XANTHURENIC ACID, AN ABNORMAL METABOLITE OF TRYPTOPHAN AND THE DIABETIC SYMPTOMS CAUSED IN ALBINO RATS BY ITS PRODUCTION

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Since 1950 Yahito Kotake *et al.* (1, 2) have performed a series of experiments on the diabetic symptoms caused in albino rats by the administration of xanthurenic acid (XA), an abnormal metabolite of tryptophan. Their studies have come to present some conclusive evidences that XA is relevant to causing human diabetes which has been generally considered to be attributable to an inadequate food ingestion and also to suggest a close relationship between the animal and human diabetes.

In recent years experimental studies of developing diabetic symptoms by administering chemical substances to animals have repeatedly been reported. Thus, Dunn *et al.* (3) have for the first time succeeded to produce experimental diabetes by the administration of alloxan. However, whether or not alloxan can really be a metabolite, remains yet to be known, although its possibility has been explained in some ways. Okamoto *et al.* (4-6) have likewise succeeded by using oxine, dithizone or nicotine in producing diabetic symptoms in animals, and presented the "zinc theory". This theory is of great interest, and has played an important part in bringing our research to a successful conclusion, since XA we employed is a substance with a close resemblance to oxine in chemical structure. Contrary to oxine, which has nothing to do with metabolism, XA, as was discovered by Musajo (7) and later identified by Lepkovsky *et al.* (8), is a metabolic product. Our experiments have confirmed it to be a substance produced in the body as a result of taking too much sodium salt of fatty acid (representing fat) and tryptophan (representing animal protein). The experimental method, which the author have employed may be a new road to the study on human diabetes.

RESULTS AND DISCUSSION

1. *Tryptophan Metabolism.*

The normal metabolic mechanism of tryptophan has so far been ascertained by Yashiro Kotake (9) to be the following: tryptophan→kynurenine→anthranilic acid→5-hydroxyanthranilic acid. He has confirmed that the enzymes involved in these chain reactions are tryptophanpyrrolase and kynu-

reninase, and that 5-hydroxyanthranilic acid thus produced has several important physiological actions, *i. e.* promoting the liver function, replenishing the blood, accerelating the bodily growth, *etc.* Another metabolic way, proposed by Beadle *et al.* (10) is as follows: tryptophan→kynurenine→3-hydroxyanthranilic acid→nicotinic acid. Braunstein has made it clear, that both of these processes fail to take place in the absence of vitamin B₆. It follows, therefore, that in albino rats of vitamin B₆ deficiency, these two series of reactions are inhibited, thus producing XA from tryptophan and excreting it in the urine as an abnormal metabolite.

In our experiment, we fed albino rats for approximately 50 days on food deficient in vitamin B₆ containing 22 % of casein and administered in addition 10 mg of tryptophan per day. The urine containing 4.5–7.0 mg of XA per day could be collected. Glazer and Mueller have also proved in human experiment that the vitamin B₆ deficiency (caused by the administration of desoxypyridoxine) promotes XA production as a result of a disturbance in tryptophan metabolism.

2. Administration of Tryptophan together with Sodium Salt of Fatty Acid and the Urinary Excretion of XA.

In the first series of experiments, we administered orally and simultaneously a large amount of both sodium salt of fatty acid and tryptophan to albino rats (for instance, 0.1 g of tryptophan and 0.4 g of sodium butyrate), and confirmed the excretion of much XA in the urine after a few hours. The same effect was observed not only by sodium butyrate alone but also by other fatty acid salts as shown in Table I (11).

Table I
The Amount of XA Excreted in 24-hour Urine.

XA was determined by Glazer method.

To albino rats, weighing 120 g, 0.1 g of tryptophan and 0.4 g each of sodium salt of various fatty acids were simultaneously administered.

Substances administered	Amount excreted
	mg
Tryptophan alone	1.61
Tryptophan plus sodium acetate	5.37
Tryptophan plus sodium propionate	8.79
Tryptophan plus sodium butyrate	9.89
Tryptophan plus sodium valerianate	9.64
Tryptophan plus sodium palmitate	9.61
Tryptophan plus sodium stearate	8.57
Tryptophan plus sodium oleate	10.49

Table I clearly shows that, compared with the administration of tryptophan alone, a remarkably increased excretion of XA was observed when one of the various fatty acids was administered together with tryptophan. From the collected urines of the albino rats, to which 0.4 g of sodium butyrate and 0.1 g of tryptophan had been simultaneously administered, we succeeded in separating and crystallizing XA. This method was found to be more convenient than that of Lepkovsky *et al.*, in which albino rats were fed for more than a month on a diet deficient in vitamin B₆. XA, when separated and crystallized, showed an aggregation of yellow, minute, needle-like crystals, and the reactions of Fe⁺⁺⁺, diazo, Millon and HCl-KClO₃ were found to be positive. We could obtain approximately 30 mg of XA from the urines of 10 albino rats.

This experiment, in consideration with that of Lepkovsky, lead us to a conclusion that the administration of a large quantity of sodium salt of fatty acid to albino rats unfailingly results in vitamin B₆ deficiency, showing that vitamin B₆ is absolutely indispensable to the oxidation of fatty acid or its related reactions.

In order to prove that the administration of sodium salt of fatty acid and tryptophan in a large amount could really cause vitamin B₆ deficiency, we administered the two substances to one group of albino rats and pyridoxine in addition to the two substances to another group. The amount of XA excreted in the urine was analyzed in both groups with the results given in Table II.

Table II
The Amount of XA Excreted in 24-hour Urine.
Albino rats weighing 150 g were employed.

Substances administered	Amount excreted (mean of 4 rats)
	<i>mg</i>
Tryptophan (0.1 g) plus sodium butyrate (0.3 g)	8.42
Tryptophan plus sodium butyrate (0.3 g) plus pyridoxine 10 mg (by injection)	2.03

As shown in Table II, the amount of XA eliminated in the urine decreased when pyridoxine was administered in addition. But the decrease in this case was not complete as was expected and some factors besides vitamin B₆ deficiency are assumed to exist which helps to produce and excrete XA after simultaneous administration of sodium butyrate and tryptophan.

The above experiment proves that by ingesting too much fat, normal tryptophan metabolism is greatly affected, leading to the formation of XA in the body. This finding is significant from the dietetic standpoint, for the ingested tryptophan is made inutile to the organism and, moreover, XA thus produced has an inimical diabetogenic action. This convinces us the importance of keeping constantly a harmonious combination of fat, protein and vitamin B₆ in food.

3. Diabetogenic Effect of XA upon Albino Rats.

A great interest has come to be focused lately on administering various kinds of chemical substances for producing diabetic symptoms in experimental animals. Thus, alloxan was first employed for the purpose, followed by zinc precipitants such as oxine, dithizone and nicotine, which led to the establishment of the "zinc theory" of Okamoto (6).

All these substances are, however, regarded to have nothing to do with metabolism. Many attempts have been made to relate alloxan and metabolism, but no direct proof in favor of it has yet been found. As for oxine and the like, it is out of question to try to consider them as in any way relevant to metabolism. XA, on the contrary, is undoubtedly a metabolite of tryptophan. We first noted its close resemblance in its chemical structure to oxine and further observed that some of the albino rats manifested cataract when fed for 150 days after several repeated administrations of sodium butyrate and tryptophan. These findings served us as a clue to the belief that XA can be a factor capable of producing diabetic symptoms in rats (2).

Effect of XA on Blood Sugar Level. We fed albino rats on a synthetic diet for two weeks consisting of in per cent : casein 22, McCollum salt mixture 6, agar-agar 3, yeast 2, butter 10, sugar 5, and starch 52.

To one group of the animals sodium butyrate and tryptophan were orally administered (Fig. 1) and in another group, an aqueous solution (pH 7.4) of crystallized XA separated beforehand from the urine of albino rats was injected intraperitoneally (Fig. 2). Changes in blood sugar level were estimated by Hagedorn-

Jensen method in each group. In all cases, 30 in all, the temporary hyperglycemia commenced about one hour after the treatment, followed by an intermediary period of hypoglycemia and finally the continued hyperglycemia, accompanied with glycosuria.

Histological Changes of Pancreas after Administering XA. We injected intraperitoneally 200 mg of XA per kg body weight to albino rats fed on the synthetic diet described above and sacrificed them after 48 hours. Pancreas tissue was histologically examined after staining by the alumen-haematoxylin-phloxin method of Gomori (12, 13). Definite pathological changes were observed in all tissues examined, namely, a remarkable drop of stain-

Fig. 1
Blood Sugar Curve

Male albino rats, weighing 150 g were employed. 0.4 g of tryptophan and 0.8 g of sodium butyrate were orally administered.

++, + indicates the amount of glucose in the urine determined by Benedict's method.

ability with hematoxylin and the resultant transparency of the cytoplasm in most β -cells of the Langerhans islets, vacuole formations and nuclear picnosis in some β -cells, *etc.* In the α -cells no such changes were detected.

It can be concluded from the results obtained that XA is unmistakably the causative substance of diabetic symptoms in albino rats. Taking further into consideration the fact that diabetic patients are found mostly in civilized countries, especially among the people ingesting good food,

we believe that this findings are highly significant. Statistics tell us that in Japan the number of diabetic patients was remarkably small during the war-time when the national food supply was extremely unfavorable, while it again started to increase with the gradual recovery of normal good living in the post-war period. Good food inevitably contains a great deal of fat and protein (rich in tryptophan), and this has an important bearing upon our experiments. From such a viewpoint, we are induced to believe that some correlated causes must exist between both animal and human diabetes.

In order to clarify further the interrelationship among food, blood sugar, body weight, XA output and diabetic symptoms, and also in order to ascertain the accumulative effect of XA upon the development of diabetic symptoms, we performed the following two experiments.

Blood Sugar Level and Body Weight after Feeding High Fat and Tryptophan Diet. Albino rats were fed on a diet containing rich fat (butter 35 %) and casein (25 %) for 240 days and the blood sugar level and body weight were plotted against time of feeding as given in Fig. 3. Even in this case, about 1 mg per day of XA was excreted in the urine, since the diet consisted of much fat and casein. In order to promote the accumulation of XA in the body, 10 mg of tryptophan daily was added to the diet mentioned above for the first 30 days of experiment.

As the result, 2–3 mg of XA were found to be excreted daily. In parallel with this, the albino rat started to gain their body weight steadily and regularly till they showed an especially remarkable increase on about the 170th day, attaining the maximum weight of 325 g manifesting obesity due to excess fat. Since albino rats fed in Japan on casein diet seldom exceed 250–300 g in body weight, these results are considered to be of particular interest. The blood sugar level was also found to rise gradually till it reached the maximum (180–200 mg/100 ml) on about the 170th day in parallel

Fig. 2
Blood Sugar Curve

Male albino rats weighing 150 g were employed.

XA 120 mg/kg was injected in intraperitoneally.

++, + indicate the amount of glucose in the urine determined by Benedict's method.

with the remarkable increase of body weight. For the determination of the true blood sugar level, King and Garner's method was adopted, using 0.02 ml of blood. By this method we invariably obtained a lower value by about 30 mg/100 ml as compared with that of Hagedorn-Jensen. On about the 200th day, they abruptly began to lose their body weight, hyperglycemia being still unchanged in the meantime, and the animals died on the 240th day.

From the results stated above, it has become clearer than ever the close interrelationship between the administered diet and the development of diabetic symptoms in these animals, and the probable nutritional etiology of the disease seems to have become more convincing.

Blood Sugar Level and Body Weight after Feeding on Vitamin B₆ Deficient Diet. Our next experiment (15) was to feed albino rats over a period of 250 days on a diet deficient in vitamin B₆ consisting of in per cent : Hammersten casein 20, McCollum salt mixture 4, salad oil 2, and sugar 74, containing in addition per gram of the diet following vitamins (in γ) : Thiamine hydrochloride 3.3, choline chloride 166.0, inositol 333.0, *p*-amino-benzoic acid 200.0 and riboflavin 6.6.

After feeding the rats under the conditions of the adequate urinary excretion of XA the blood sugar level and body weight were plotted against the experimental days as shown in Fig. 4.

On the 20th day, 10 mg of tryptophan was added daily to the diet for 60 days in order to promote the production of XA in the body. The albino rats, each weighing about 280

Fig. 3
Body Weight and Blood Sugar Level after Administering High Fat and Casein Diet. Albino rats were fed for a long period on a diet containing much fat and casein.

Fig. 4
Body Weight and Blood Sugar Level after Feeding on a Vitamin B₆ Deficient Diet. Albino rats were fed for a long period on a diet deficient in Vitamin B₆.

g at the outset, gained gradually the body weight for the first 30 days, when it began to lose and showed a remarkable drop on about the 100th day and died on the 230–250th day. In the meantime, a rapid rise in the blood sugar level was noticed on about the 35th day, at about the same time vitamin B₆ deficiency was noticed, and the blood sugar level rose thereafter to 160–200 mg/100 ml. On about the 140th day, a sudden and remarkable drop of the curve was observed, then, after a period of temporary hypoglycemia (40–50 mg/100 ml), it rose to 200 mg/100 ml again, and the rats finally died. During the whole course of the experiment, the true blood sugar level of above 400 mg/100 ml was observed in some cases. The urinary excretion of XA started on about the 30th day, amounting usually to 4–7 mg a day.

Glucose Tolerance. Employing the same animals fed on a diet rich in fat and deficient in vitamin B₆, the glucose tolerance was examined on the 190th day and a remarkable drop was detected as shown in Fig. 5.

From the above experiments it is quite evident that XA can have more accumulative effect upon the development of diabetic symptoms when it constantly stays in the body than when it is administered merely once or twice. Dietetically this is believed to be highly significant, for it suggests the harm of excessive fat ingestion. The amount of acetone bodies (16) excreted in the urine of each experimental rat was examined at its hyperglycemic and vitamin B₆ deficient period and was found to be 8.63 mg *per diem*, a value about 8 times as high as the value usually obtained in normal rats (1 mg).

From these findings it can be concluded that unlike alloxan, XA has an accumulative effect upon the development of diabetic symptoms. It is worthy of note, considering the fact that human diabetes generally progresses chronically.

Histological Findings. Albino rats were fed over a period of many days on a diet containing much fat and casein as well as the one containing casein, deficient in vitamin B₆. They were sacrificed when they manifested hyperglycemia and the pancreas tissue was examined histologically after staining by Gomori's alumen-hematoxylin-phloxin method.

As shown in Fig. 6 a remarkable drop of stainability, a decrease of granula, many vacuole formations, and intensive collapse of the protoplasm, etc. were noticeable in the β -cells of the Langerhans islets. In the tissue preparations of the animal fed on a diet of casein free from vitamin B₆, the

Fig. 5
Glucose Tolerance Curve

2 g/kg of glucose was orally administered.

*Fig. 6**Pathological Changes of Langerhans Islet.*

Stained by Gomori's alumen-hematoxylin-phloxin method.

(1)

(1) Normal healthy albino rats.

(2) Rat killed after feeding on a diet containing 22 % of casein deficient in vitamin B₆.
(10 mg of tryptophan was daily administered for the first 60 days)

(2)

(3)

(3) Rat killed after feeding for 200 days on a diet containing much fat (butter 35 %) and casein (25%).

(10 mg of tryptophan was daily administered for the first 30 days.)

With regard to the β -cells, notice vacuole degeneration, marked collapse and remarkably decreased granula of the protoplasm, indistinguishable cell-membrane and drop of stainability.

No such changes are to be detected in the α -cells and others.

pathological changes were especially marked, and besides, edema of the islets was observed. Changes of the nuclei, however, were but slight, if any. Neither in the α -cells of the islets nor in the parenchymatous cells any distinct changes could be seen. All these changes and features are considered to be the symptoms of pancreatic diabetes.

Relationship between XA and Glucose Metabolism. In order to confirm the close relationship between XA and glucose metabolism more thoroughly, 200 mg/kg of XA was injected to rats intraperitoneally. They were killed group by group at each period of hyperglycemia, hypoglycemia and continued hyperglycemia, respectively, and the glycogen amount in the liver, heart and skeletal muscle was estimated, in comparison with each control values obtained from normal healthy animals (17).

In Fig. 7 are given the mean values of the results of 5 rats. The glycogen level decreased most remarkably in the liver, decreasing to one-half that of the control value in the primary hyperglycemia period, increasing a little in the hyperglycemia period and decreasing again so markedly in the continued hyperglycemia period till it fell to less than one-third. In the continued hyperglycemia period it decreased remarkably in the skeletal muscle, too, but it invariably had a rising tendency in the heart muscle. These results agreed in general with those found in the experiments with alloxan, and the action of XA upon glucose metabolism may be regarded as established by the above observations.

4. Xanthurenic Acid in the Urine of Diabetic Patients.

For establishing the relationship between human diabetes and diabetic symptoms caused in albino rats by XA, eleven diabetic patients, under treatment in the hospital attached to the Wakayama Medical College were examined. One litre of urine was collected from each patient, filtered and concentrated. The concentrated filtrate was treated with mercuric acetate, and the precipitate thus obtained was suspended in water. The precipitate was completely decomposed with H_2S and the resultant filtrate was concentrated *in vacuo* to 5–10 ml. In parallel with this procedure, the urine of normal healthy people was treated exactly in the same way as a control and each solution was subjected to paper chromatography, using as a developing solvent a mixture of butanol, acetic acid and water (4 : 1 : 1). The results obtained (18) are shown in Fig. 8.

The color reactions and R_F value of each spot presented are shown in

Fig. 7
The Amount of Blood Sugar and Glycogen.

Table III.

F₄ of column B undoubtedly corresponds to XA of column C on the basis of color reaction and *R_F* value, and was identified as XA. In the same way F₃ is considered to be kynurenine. The urines of all the eleven diabetic patients, without a single exception, were proved to develop these chromatographic spots. The spot corresponding to XA (F₄) was especially conspicuous when the urine of the patient without preliminary treatment was examined. Out of three cases of normal healthy people, F₃' alone could be identified as kynurenine, no other spot being recognized, except those which are considered to represent minute quantities of substance in conjugated forms. XA in a free form was presented exclusively by the urine of diabetic patients, a fact from which we conclude the necessary etiological relationship of free XA to human

Fig. 8
The Paper Chromatogram of the Urine of a Diabetic Patient.

Table III
Spots Detectable by Paper Chromatography.
A : the urine of normal healthy subject.
B : the urine of diabetic patient.
C : mixed solution of kynurenine (K), and XA.

Color reaction	A			B			C	
	F ₁ '	F ₂ '	F ₃ '	F ₁	F ₃	F ₄	K	XA
<i>R_F</i> value	0.06		0.40	0.06	0.41	0.50	0.41	0.50
Bratton-Marshall	—	+	—	—	+	—	+	—
Ehrlich	+	—	—	+	—	+	—	+
Pauly	+	—	—	+	—	+	—	+
Millon	—	—	—	—	—	+	—	+
Fe ⁺⁺⁺	—	—	—	—	—	+	—	+
Ninhydrin	+	+	—	+	+	—	+	—

diabetes. As a matter of fact, it is to be noted that the urine of an untreated patient presented a remarkably conspicuous XA spot and that in the course of the treatment, the spot faded progressively till finally it disappeared completely.

5. Effect of Insulin and Methionine upon the Excretion of XA.

XA being proved to be a toxic substance, it is possible to imagine that there must be some anti-toxic action going on in the body against it so as to inhibit its formation or detoxicate its toxicity either by decomposing or conjugating. Pyridoxine was found to be somewhat effective in preventing its production in the body. In favor of this view, a decreased excretion of XA in the urine of the albino rats was recorded when pyridoxine was added

to the diet containing tryptophan and sodium butyrate, yet it must be noted that any amount of pyridoxine could not completely inhibit XA production from tryptophan. And this induces us to surmise that there may also be some substance or factor other than pyridoxine, which shows an inhibitory action.

Inhibitory Effect of Insulin on XA Production.

To one group of albino rats, each weighing about 150 g, tryptophan and sodium butyrate were administered orally at the same time, so as to excrete XA in the urine and some of them were employed as controls. To the rest of them, insulin was further injected. The 24-hour urine from each group was collected and subjected to paper chromatography. For this purpose the urine was treated with mercuric acetate, the precipitate was decomposed with H_2S and the filtrate was concentrated to 1 ml. For development a mixture of butanol, acetic acid and water (4 : 1 : 1) was used. The results obtained (19) are shown in Fig. 9.

The results of the color reaction test of each spot are shown in Table IV. It may be seen that both F_5 and F_6' correspond to XA, in color reactions and R_F value. F_6' was observed to be much weaker in all its color reactions and smaller in size than F_5 and in this regard, there was not a single exception in all the six cases examined.

Fig. 9
The Paper Chromatogram Showing the Effect of Insulin.

S : tryptophan plus sodium butyrate

I : tryptophan plus sodium butyrate plus insulin

Table IV
The Color Reactions of the Chromatogram.

Color reaction	S					I					
	F_3	F_4	F_5	F_6	F_7	F_4'	F_5'	F_6'	F_7'	F_8'	F_9'
R_F	0.32	0.43	0.50	0.71	0.89	0.32	0.43	0.50	0.71	0.30	0.89
Bratton-Marshall	—	+	—	+	+	—	+	—	+	+	+
Ehrlich	+	—	+	—	—	+	—	+	—	—	—
Pauly	+	—	+	—	—	+	—	+	—	—	—
Millon	—	—	+	—	—	—	—	+	—	—	—
Fe^{+++}	—	—	+	—	—	—	—	+	—	—	—
Ninhydrin	+	+	—	—	—	+	+	—	—	—	—

From the above experiment it is evident that insulin is capable of inhibiting XA production. For further establishment of the insulin effect, the urinary XA was estimated quantitatively.

To a group of albino rats, 0.1 g of tryptophan and 0.4 g of sodium butyrate were administered, so as to excrete XA in the urine, several animals being used as a control. In parallel with this, we injected various quanti-

ties of insulin to the rest of them. The 24-hour urine from each rat of the two sub-groups was collected and the amount of XA was estimated. The results shown in Fig. 10 indicate that only an appropriate amount of insulin could remarkably depress the excretion of XA and inhibit its production in the body. Furthermore, we succeeded in separating and crystallizing a substance produced presumably in place of XA after administering 0.1 unit of insulin together with tryptophan and butyrate. The amount obtained from the urine of rat was 10 mg, having mp 258–269°. Since both tests of Kretchy and Jaffe proved to be positive, it is considered to be kynurenic acid. For its identification an absorption spectrum was estimated, comparing with the authentic sample of kynurenic acid of Roche. As shown in Fig. 11, the curve was proved to be practically identical with the synthesized preparation¹. This experiment is of significance, considering the experiments by Yashiro Kotake and Ichihara (20) showing that kynurenic acid is nothing but a non-toxic, non-effective final product of tryptophan. Presumably kynurenic acid is the only non-toxic metabolite possessing a quinoline ring, and less XA is produced after insulin administration, while kynurenic acid possessing also a quinoline ring is produced in its place, probably as a result of a sort of anti-toxic action.

Inhibitory Effect of Methionine on XA Production.

It is conceivable that our prescription of a large amount of sodium butyrate may result in suppressing the liver function and producing acetone bodies, and thus leading to anomalous tryptophan metabolism.

Suspecting that methionine may have an inhibitory action against XA production as a result of abnormal tryptophan metabolism, we conducted the following experiment.

Twenty milligrams of methionine were injected to albino rats fed on tryptophan and sodium butyrate. The 24-hour urine was collected and the amount of XA in the urine was estimated. As a control, the 24-hour urine of the animal similarly treated but without methionine injection was like-

Fig. 10
The Amount XA Excreted in 24-hour Urine.

Fig. 11
Absorption Spectrum of Kynurenic Acid.

¹ Performed by Dr. Sakan, the Science and Engineering Department, Osaka City University.

wise analyzed.

As shown in Fig. 12, a remarkable inhibition of XA excretion by methionine was observed. When both methionine and pyridoxine were simultaneously injected, no urinary XA was detected. In this case, no continued hyperglycemia was detected either.

It is certain that methionine, insulin and pyridoxine are three substances capable of controlling an abnormal tryptophan metabolism.

6. Decomposition of XA in the Liver.

Remarkable is the fact that XA produced in the body, is easily excreted in the urine. According to our experiment (20), some portion of XA injected to an albino rat is excreted in the urine in a few minutes. We further surmised that XA might be decomposed by some enzymic process in the liver (21). For testing this view, 1 g of liver tissue was extracted with 1 ml of water and treated with ammonium sulfate. By using kaolin and sodium carbonate, we adsorbed and eluted the globulin fraction thus obtained. XA^2 (mp 296.5°) was added to the enzyme solution (pH 7.2) and the whole was kept at 38°. The content of XA was estimated periodically, with the results shown in Fig. 13.

As shown in Fig. 13, XA was decomposed with the progress of time. This decomposing action is most conspicuous at the optimum temperature (38°) and pH (7.2) and disappears after short heating. From these findings, it is concluded that this decomposing action is due to an enzyme. We call it "xanthurenase" hereafter.

We are now under examination of the enzymic decomposition product of XA. By using paper chromatography and estimating absorption spectrum, we are inclined to assume a phenol derivative to be formed by splitting of the pyridine ring of XA, but no clear-cut identification has yet been achieved. It is considered that this enzymic action is the most potent of all the

Fig. 12
The Amount of XA Excreted in 24-hour Urine.

The mean values of 4 albino rats.

Fig. 13
Decrease of XA.

(The enzymic action of "Xanthurenase")
Enzyme : 5 g globulin fraction of rabbit liver. XA : 1 mg. 38°, pH 7.2.

² Synthesized by Dr. Sakan.

anti-toxic actions going on in the liver against XA, and that if and when this enzyme action is weakend, XA is stored, giving rise to diabetic symptoms.

7. On the Conjugated Compounds of XA Formed in the Body.

There remains another anti-toxic action that should be considered, *i.e.* the excretion of XA in conjugation with some other substances. We have experimentally succeeded (22) in separating two conjugated forms of substances from the urine of XA injected albino rats. One of them is considered to be a conjugate of OH radical of 4-position of the quinoline ring with glucuronic acid and the other, which we have recently succeeded in separating in a crystalline form to be a conjugated substance at OH radical of 8-position of quinoline ring with sulfuric acid. Of these two, we are inclined to consider the latter to have an anti-toxic action against the development of diabetic symptoms.

SUMMARY

1. After administering tryptophan to albino rats fed on a B₆ deficient diet containing casein, xanthurenic acid is excreted in the urine.

2. Feeding rats on a diet containing much fatty acid salt and tryptophan, they become deficient in vitamin B₆ and a remarkable amount of xanthurenic acid is excreted in the urine, which was isolated and identified. After the administration of pyridoxine, the urinary excretion of xanthurenic acid remarkably decreased but never completely disappeared.

3. After administering orally both sodium butyrate and tryptophan to albino rats, the blood sugar curve show primary hyperglycemia, followed by temporary hypoglycemia, and finally definite hyperglycemia, accompanied by glycosuria owing to the production of xanthurenic acid.

4. Administration of xanthurenic acid to albino rat leads also to the development of diabetic symptoms.

5. Xanthurenic acid, when given continuously, shows an accumulative effect on diabetic symptoms. In the urine of the rats in hyperglycemia and vitamin B₆ deficiency, a definite increase of acetone bodies is detected.

6. The pancreas of rat showing hyperglycemia after feeding for a long period on high fat and casein diet or vitamin B₆ deficient one, showed histologically degenerative changes of β -cells of Langerhans islets. It was especially conspicuous in vitamin B₆ deficiency.

7. In the urines of diabetic patients, the spot corresponding to free form of xanthurenic acid was detected by paper chromatography, contrary to the healthy subjects.

8. Insulin was found to prevent the urinary excretion of xanthurenic acid of experimental animals as detected by paper chromatography. The product excreted in the urine instead of xanthurenic acid was proved to be kynurenic acid.

9. Methionine inhibit the formation of xanthurenic acid. By simultaneous application of methionine and pyridoxine to rats, the urinary xanthurenic acid disappeared completely.

10. The xanthurenic acid injected to rat is readily excreted in the urine. An enzyme, xanthureninase, was found in rabbit liver.

11. The injected xanthurenic acid is excreted in conjugated forms as non-toxic substances.

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