Studies of Metabolism in Tuberculous Lesions

IV. Studies on Tyramine and its Derivatives in Tuberculous Animals and Patients.

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The author has continued to present a series of the report as regards the cultivation experiment that tyrosine and its derivatives were decomposed on the definite process by mycobacterium tuberculosis. Practically on tuberculous animals or patients, I carried out this experiment for the purpose of knowing whether these substances (Tyrosin and its derivatives) would be decomposed or not through the same process as my previous experiments, and therefore, I tried to the isolated tyramine and its derivatives in the lung of the tuberculous patients. At the same time, after tyramine injection into rabbits, and also sought out its transformed substance.

EXPERIMENT

Materials

1) The lung was taken out from tuberculous patients by the operation : It was devided into necrotic, tuberculous and normal parts in aseptic state.

2) The blood and lung of the experimental tuberculous rabbits : the killed-bacillar vaccine 1.0cc (wet 1mg./L paraffin oil) of mycobacterium tuberculosis avium (heat for 20 minutes at 100°C) was injected into the thigh part of the rabbit and after one week again, injected into the similar part of opposite leg. After 3 weeks, the living-bacilli (0.1 cc) were directly injected into the lung of the rabbit, and the formation of disease-focus was comfirmed after 5 weeks from its injection by X-ray photograph.

a. Blood : —After 2 hrs from tyramine injection into V. auricularis of the rabbit, the blood was collected from the heart.

b. Lung : —The tuberculous lung, especially the necrotic parts including their surrounding tissues.

As the control, the blood of the tuberculous rabbit and the normal lung before tyramine injection.

Method.

1) Blood :

a. Five cc of blood was extracted directly from the heart of the tuberculous

rabbit same as the control.

b. After 2 days from the above-mentioned treatment, 0.1 gm. of tyramine was injected into V. auricularis, and after 3 hrs, 5cc of the blood was collected directly from the heart, and the isolated blood was immediately added to absolute alcohol. After heating, the solution was filtrated through the Toyo Filter Paper No. 51. The filtrate was treated with the same method to the under mentioned treatment of the isolated lung.

2) The lung of experimental tuberculous rabbits :—

For the purpose of extirpation of the lung, after 1 hour, the rabbit of which the blood had been extracted was killed and its lung were treated with undermentioned methods.

3) The treatment of the extracted lung : —

After the extirpation of the lung by the operation, immediately the necrotic part and the tuberculom were separated from the normal part and they were homogenized by homogenizer, and were divided into two portions. One portion of them was put in cellophane menbrane, and dialysed for 10 hrs in distilled water, stirring and chilling in the ice, and then, evaporated on waterbath to concentration.

On the other hand, the other rest portion was put in absolute alcohol, and after heating, it was filtered through Toyo Filter's Paper No. 7. After removing alcohol by evaporation, once more the distilled water was added in it, followed by heating, filtration and concentration.

The upper division of the lung in the control rabbit was treated with same method to the above-mentioned experiment, too.

4) The concentrated sample was analysed through chromatography. Also, its composition in ether or alcohol was followed by fractionation, and at the same time the isolated each fraction was analysed through chromatography.

5) Chromatography

1. Paper-chromatography

Buffer : — Butanol : 2.5N NH_4OH = 4 : 1
Pyridine : 2.5N NH_4OH = 4 : 1
Phenol : dist. water = 4 : 1
Propionic acid : Benzene : water = 2 : 2 : 1

2. Papar-electrophoresis

Buffer : — 0.2 mol Acidic potassium phtalic acid 80 cc.
0.2 mol NaOH 43cc.
Aqua ad 400cc.

Carrent :—10 mA/cm.
Stating voltage : 600V
Phoresis-time : 2 hrs.

Color-Reagent : Ehrlich's Diazo-Reagent
Millon's Reagent
Ninhydrine Reagent
Orcinol Reagent
Iodine Reagent

RESULTS

Table 1. Blood

Case	Substance	Tyramine	Tyrosol or N-ac.	P-hydroxyphen-ylpyruvic acid	P-hydroxyphen-yl lactic acid	P-hydroxy-phenyl-acetic acid
Experimental tuberculous rabbit	1	++	−	±	−	+
	2	+	±	−	−	±
	3	+	−	−	±	+
	4	−	−	−	−	±
	5	±	−	−	−	−
Control	1′	±	−	−	−	±
	2′	−	±	−	−	−
	3′	−	−	−	−	±
	4′	−	−	−	−	−
	5′	−	−	−	−	−

Table 2,

Case	Fraction	Amine Fr.	Tyr. Fraction	N-ac. Fraction	Acid Fraction
Experimental tuberculosu rabbit	1	+	−	−	+
	2	±	+	−	±
	3	−	−	−	±
	4	−	−	−	−
	5	−	−	−	−
Control	1′	±	−	−	±
	2′	−	−	−	−
	3′	−	−	−	−
	4′	−	−	−	−
	5′	−	−	−	−

Table 3. The Lung of Rabbits.

Substance / Case		Tyrosine	Tyramine	N-ac. or Tyrosol	P-hydroxy-phenyl-pyruvic acid	P-hydroxy-phenyl-Lactic acid	P-hydroxy-phenyl-acetic acid
Tuberculous rabbit Tyramine injection	1	+	±	−	±	−	−
	2	+	+	±	−	+	−
	3	±	−	±	−	−	±
	4	−	±	−	−	−	−
	5	−	−	−	−	−	+
Tuberculous rabbit non injection	6	+	±	−	−	±	−
	7	±	−	−	−	−	−
	8	−	−	−	−	−	−
	9	−	−	−	−	−	±
	10	−	−	−	−	−	−
Non tuberculous rabbit Tyramine injection	11	+	−	−	±	−	±
	12	±	±	±	−	−	+
	13	−	±	−	−	−	±
	14	−	−	±	−	±	−
	15	−	−	+	−	−	−
Non tuberculous rabbit non injection	16	±	−	−	−	−	−
	17	±	−	−	−	−	−
	18	−	−	−	−	−	−
	19	−	−	−	−	−	−
	20	−	−	−	−	−	−

Results.

Tab. 1 : The results that the samples were analysed through chromatography before fractionation.

In case 1, the injected tyramine remained and was shown all positive both on paper and electro-chromatography. The positive reaction for p-hydroxyphenyl-pyruvic acid was obtained only in butanol buffer. P-hydroxyphenyl-acetic acid was positive only when paper chromatography was used.

Also, in case 2, tyramine was shown positive on paper chromatography, and other substances were uncertain.

The signs in case 3, 4, 5 and the control are written in accordance with case 1,2.

Tab. 2 : Though I tried to crystallize any substances from the blood by frac-

tionation, but I could not get the crystal at all. However, chromatography to analyse each fraction was used, and consequently, tyramine in amine fraction, tyrosol and one portion of p-hydroxyphenyl-pyruvic acid in tyrosol fraction and all acid in acids fraction were confirmed.

Tab. 3 : Case 1~5 are the occasions that tyramine was injected into the experimental tuberculous rabbit. The necrosis was found in the lung of its rabbit. Case 6~10 : The results from the occasion that the lung of tuberculous

Table 4. The Lung of Human.

materials	case	Tyrosine	Tyramine	Tyrosol or N-ac.	P-h-Phenyl -pyruvic acid	P-h-phenyl -lactic acid	P-h-phenyl -acetic acid
Necrotic part	1	++	−	−	−	−	+
〃	2	+	−	−	−	−	−
〃	3	+	−	−	−	±	−
〃	4	±	−	−	−	−	−
〃	5	±	−	−	−	−	−
〃	6	±	−	−	−	−	−
〃	7	±	−	−	−	−	−
〃	8	±	−	−	−	−	−
〃	9	−	−	−	±	−	−
〃	10	−	−	−	−	−	−
〃	11	−	−	−	−	−	−
〃	12	−	−	−	−	−	−
〃	13	−	−	−	−	−	−
〃	14	−	−	−	−	−	−
〃	15	−	−	−	−	−	−
〃	16	−	−	−	−	−	−
Tuberculom part	17	+	−	−	−	−	−
〃	18	+	−	−	−	−	−
〃	19	−	−	−	−	−	−
〃	20	−	−	−	−	−	−
〃	21	−	−	−	−	−	−
〃	22	−	−	−	−	−	−
Pleural Exsudat.	23	++	−	−	−	+	±
Pleural-Exsudat. Tyramin	24	+	++	−	−	−	+

rabbit was extracted in non-treatment state.

Case 11～15 : The cases that tyramine was injected into the normal rabbit. Cases 16～20 : The cases that the lung of normal rabbits was extracted in non-treatment state.

Tab. 4 : The results before and after fractionation were put together into this Table.

Case 1～16 : The necrotic lung tissue was used in the most parts of these cases.

Case 17～22 : In these cases, the tissue are used thought as tuberculom for the most parts of the tissue.

Case 23 : The sample of this case was pleural exsudate of empyema thorax patient, and its exsudate was almost in pus-state.

Case 24 : After 24 hrs from the infusion of tyramine 0.1 gm. into the pleural cavity of the case 23-patient, the pleural exsudate was extracted and treated in similary to case 23.

It was impossible for all cases to be crystallized by fractionation.

COMMENT

Tyrosine and tyramine were decomposed not only in the cultivation medium by the bacilli or the bacillary enzymes but in the animal and human lungs. This experiment was expected that the enzymes in the body would give the great influence upon the decomposition.

In the first place, according to the experimental results of the blood, it is impossible to get out the crystal from the blood of the injected tyramine. As for the reason, it is considered that the injected tyramine was too small amounts, it is diluted by body fluied, and, at the same time, it was decomposed.

Although the formation of the substances (tyrosol, N-acetyltyramine, p-hydroxyphenyl-pyruvic acid and p-hydroxyphenyl-lactic acid) was usually found as uncertainty, but it was negative in several cases. Comparing these results with the control, tyramine and tyrosol or N-acetyltyramine were recognized as uncertainty even in the control case, too. It is considered for these results to be dependend upon the reasons that these substances were formed by the decomposition of tyrosine owing to the wide range of lesion.

As regerds the question whether it is tyrosol or N-acetyltyramine, it is apparently considered to be tyrosol from the results of the fractionation in Tab. 2, but it is uncertain that this tyrosol is formed directly from tyramine or tyrosine.

As to p-hydroxyphenyl-acetic acid, almost the similar results are found in the case of tyramine injection of thec ontrol. From the results of Tab. 2, it is recognized that p-hydroxyphenyl-acetic acid is formed in larger amounts in the case of tyramine injection than in the control.

In the experiments on the lung of rabbits, when tyramine was injected, it is undoubtedly obvious that the tyramine would be recognized in the isolated lung and in the case of non-tyramine-injection, tyramine was uncertainly found in only one of the tuberculous rabbit.

The substances, such as tyrosol or N-acetyltyramine, were recognized only in the tyramine-injection case, and these substances were observed to be tyrosol by the similar analytical method of these series II., but the such substances could not be recognized in non-injection case. Owing to its above-mentioned results, these substances would be probably formed by the decomposition of tyramine.

The formation of p-hydroxyphenyl-pyruvic acid and p-hydroxyphenyl-lactic acid, was doubtful, but it is considered that, owing to the results on the healthy-non-treated rabbit, its formation is caused by the decomposition of tyrosine within the decomposed tissue of the rabbit with such wider lesion as the rabbit which was used in the experiment on the blood, and that its formations were not caused directly from the decomposition of tyramine.

In the experiment of the human lung, tyrosine is recognized in the several cases of its experimental lungs. Its tyrosine amount became the higher in the lung including the larger necrotic part. As regards the cause of its formation, its tyrosine formation would be caused by the decomposition of tissue protein.

Although the individual formation of p-hydroxyphenyl-pyruvic acid and lactic acid was doubted in one example, its formation would not procced on the course, tyrosine→tyramine, but its course would be tracted ontyramine→p-hydroxyphenyl-pyruvic acid→p-hydroxypheny-lactic acid.

As regards this process, it is resonable to consider as follows—this is not the process that tyramine would be decomposed by tubercule bacilli themselfs, but this process would be effected by enzymes which existed in the body.

Ewins & Laidlaw[1] prescribed tyramine to dog through its mouth and recognized p-hydroxyphenyl-acetic acid from the dog's urine, and moreover obtained the same results as above-mentioned in the reflex-test of the liver and the uterus.

The such results prove that this process is similar to my cultivation experiment as follows; Tyrosine→tyramine→tyrosol→p-hydroxyphenyl-acetic acid.

According to my experimental dates for rabbits, tyrosol formation was recognized in the case of tyramine injection, this formation proved that tyramine would be decomposed through the following process (Tyrosine→tyramine→p-hydroxyphenyl-acetic acid) and that the decomposition would not be caused by mycobacterium tuberculosis, but be caused by body enzymes similarly to Ewins's experiment. On the account of the experimental results of Salkowski[3] et al[4]., etc.[5][6], Ichikawa[2] explained that the decomposition process (phenylalamine→phenyl pyruvic acid→phenyl acetic acid) might be caused by tubercle bacilli, but

phenyl pyruvic acid in the body, and similarly in the case of tyrosine, the decomposition, such as above-mentined process, would be caused.

On the experiment fo the tuberculous rabbit, the unknown spot was revealed in the case of using butanol in paper chromatography, but I did not the determination of this spot although this spot was considered to be corresponded to homogentidic acid,

Matsumoto[7] obtained homogentidic acid by decomposing tyrosine with liver's enzyme, therefore the unknown substance in the experimental case of rabbits may be corresponded to this homogentidic acid.

Marshall et[8][9] al, obtained complex compound by prescribing sulfamine to the dog through its mouth or by acting sulfamine on a piece of liver, and moreover Du Vigneaud[10] gave phenyl-amino-lactic-acid to dog and obtained acetyl-phenyl-amino-lactic acid.

However, in my experiment, although p-hydroxyphenyl-pyruvic acid formation was doubted, N-acetyltyramine could not be found at all.

I recognized tyrosine in larger amounts in the pleural exsudate, and also at the same time, rarely found the formation of p-hydroxyphenyl-acetic acid in the case of tyramine infusion.

These results proved that either tyrosine or tyramine would be decomposed to p-hydroxyphenyl-acetic acid.

It is probable consideration that, in the animal tuberculous lesion, tyramine and its derivatives would proceed the decomposition process passing through p-hydroxyphenyl-acetic acid by the simultaneous action of both tubercle bacilli and body enzymes.

SUMMARY

1) the blood and the lung after tyramine injection into the tuberculous and non tuberculous rabbit, were isolated and the transformation of the substance was investigated. According to the results, it is considered to be reasonalbe that the substance would be decomposed on the process passing through p-hydroxyphenylacetic acid.

2) By tyramine injection, tyrosol is considered to be formed within the lung having the tuberculous lesion. But it is not clear whether tyrosol was transformed from tyramine or not.

3) In the 20 tests on the isolated human lung, tyrosine was recognized the several cases of these tests, but its tyrosine derivatives were not recognized in any test with the exception of only one case having the recognition of p-hydroxyphenyl-acetic acid.

4) In the pleural exsudate (pus), large amounts of tyrosine were recognized, and also p-hydroxyphenyl-acetic acid was recognized after tyramine infusion.

5) In all tests, it was impossible to obtain the crystal of any substance by the fractionation.

6) It is considered that tyramine would be decomposed within the body of the experimental rabbit and then would pass through p-hydroxyphenyl-acetic acid.

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