15. Research of Xanthurenic Acid. I.

A New Method of Isolating Xanthurenic Acid, and Several Researches in Relation to It.

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Introduction: In 1935, Musajo (1) fed white rat with fibrin, and isolated a yellow crystal compound naming it xanthurenic acid. Later this compound was confirmed as 4,8-dihydroxyquinoline-2-carboxylic acid (2) and built from tryptophane passing kynurenine in vivo (3, 4). In 1943, Lepkovsky, Roboz and Haagen-Smit (5) noticed the appearance of a large amount of xanthurenic acid in the urine of a white rat which has become pyridoxine deficient by feeding with a pyridoxine deficient diet for a long time, given tryptophane or protein containing much tryptophane, and succeeded relatively easily isolating xanthurenic acid from this urine by the help of a paper packed chromatographic column (6). Lepkovsky et al. concluded that xanthurenic acid is a certain abnormal metabolite of tryptophane metabolism when the body is pyridoxine deficient, from the fact that xanthurenic acid appears in the urine of a pyridoxine deficient white rat and quickly disappears from the urine when given pyridoxine. Further this compound is excreted in the urine of white rat, rabbit, dog, guinea pig, pig, human being (7, 8) etc., but not in that of chickens.

We accidentally succeeded in occurring pyridoxine deficiency very simply in a short time by giving sodium salt of fatty acid orally to white rat, and succeeded in easily isolating xanthurenic acid in a crystal form by giving tryptophane at the same time in this condition and collecting the 24 hours urine, and as we have done several experiments accompanying it, so we will report them here.

Experiment.

(I) Make a body weight 120–150 g white rat hungry for one day, and give sodium butyrate 0.3 g and tryptophane 0.1 g orally at the same time, then after several hours is excreted a thick yellow urine, which gives color reaction of xanthurenic acid by ferric iron, that is, it develops a remarkable green color when added a minute amount of ferric chloride, and excretion of a large

amount of xanthurenic acid is noticed. Purifying the clear yellow urine, which is obtained by collecting the 24 hours urine of 10 white rats managed as above mentioned, by using the paper packed chromatographic method following the method of Lepkovsky et al, we succeeded in easily isolating the xanthurenic acid in crystal form. We obtained 30 mg of crystal xanthurenic acid from 1 g tryptophane which is for 10 rats. The obtained crystal was ferric iron reaction, diazo reaction, Millon reaction, HCl- and KClO_3 reaction all respectively positive (5).

(II) The excretion amount of xanthurenic acid when given many kinds of fatty acids.

We compared the quantitative determination of xanthurenic acid in the urine of white rat when given only tryptophane and when given one of many kinds of fatty acids at the same time with tryptophane. The xanthurenic acid amount was measured by colorimeter which was obtained by acting ferric chloride on it in pH 2-3 according to Miller and Baumann's method (9). The results are shown in the following table.

xanthurenic acid mg/24 hrs when given tryptophane 0.1 g plus one of many sorts of fatty acids 0.4 g (body weight 120 g)	xanthurenic acid mg/24 hrs when given tryptophane 0.1 g plus each mol of fatty acid as sod. propionate 0.3 g (body weight 200 g)
only tryptophane	1.61
tryptophane plus sod. acetate	5.37
trypt. plus sod. propionate	8.79
trypt. plus sod. butyrate	9.89
trypt. plus sod. valerianate	9.64
trypt. plus sod. palmitate	9.61
trypt. plus sod. stearinate	8.57
trypt. plus sod. oleate	10.49

When tryptophane 0.1 g is orally administered together with each fatty acid 0.4 g, each fatty acid excretes almost the same amount of xanthurenic acid. Consequently, we have made it clear that fatty acids besides butyric acid have the same effect as butyric acid.

(III) Experiment administering sodium salt of fatty acid and tryptophane with pyridoxine.

From the fact that the appearance of high xanthurenic acid amount is seen in the urine when administered tryptophane and fatty acid, we considered that acute pyridoxine deficiency is occurred in the body of the white rat when fatty acid is administered. Now a phenomen to prove the fact, the occurrence of hair redness, which is the characteristic of pyridoxine deficiency, was con-

firmed. So making the white rat administered sodium butyrate and tryptophane as a control, we compared the excretion amount of xanthurenic acid when pyridoxine is administered together with sodium butyrate and tryptophane. The results are shown in the following table.

Rat (male) 150 g. body weight. Administered compound	Xanth. acid mg/24 hrs
tryptophane plus sod. butyrate 0.3 g	10.40
tryptophane plus sod. butyrate 0.3 g plus pyridoxine 10 mg (injection)	6.73

As seen in the table, decrease of xanthurenic acid excretion was surely noticed by pyridoxine administration. Consequently, we consider it probably correct that acute pyridoxine deficiency is occurred by fatty acid administration. In another words, it shows that pyridoxine is necessary in the fatty acid oxydation process in vivo. We wish to prove it further with more experiments.

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