of its glucose group. Moreover, I could presume that the substance formed at the same time was nearly the same as in glucose-media.

(In addition to the fact that tyrosol formation was recognized in glycerin-media).

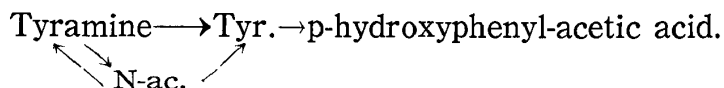
Perhaps, it may be thought that the glucose group has a special effect on N-acetyltyramine, and the occurrence of tyrosol formation was doubtful in glucose-media, while it was indicated that the quantity of the formative substance might be closely connected with the C-source.

In the experiment (2) using another substance, pyruvic acid as C-source, a large amount of tyrosol was obtained as in the case of using glycerin as C-source, but in pyruvic acid-media, tyrosol quantities were less than N-acetyltyramine.

It was considered from this experiment that tyrosol was not always formed in the substance of small carbon number, and N-acetyltyramine might be formed even in the case of using these substances.

The amounts of N-acetyltyramine were larger than those of tyrosol and the remaining amine. This indicated that the decomposition rate was slow, and a large amounts of the added amine were still unchanged during this period, from Table 3 and 4, tyramine was obtained in glucose media but not in glycerin-media. In the previous report, I suggested that the decomposition process was as follows: Tyramine \rightarrow N-ac. \rightarrow Tyr. \rightarrow p-hydroxyphenyl-acetic acid.

From these experiments, the following process is more reasonable.



The fact that such a substance as tyramine was obtained in glucose media, proved that there was a reversible relation between tyramine and N-ac., and this fact is also logically considered to be possible.

The result that I could not confirm tyramine formation in glycerin-media, was understood by considering the decomposition in the process of N-ac. \rightarrow Tyr.

Though the courses, such as N-ac. \rightarrow Tyramine \rightarrow Tyr., is naturally understood,

the possibility of the decomposition on the process (N-ac.→Tyr.) is larger, when the large amounts of N-ac. are added or when few or no tyramine is proved.

(Still now, I can not completely deny the process : tyramine→tyrosol.)

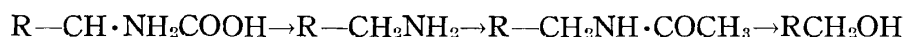
The methods of decarboxylation converting amino acid to amine, have been already made public by the following experiments.

Ellinger²⁾ obtained cadaverine and ptorestine from lysine and arginine respectively. Sasaki³⁾ and Shirai¹¹⁾ obtained tyramine from tyrosine, too. Especially Shirai obtained amine by affecting mycobacterium tuberculosis on tyrosine. Therefore depending on these experiments, the decomposition process leading from tyrosine to tyramine is considered to be correct.

Formerly, Courmont et al⁵⁾ reported that tyrosine and phenylalanine could not be affected by mycobacterium tuberculosis, and that those substances had rather repressive action upon the growth of mycobacterium tuberculosis. Campbell¹⁾ reported that an amine could not be made by decarboxylation of amino acid by mycobacterium tuberculosis.

In regard to the process of the conversion from tyramine, Kurono¹⁾ obtained the next lower alcohol by acting yeast upon tertiary amine. Ichihara⁷⁾ reported that the tyrosine decomposition would take place through decarboxylation, deamination and reduction.

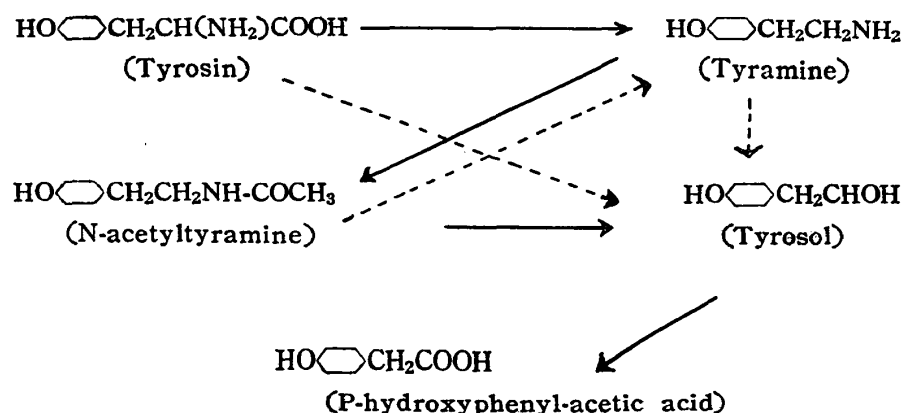
From the results of my experiments, the process of the decomposition through "the way to acetylation" was presumed as follows :



I can not deny the process that the decomposition proceeds through "decarboxylation, deamination and reduction" by mycobacterium tuberculosis.

Tyrosol was obtained in larger amounts than tyramine, in the case of using glycerin as C-source.

On the other hand, the following hypothesis is supposed to be reasonable, and therefore, the process which the decomposition was proceeded through the course (N-acetyltyramine→acid), would be necessarily changed into the process (N-ace-



tyltyramine→tyrosol).

Judging from above mentioned results based on my experiments, tyrosin and its derivatives could be decomposed by mycobacterium tuberculosis avium (Takeo) as the above figure.

SUMMARY

By the action of mycobacterium tuberculosis avium (Takeo),

(1) The author recognized and detected the formation of N-acetyltyramine on the decomposition of tyramine in Lactose-Media by the same methods as in Glucose-Media, and at the same time, tyrosol was also formed.

(2) In Pyruvic-Media tyramine was decomposed and small amounts of N-acetyltyramine can be obtained in a crystalline form.

(3) By use of N-acetyltyramine which was formed from tyramine by mycobacterium tuberculosis avium (Takeo), the following experiments were performed.

a) In Glucose-Media, although N-acetyltyramine was decomposed by mycobacterium, the formations of tyramine and tyrosol were observed obscurely and the formation of p-hydroxyphenyl-acetic acid was detectable.

b) In Glycerin Media, the N-acetyltyramine was decomposed in the same way. In this case, the tyramine formation was doubtful but tyrosol was obtained in small quantities and p-hydroxyphenyl-acetic acid formation was appeared.

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