138. Research on Xanthurenic Acid. XIV
Effect of 5-Hydroxy-anthranilic Acid on the Formation and
Conjugating Reaction of Xanthurenic Acid in Albino Rat

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Introduction. As confirmed by Yahito Kotake et al.¹⁾ in their preceding paper, simultaneous administration to an albino rat of sodium butyrate and tryptophan in fairly large quantities causes an excretion of xanthurenic acid in its urine, and animal shows pancreas diabetic symptoms. But in such case, insulin,²⁾ V.B.₆ or methionine³⁾—the last one above all—can inhibit the output of xanthurenic acid to be excreted.

The present paper deals with an other discovery in this connection that 5-hydroxy-anthranilic acid, a tryptophan metabolite pointed out by Yashiro Kotake⁴⁾ as one of biocatalysators, has not only an inhibitive action against the formation of xanthurenic acid in the body of an albino rat but also increases its conjugating reaction. The effect of 5-hydroxy-anthranilic acid on xanthurenic acid in the body, therefore, can inevitably be regarded to be a sort of detoxication.

Experimental and Results. (I) Inhibitive action of 5-hydroxy-anthranilic acid against the formation of xanthurenic acid and the consequent development of diabetic symptoms in an albino rat administered with sodium butyrate and tryptophan.

(1) Materials and procedure. Albino rats fed for more than two weeks on a casein diet (casein 22%, McCollum salt 6%, agar-agar 3%, yeast 2%, butter 10%, sugar 5%, and starch 52%) and weighing around 200 g were used.

Those orally administered with sodium butyrate 0.4 g and tryptophan 0.1 g were set aside as the control group, while those further injected with 0.2 mg/kg of 5-hydroxy-anthranilic acid twice, that is, immediately after the said administration and at an interval of one day after the first injection, were employed as the test group. The 24-hour urine collected from each group was subjected to our examination.

(2) Quantitative determination of xanthurenic acid. In determining xanthurenic acid, Glazer-Mueller's method⁵⁾ was employed. As shown in Table I, the excreted amount of xanthurenic acid remarkably decreased in the urine of the test group, as compared

with the control group. It is certain, therefore, that 5-hydroxy-anthranilic acid can remarkably diminish the formation and excretion of xanthurenic acid in the body.

Table I. Excreted xanthurenic acid

Exp. no.	Control group mg	Test group mg
1	9.8	2.1
2	8.24	1.4
3	7.52	1.85
4	8.19	2.38
5	7.73	3.6
6		1.8
7		1.22
8		2.5
9		2.4
10		1.52
Average	8.29	2.07

reported by Yahito Kotake and T. Inada in their paper, revealing the continued hyperglycemia 24 hours after the initial hyperglycemia. In this continued hyperglycemia period, sugar was detectable in its urine and diabetic symptoms were noticeable. But on examination of the test group, it was discovered that the formation of xanthurenic acid was inhibited by the action of 5-hydroxy-anthranilic acid, and although the blood sugar curve varied more or less at the initial state, it was restored to normality 24 hours later without developing any diabetic symptoms at all.

(4) Paper-partition-chromatography. The above experiment well testifies that 5-hydroxy-anthranilic acid blocks the output of xanthurenic acid in the body of an albino rat administered with sodium butyrate and tryptophan and as the result the development of continued hyperglycemia does not occur, but in order to further clarify the action of 5-hydroxy-anthranilic acid with regard to its effect on the tryptophan metabolism caused by administration of sodium butyrate, paper-chromatographical examination was further taken up.

Method: One-dimensional and ascending method.

Developer: Methanol : n-butanol : benzene : water (2:1:1:1).

(3) Blood sugar measurement. The measurement was conducted in accordance with King-Garner's method.⁶⁾ Two different group of albino rats, such as mentioned above, were employed for the purpose. The results are shown in Fig. 1 (one typical example with regard to each group).

The blood sugar curve of control rat, as shown here, coincided with the one previously

Fig. 1. Blood sugar curve

— Control rat (Na-butyrate 0.4 g+tryptophan 0.1 g)
 - - - Test rat (Na-butyrate 0.4 g+tryptophan 0.1 g+5-hydroxy-anthranilic acid)

Developing time: 15 hours.

On each sheet of the filter paper employed more than 10 spots were obtained and the identification of each spot was attempted on the basis of its R_f value, fluorescent color, color-reaction, and its ultraviolet spectrum.

As a result, 3-hydroxy-kynurenine, its conjugated compounds and xanthurenic acid were detected to be on the decrease remarkably in urine of the test group of albino rats, as compared with the control group. On the contrary, however, there could be found such fluorescent spots as were identifiable to be the conjugated compounds of xanthurenic acid, glucuronide of anthranilic acid and its derivatives, along with a fairly large amount of N^{α} -acetyl-kynurenine. It is possible to conclude, therefore, that the tryptophan metabolism abnormalized by administration of sodium butyrate came back to normality again by the action of 5-hydroxy-anthranilic acid.

(5) Quantitative determination of kynurenic acid. The above-stated decreased excretion of xanthurenic acid and increased output of kynurenic acid can be considered to be a sort of detoxication against xanthurenic acid, as in the case of insulin previously studied by Yashiro Kotake, Yahito Kotake, M. Hishikawa, T. Sakan, and M. Yamaguchi. In order to further ascertain it, an attempt was made this time to determine the content of kynurenic acid excreted in the urine of both groups.

The fluorescent spots representing kynurenic acid from above-mentioned paper-partition-chromatogram (pH of urine employed is 8.0), were cut out and extracted in phosphate buffer (pH 7.4). By employing Beckman's spectrophotometer to obtain the optical density at $332\text{m}\mu$ of the extraction in accordance with Knox's method,⁷⁾ the amount of kynurenic acid was calculated.

The results are shown in Fig. 2. In comparison with the urine of the control rats, kynurenic acid amount of test rats was found to increase remarkably by the action of 5-hydroxy-anthranilic acid.

(II) Effect of sodium butyrate on the conjugating reaction of xanthurenic acid.

Albino rats fed for more than two weeks on a casein diet and weighing around 200 g were set aside as the control group. Those employed as the test group were further administered orally with 0.4 g of sodium butyrate. To each rat of these two groups 10 mg of xanthurenic acid (m.p. 296.5°C) in ammoniacal solution (pH 7.2–

Fig. 2. Excretion of kynurenic acid (mean value of 3 rats)

Table II. Excreted ethereal sulfate (mean value of 3 rats)

	Excreted ether sulfate pro 10 mg xanthurenic acid	
	*The amount of SO ₃ mg	% of conjugating xanthu- renic acid with H ₂ SO ₄
10 mg xanthurenic acid injected group	0.8877	22.74
Oral administration with 0.4g sodium butyrate and xanth. a. inj.	0.415	10.63

* This indicates the absolute increased amount of conjugated SO₃ by injection of 10 mg xanthurenic acid.

Table III. Quantitative determination of xanthurenic acid
(mean value of 3 rats)

		Hydrolysis		Conju- gated xanth. acid mg	% of con- jugation to excre- tion of total xanth. a.	Relative % of con- jugated xanth. a.
		Xanth. a. before hydrolysis	After			
1	Xanth. acid (inj.)	6.02	8.52	2.5	29.3	100
2	Sod. butyr. (per os) xanth. acid (inj.)	6.53	7.44	0.91	12.2	36.4
3	Methionine (inj.)+ sod. butyr. + xanth. acid	5.95	7.50	1.55	20.6	62.0
4	5-Hydroxy-anthranilic acid (inj.) sod. butyr. +xanth. acid	5.95	7.67	1.72	22.4	68.8

7.4) was injected hypodermically and the 24 hours urine was collected.

(1) Quantitative determination of ethereal sulfate of xanthurenic acid.⁸⁾ The determination of ethereal sulfate of xanthurenic acid was conducted in accordance with Sperber's method,⁹⁾ by use of turbidity measurement with regard to barium sulfate. Beckman's spectrophotometer was employed at the wave length of 650 mμ.

On examination of the urine of the test group orally administered with sodium butyrate and injected with xanthurenic acid, the content of ethereal sulphuric acid, as shown in Table II, were clearly diminished as compared with the control group simply injected with xanthurenic acid.

(2) Quantitative determination of conjugated xanthurenic acid. In determining xanthurenic acid, the afore-mentioned Glazer-Mueller's method was employed. The collected urine was mixed with hydrogen chloride until 5N was reached and then hydrolyzed in a sealed tube at 100°C. The contents of xanthurenic acid both before and after the hydrolysis were determined. As shown in Table III, the per-

centage of conjugated xanthurenic acid was found to be about one-third when sodium butyrate was administered to albino rats.

(III) Effect of methionine and 5-hydroxy-anthranilic acid on the conjugating reaction of xanthurenic acid.

The experimental procedure stated above was again employed. At 24 hours later, after the first injection of 10 mg of methionine, another 10 mg of it was hypodermically injected simultaneously with injection of xanthurenic acid, 100 γ of 5-hydroxy-anthranilic acid was also injected twice in the same way as above. The results are shown in Table III. In the two experiments, the decreased conjugative reaction of xanthurenic acid brought about by administration of sodium butyrate was remarkably restored and approximately normalized by the action of methionine or 5-hydroxy-anthranilic acid.

Consideration. From the experiment stated above, it is now clear that 5-hydroxy-anthranilic acid, a tryptophan metabolite discovered in vivo by Yashiro Kotake as one of biocatalysators is remarkably inhibitive against the output of xanthurenic acid, an abnormal tryptophan metabolite produced consequent upon excessive administration of sodium butyrate (or fat). Both methionine and 5-hydroxy-anthranilic acid are capable of restoring the weakened conjugating reaction of xanthurenic acid by administration of sodium butyrate in the body.

From these findings, it is possible to conclude that 5-hydroxy-anthranilic acid inhibits the formation of xanthurenic acid and accelerates its conjugating reaction and as the results the accumulation of xanthurenic acid in the body is ceased. This fact prevents simultaneously the diabetic symptoms caused by xanthurenic acid.

In concluding this report, we wish to express our heart-felt gratitude to Prof. Yashiro Kotake for his helpful suggestion and guidance.

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