Über die Hemmbarkeit der Ophio-*l*-Aminosäureoxydase durch Antiserum

Von verschiedenen Autoren wird die *l*-Aminosäureoxydase in den Giften von *Bothrops jararaca, Crotalus terrificus* und *Vipera aspis*[1], in Gift von *Echis carinatus*[2] und anderen Viperiden-Giften[3] als durch Antiserum nicht hemmbar beschrieben; bisweilen scheint jedoch Hemmung möglich zu sein[4].

Lässt man im Geldiffusionstest Schlangengifte und Anti-Schlangengift-Seren reagieren, so lassen sich stets Präzipitationslinien mit *l*-Aminosäureoxydase-Aktivität nachweisen[5], was auf Vorhandensein von präzipitierendem Enzymantikörper schliessen lässt; fermentaktive Präzipitate lassen sich jedoch aus Lösung mit polyspezifischen Pferdeantiseren nicht gewinnen. Durch Immunisierung von Kaninchen mit gereinigter *l*-Aminosäureoxydase[6] (Zehenballeninjektion und Verwendung von Bajol/Arlacel als Adjuvans) wurde nun monospezifisches Antiserum mit ausreichendem Titer gewonnen[7], mit dem die Hemmbarkeit der *l*-Aminosäureoxydase in Schlangengiften quantitativ untersucht werden konnte (Ergebnisse siehe Tabelle).

Gift von	Eingesetzte Aktivität Qo_2	Quantitative Präzipitation durch Antiserum (ml)	Aktivität im Überstand	Aktivität im Präzipitat	% Hemmung
Vipera ammodytes (0,1 mg)	44	0,35	0	28	36
Vipera ursinii (0,12 mg)	44	0,37	0	28	36
Vipera lebetina (0,08 mg)	44	0,41	0	28	36
Bitis gabonica (0,21 mg)	44	0,45	0	28	36
Cerastes cerastes (0,09 mg)	44	0,85	0	28	36
Echis carinatus (0,07 mg)	44	1,25	0	28	36

Inkubation der angegebenen Giftmenge mit gestaffelten Mengen Antiserum 1 h bei 37 °C und 4 h bei 4 °C im Endvolumen von 1,5 ml in 0.06 *M* Phosphatpuffer pH 7,3; Zentrifugation und dreimaliges Waschen der Überstände, Suspendieren der Präzipitate mit Vortex jr. Mixer und Bestimmung der Aktivität in den Überständen und Präzipitaten nach[5].

Wie die Tabelle zeigt, genügt zur quantitativen Präzipitation des homologen Enzyms die geringste Antiserum-Menge, während das Ferment in den Giften verwandter Spezies grössere Mengen zur Präzipitierung benötigt (Abhängigkeit vom Verwandtschaftsgrad der Spezies?). Die Hemmbarkeit der *l*-Aminosäureoxydase beträgt bei den untersuchten Viperidengiften jedoch in jedem Fall 36%; Kreuzreaktionen der *l*-Aminosäureoxydase in Giften von Tieren der Familien Elapidae und Crotalidae konnten nicht beobachtet werden.

Summary. 36% of the *l*-amino acid oxidase activity in the venom of *Vipera ammodytes* and related species has been inhibited by monospecific antiserum.

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Behringwerke AG, Marburg, Lahn (Deutschland), 19. April 1966.

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5-Hydroxylation of Kynurenine in Animals

We formerly reported the detection of 5-hydroxykynuramine (V) in the urine and the brain of mouse[1,2], and then found in 1963 a small amount of 5-hydroxykynurenine[3] (IV), the precursor of 5-hydroxykynuramine[3], in a human urine (a seemingly normal male adult) after treatment with a large amount of the latter.

In this paper we describe our attempt to detect 5-hydroxykynurenine in the urine of chickens and mice administered kynurenine (III) in order to establish the formation in animals of the above amine and 6-hydroxykynurenine in connection with tryptophan metabolism. Urine of 2 chickens (White Leghorn), each given orally 0.3 g of DL-kynurenine sulphate daily for 7 days, was collected for a total of 10 days during and after kynurenine administration, and then acidified with HCl to 6 *N* and heated in a boiling water bath for 2 h in order to hydrolyse some conceivable conjugates of hydroxylated kynurenine derivatives. After cooling, it was shaken with a mixture of *n*-butanol and benzene (1:1) to remove resinous dark-coloured product. The aqueous layer was concentrated under reduced pressure to a thick syrup and was dissolved in 0.1 *N* HCl, and then passed through a Dowex 50W-X4 (hydrogen form) column (2 · 32 cm). In another experiment, using 96 mice of DD-strain each weighing ca. 25 g, each animal was injected with 2.0 mg of DL-kynurenine sulphate daily for 3 days and their urine was collected for 5 days by blotting with sheets of filter paper (No. 514, Toyo-Roshi Co., Tokyo), which

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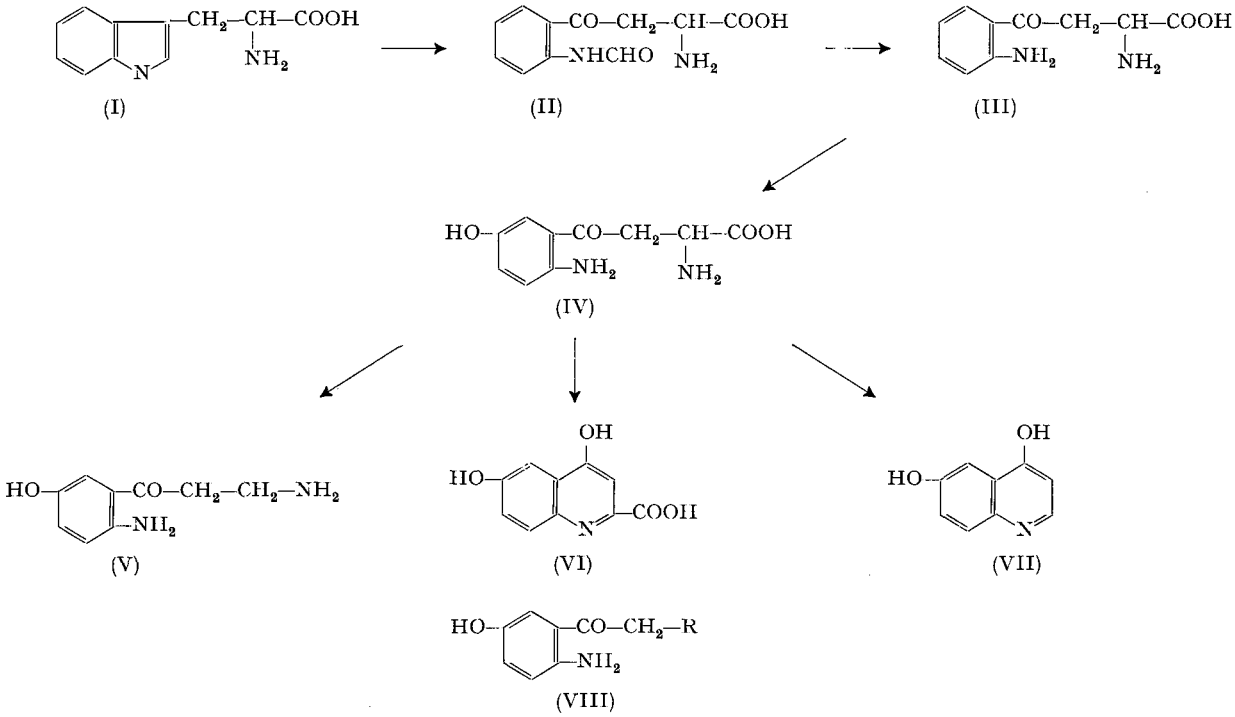

Solvent systems	Rf					
	Natural 5-hydroxy-kynurenine (OKN)	Authentic 5-hydroxy-kynurenine	Incubation product (A)	Authentic 6-hydroxy-kynurenic acid	Incubation product (B)	Authentic 4,6-dihydroxy-quinoline
a	0.13	0.13	0.50	0.50	0.74	0.74
b	0.23	0.23	0.38	0.38	0.81	0.83
c	0.26	0.26				
d	0.35	0.35				
Pauli-Monda reagent	Dark purple (violet)	Dark purple (violet)	Red	Red	Red	Red
Fluorescence under UV-light	White yellow	White yellow	Reddish brown-white	Reddish-brown white	Green	Green
Fluorescence under UV-light when exposed to ammonia			White yellow	White yellow	Bright blue	Bright blue

a = *n*-Butanol, acetic acid, water (4:1:5). b = Methanol, *n*-butanol, benzene, water (2:1:1:1) = Mason-Berg. c = Mason-Berg with 1% NH_4OH. d = 70% isopropanol.

were extracted with 0.1 *N* HCl and then passed through the same column as described above.

The column which was treated with chicken or mice urine was washed with 0.2 *N*, 1.5 *N* and 2.4 *N* of HCl successively, and finally eluted with 5 *N* HCl. The eluate was concentrated under reduced pressure of nitrogen at 40 °C to a thick syrup, which was applied in a band form to filter paper No. 51 (40 · 40 cm) of the Toyo-Roshi Co., Tokyo, and developed with ascending technique using a mixture of *n*-butanol, acetic acid and water (4:1:5). The chromatogram was sprayed with Pauli-Monda reagent[1]. While under UV-light, the fluorescence of the residual kynurenine was the strongest in the region of paper chromatogram comprising 3 kinds of kynurenine bands. Among the colours given by spraying Pauli-Monda reagent, that of 3-hydroxykynurenine was the strongest. Kynurenine itself gave no colour, but at the same Rf as that of 5-hydroxykynurenine there appeared a band (OKN) (though weaker than that of 3-hydroxy derivative) giving a distinct violet colour; the colour, which is strikingly characteristic of 5-hydroxykynurenine and its amine[1], having 2-amino-5-hydroxyacetophenone structure (VIII)[4]. The conceivable 5-hydroxy derivative mentioned above showed the same Rf's on the paper chromatograms developed by using 4 different kinds of solvent systems as those of synthetic 5-hydroxykynurenine, as indicated in the Table, and gave, on treating with a crude

[4] Y. JOH, K. MURAKAMI, and K. MAKINO, unpublished data.

enzyme (or homogenate) of mouse liver, 2 degradation products A and B, which were identified as 6-hydroxykynurenic acid and 4,6-dihydroxyquinoline respectively (VI and VII) by comparing with the corresponding synthetic samples on paper chromatograms in their Rf's, their colours of fluorescence under UV-light with their characteristic colour change when exposed to ammonia, and the colours given by spraying Pauli-Monda reagent (Table). As the mere incubation of non-hydroxylated kynurenine gave neither VI nor VII, the authors consider that the reaction in the scheme is one of the best ways for identifying a metabolite with 5-hydroxykynurenine[5]. The extent of 5-hydroxylation of kynurenine roughly estimated by fluorometric method in chicken amounted to ca. 0.5%. As controls, the urine of chickens and mice not treated with kynurenine was collected and worked up in the same way as described above but contained only a trace of 5-hydroxykynurenine or none.

Attempts to purify the 5-hydroxykynurenine spots isolated from the original paper chromatogram by repeated developments on filter paper for identification purposes, resulted very often in obtaining only spots showing UV-absorption spectra deviating from the typical one, presumably owing to some oxidation even when the synthetic 5-hydroxykynurenine was used as the test material and taking precautions such as preventing oxidation. Therefore, the present authors prefer the enzymatic identification as described above to the optical method.

This detection of 5-hydroxykynurenine never means its artificial formation from kynurenine in the isolation procedure, for the same treatment of urine as described above after the addition of kynurenine has never led to the detection of 5-hydroxykynurenine.

The above results show for the first time that kynurenine is converted in animals to 5-hydroxykynurenine, and thus the origin of 5-hydroxykynurenine and a new route in the tryptophan metabolism through 5-hydroxykynurenine suggested by MAKINO and TAKAHASHI[6,7] 12 years ago are now established (scheme).

Formerly H. TAKAHASHI told one of the authors (K.M.) personally that he once detected 6-hydroxykynurenic acid in the urine of a pregnant woman[8]. ROY and PRICE[9] reported the isolation of a small amount of the same acid from pig urine[9]. Recently KIDO et al.[10,11] detected IV, VI and VII in the urine of chickens and human beings administered tryptophan. These findings all coincide with our present scheme.

Zusammenfassung. 5-Oxykynuramin (Vorläufer von Mausamin aus Maushirn) wurde aus Urin von Huhn und Maus nach Verabreichung von Kynurenin durch Kombination der Ionen-Austausch-Chromatographie mit Papier-Chromatographie isoliert. Durch Vergleich mit dem synthetischen Präparat wurde es papierchromatographisch und durch Spaltung mittels Mäuseleberhomogenat in 4,6-Dioxychinolin und 6-Oxykynurensäure identifiziert.

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Development of Hyperphagia in Female Rats with Ventromedial Hypothalamic Lesions Placed at Four Different Ages[1]

Most studies on the hypothalamic control of food intake and the factors influencing it have been conducted on young-adult and adult rats[2] but few have investigated food intake patterns in weanling rats[3–5]. In the latter studies it was observed that young growing rats with ventromedial lesions did not show the hyperphagia observed in similarly treated adult rats. This may be related not only to metabolic differences between fast-growing young rats and adult animals but also to a differential response of the satiety mechanisms to destructive lesions.

Food intake is generally expressed in g/day, kilocalories/day or grams relative to body weight. The present study was undertaken to not only investigate postoperative food intake patterns in rats with ventromedial hypothalamic lesions placed at 4 different ages but also to relate this parameter to 'metabolic size' (KLEIBER unit, i.e. $kg^{3/4}$)[6]. The duration of the experiment was chosen to be 6 weeks since during this time all animals were probably still in their dynamic state of obesity[7].

Female Holtzman rats were divided into 8 groups and treated in the following manner: Groups 1, 3, 5 and 7 received bilateral electrolytic lesions in the ventromedial hypothalamic area when 27, 59, 75 and 140 days old, respectively. Groups 2, 4, 6 and 8 served as corresponding controls. The lesions were placed with a Horsley-Clarke stereotaxic instrument using an enamel-coated stainless steel electrode of 0.37 mm diameter. An anodal current of 1.5 mA was used in the youngest rats (Group 1) while for the older animals 2.0 mA were used; the current was allowed to flow for 10 sec. The coordinates were obtained from previously established charts[8]. The animals were housed in individual cages in a room kept at 25 °C with 12 h light and 12 h dark and given a synthetic diet (4.2 Cal/g) and tap water ad libitum. Food intake was meas-

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