explains why in the present study methylxanthines and instant tea stimulated microsomal enzyme activity measured in vitro, but since the level of aniline in the blood remained unchanged the in vivo metabolism was not altered. It was also shown that caffeine's shortening of sleeping time[13] was not due to the influence on drug metabolism but rather to an interaction at the brain level[14]. Furthermore, consumption of at least 6 cups of coffee or tea per day by humans did not induce liver microsomal enzyme activity[5].

Methylxanthines only caused an induction in vitro when given in concentrations of 75 mg/kg or higher, as confirmed by other workers[4,5]. In lower concentrations, methylxanthines did not change in vitro enzyme activity, however other authors[6] have claimed that caffeine given at 20 mg/kg inhibits microsomal enzyme activity. But their results were contradictory, since one of their substrates used indicated an inhibition, the other an induction. On the other hand, the cytochrome P-450 level was not changed by methylxanthines in the present study nor in the in vitro studies of the workers mentioned above[5,6].

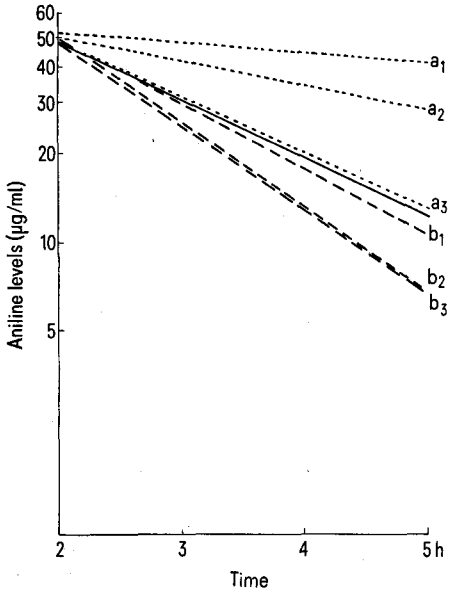

Fig. 2. Induction or inhibition of in vivo metabolism. Phenobarbital was injected i.p. daily for 3 days and SKF 525-A once. Animals were then injected i.p. with 50 mg/kg of aniline. Aniline disappearance from blood was measured using 6 male rats per group. Decline of aniline levels in serum was presented as regression lines on a semi-log scale. Significant difference between controls is indicated as $p < 0.01^a$ or NS if not significant. ————, Control group. - - - - - - -, a_1 SKF 525-A 100 mg/kg[a]; a_2 SKF 525-A 56 mg/kg[a]; a_3 SKF 525-A 32 mg/kg NS. – – – – – – –, b_1 Phenobarbital 27 mg/kg NS; b_2 Phenobarbital 48 mg/kg[a]; b_3 Phenobarbital 75 mg/kg[a].

Since cytochrome P-450 plays an important role in drug metabolism[15,16] and its level is normally increased by enzyme induction, it could be concluded that an induction of in vivo drug metabolism can only be expected if the cytochrome P-450 level is elevated. Especially since a dose-dependent induction of microsomal metabolism, caused by phenobarbital, showed a very good correlation between the P-450 level and the in vivo aniline metabolism. Whereas, in vitro aniline hydroxylation was always more pronounced than the in vivo metabolism.

However, no such correlation between in vivo and in vitro drug metabolism was observed when inhibition occurred, which suggests the involvment of a different mechanism. Aniline hydroxylation was only inhibited when SKF 525-A was administered to animals in a high concentration (100 mg/kg) which confirms other workers' findings[17]. But already at a lower concentration of SKF 525-A (46 mg/kg), in vivo aniline metabolism was inhibited which again agrees with other workers[18]. Provided that the microsomal enzyme system is relatively unspecific[19] it can be concluded that compounds, which induce in vitro drug metabolism might not always have an effect on microsomal metabolism when measured in vivo. Hence a normal coffee or tea consumption of 5 cups per day by a 70 kg man, resulting in an intake of about 7 mg/kg of caffeine and traces of theobromine, would not have harmful consequences through changes in microsomal enzyme activity.

Zusammenfassung. Eine Induktion der Mikrosomalenzyme der Leber, gemessen in vitro, wurde beobachtet, wenn hohe Dosen von Methylxanthinen an Ratten verabreicht wurde. Wenn den Versuchstieren gleich hohe Dosen von den obengenannten Substanzen verabreicht wurden, die Aktivität der arzneimittelabbauenden Enzyme jedoch in vivo gemessen wurde, so war kein Unterschied zur Kontrollgruppe festzustellen.

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Characterization of Myxovirus Sialidase

In myxoviruses, the sialidase as well as haemagglutinin is localized on the outer-envelope of the virion particle. It has been reported that the antibody against virus sialidase was effective in preventing virus infection[1]. Recently the virus sialidase has been considered to play an important role in the process of virus multiplication[2,3]. The characterization of the sialidase will be useful for a better understanding of virus infection.

In the present communication, we compared the substrate specificity of the sialidase in several species of myxoviruses. Previously, we discovered an inhibitor against bacterial sialidase called siastatin[4]. The inhibitor

Table I. Relative rate of hydrolysis of various substrates by sialidases

Virus	BSL	Glycolipid		Glycoprotein					
		Ganglioside	Hematoside	Fetuin	Mucin	A	B	E	Bovine glycoprotein
PR-8	1.00	0.08	0.05	0.08	0.07	0.06	0.08	0.13	0.04
NWS	1.00	0.06	0.01	0.07	0.06	0.07	0.08	0.11	0.04
Swine	1.00	0.02	0.0	1.00	0.23	0.84	1.63	1.86	0.40
Jap-305	1.00	0.23	0.21	0.90	0.06	1.25	2.70	3.20	0.47
Aichi	1.00	0.03	0.05	0.30	0.07	0.52	0.60	0.72	0.23
Singapore	1.00	0.07	0.17	0.35	0.09	0.24	0.60	0.81	0.15
Hong Kong	1.00	0.09	0.22	0.40	0.11	0.50	0.70	0.11	0.26
Lee	1.00	0.0	0.0	0.10	0.07	0.06	0.06	0.14	0.0
Sato	1.00	0.0	0.02	0.50	0.06	0.20	1.35	1.02	0.43
Narashino	1.00	0.10	0.32	0.75	0.08	0.26	5.33	1.20	1.00
Ishii	1.00	0.07	0.19	0.30	0.0	0.20	0.40	0.45	0.31
B1	1.00	0.0	0.08	0.25	0.0	0.17	0.80	1.00	0.15
HVJ	1.00	0.06	0.13	0.55	0.04	1.90	5.50	6.90	0.50
Cl. perfringens	1.00	7.60	2.0	11.90	1.92	4.50	8.10	10.30	3.82
Streptomyces (MB 503-Cl)	1.00	0.10	0.05	0.80	0.29	0.92	0.82	1.53	0.73

behaves competitively to bovine sialyllactose (BSL). The effect of siastatin on virus sialidase was also compared with that on sialidases from other sources. It was clearly demonstrated that the virus sialidase does not originate from the host cells.

BSL, ox brain ganglioside, horse erythrocyte hematoside, calf serum fetuin, bovine submaxillary mucin, bovine glycoprotein Cohn Fraction VI and human α_1-acid glycoproteins were employed as the substrate. Influenza viruses, A/PR/8/34 (HON1), A/NWS (HON1), A/Swine/Wisconsin/15/30 (Hsw1N1), A/Jap/305/57 (H2N2), A/Aichi/2/68 (H3N2), A/Singapore/1/57 (H2N2), A/Hong Kong/1/68 (H3N2), B/Lee/40, and Sato, Narashino, Ishii, B1 of Newcastle disease virus (NDV), and the hemagglutinating virus of Japan (HVJ), and sialidase prepared from *Cl. perfringens* and *Streptomyces* were employed. The initial velocity of the hydrolysis of the various substrates was compared to that of BSL. The results are summarized in Table I. The procedures were the same as described in our previous paper[5]. Swine, Jap-305, Aichi, Singapore, Hong Kong, Sato, Narashino, B1 and HVJ hydrolyzed glycoprotein rather effectively, but PR-8, NWS and Lee showed as slower rate of hydrolysis against glycoprotein.

The amount of various substrates in each reaction mixture was normalized to that which contained 0.14 μmoles of glycosidically bound N-acetylneuraminic acid. This corresponded to 80 μg of BSL, 363 μg of human α_1-acid glycoproteins, 460 μg of ox brain ganglioside and horse erythrocyte hematoside, 572 μg of bovine glycoprotein, 520 μg of mucin and 1.21 mg of fetuin. The substrates, except ganglioside and hematoside, were dissolved in 0.02 *M* citrate-phosphate buffer, pH 6.0. Ganglioside and hematoside were dissolved to 2 mg/ml in $CHCl_3$:MeOH (1:2) and added to testing tubes. After the evaporation of organic solvent in vacuo 0.02 *M* citrate-phosphate buffer was added and a suspended condition was made by sonication. The enzymes were prepared as described in the previous paper, and 0.2 unit of each enzyme was included in the reaction mixture[5–9]. The total volume of the reaction mixture was adjusted to 0.5 ml. After 30 min at 37 °C, released sialic acid was determined by the thiobarbituric acid method[10]. One unit of sialidase activity was defined as the amount of enzyme which released 1.5 nmoles of N-acetylneuraminic acid per min from BSL.

A, B, E: 3 kinds of α_1-acid glycoproteins prepared from human normal serum, ascites of cirrhosis and ascites of stomach cancer were gifts from Dr. K. Hotta of Kitasato Institute, Tokyo[11] (Table I).

Glycolipid was not easily hydrolyzed by virus sialidase except by Jap-305 and Narashino. Ignoring the trivial differences, we can conclude that the virus sialidase hydrolyzes sialoglycoprotein rather effectively, but the rate of hydrolysis of sialoglycolipid is rather slow. When compared with the sialidase from bacteria and actinomycetes, which we reported previously, the virus sialidase is similar to that from actinomycetes in regard to the substrate specificity.

Siastatin was discovered from the culture filtrate of streptomyces to be an inhibitor of sialidase from *Cl. perfringens*[4]. It was separated into siastatin A and B, and the both components were purified. The structure of siastatin B was determined as 2(S/R)-acetamido-3(S/R), 4(R/S)-dihydroxypiperidine-5(R/S)-carboxylic acid. Chemical studies on siastatin A are now in progress.

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Inhibitory effects of siastatin A and B are shown in Table II.

Sialidases were prepared from rat mammary gland, brain and liver, *Clostridium perfringens* and *Streptomyces*, and purified virus was prepared as described in the previous paper[5-9]. Soluble virus sialidase was isolated from purified Aichi strain by incubation at 37 °C for 120 min with pronase (1 mg/ml). Reaction mixture was centrifuged at 28,000 rpm for 120 min and the supernatant was concentrated by Ficoll at 4 °C and purified by 3 to 20% linear sucrose gradient centrifugation for 5 h at 60,000 rpm. The active fraction obtained by dialysis with phosphate buffer saline (pH 7.2). Sialidase of CAM was purified by the method described by ADA[12]. *Vibrio cholerae* sialidase was purchased from General Biochemicals, U.S.A. Inhibitory effects of siastatin A and B were determined according to the method described for Table I.

Table II. Inhibitory effects of siastatin A and B against various sialidase

Enzymes	Substrates	ID_{50} (μg/ml) Siastatin A	Siastatin B
Aichi	BSL	> 250	> 250
	Fetuin	> 250	> 250
Aichi	BSL	> 250	> 250
(Soluble)	Fetuin	> 250	> 250
Jap-305	BSL	> 250	> 250
	Fetuin	> 250	> 250
Narashino	BSL	> 250	> 250
	Fetuin	> 250	> 250
Sato	BSL	> 250	> 250
	Fetuin	> 250	> 250
B1	BSL	> 250	> 250
	Fetuin	> 250	> 250
CAM	BSL	3.4	110
Rat mammary gland	BSL	> 500	220
brain	BSL	> 500	800
liver	BSL	> 500	340
Cl. perfringens	BSL	0.7	6
	Fetuin	2.7	21
	Ganglioside	1.7	22
V. cholerae	Fetuin	> 500	> 500
	Ganglioside	> 500	> 500
Streptomyces	BSL	720	20
	Fetuin	600	10

Against sialidase from chorioallantoic membrane (CAM) or *Cl. perfringens*, siastatin A shows a stronger inhibition than siastatin B. While the sialidases obtained from *Streptomyces*, mammary gland, brain and liver of rats were inhibited more strongly by siastatin B than siastatin A. However, siastatin A and B did not inhibit myxovirus sialidase and *V. cholerae* sialidase. Virus sialidase and CAM sialidase behave quite differently towards these inhibitors; virus sialidase is completely free from the effect of siastatins, whether sialidase is in the form of virion particle itself or in the solubilized form. It is already known that viral and cellular sialidases are different in antigenic specificity. By employing a new inhibitor against sialidase, we can present new evidence demonstrating that viral sialidase has a completely different origin from that of host cells, and that it is made de novo in the infected host cells.

Zusammenfassung. Die Sialidasen von Myxoviren zeigten eine ähnliche Substratspezifität wie die Streptomyces-Sialidase. Siastatin A und B, Produkte von Streptomycesarten, wurden auf ihre hemmende Wirkung gegen Sialidasen verschiedenster Herkunft untersucht und als spezifische Hemmstoffe gegen bakterielle Sialidasen erkannt. Siastatin A und B hemmen die Sialidase der Chorioallantoismembran, nicht aber die Sialidasen von Myxoviren. Daraus folgt, dass virale Sialidasen in infizierten Wirtszellen de novo zusammengesetzt werden.

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Trehalose of *Culex pipiens fatigans*

The presence of high levels of trehalose was reported in many insects[1,2]. This disaccharide has been recognized to be the major carbohydrate in insect blood[3]. Studies conducted in *Bombyx mori*[4], *Gelerio*[5] and *Calliphora*[6] suggested considerable variation in trehalose content in different developmental stages of the insects. The utilization of trehalose during flight of diptera was studied in the blowfly[7]. However, information on the trehalose content and metabolism in mosquitoes is scanty. Recently, a highly active trehalase was identified in *Culex pipiens fatigans*[8]. The present investigation deals with the trehalose content in the *Culex* mosquito and its role as a lipid precursor and a nutrient to the insect.

Materials and methods. Eggs, larvae, pupae and adults of *Culex pipiens fatigans* were processed as described elsewhere[9].

Extraction of free sugars. The free sugars were extracted from the insect material by the method of JOHNSTON and DAVIES[10] and quantitated by the anthrone method[11]. Fourth instar larvae were fasted for 24 h in distilled water. 1 group each from the larvae and pupae (12 h old) was kept as controls. The second group was subjected to mechanical stirring for 30 min to keep them continuously

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