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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moving. Freshly emerged adults (24 h old) were subjected to continuous flight for 30 min by rotating and tapping the cage mechanically. A portion of the mosquitoes was collected before the agitation as control. The control and experimental groups were processed for sugars.

Chromatography of free sugars. Carbohydrates were separated on thin layer chromatography (TLC) on Alusil plates[10] (Silica gel G – Aluminium oxide, 1:1) impregnated with 0.1 *N* boric acid, with 1-propanol-ethylacetate-water-ammonia (25%), 60:10:30:10 and on Silica gel G plates impregnated with 0.02 *M* sodium acetate and developed in 1-propanol-ethylacetate-water, 7:1:1.

Identification of trehalose. Trehalose was identified on TLC plates with a marker by spraying the chromatograms with anisic aldehyde reagent[12]. The sugar was eluted from the spots with 80% ethanol and further characterized by acid hydrolysis and identification of products. Glucose after separation by TLC and elution was estimated by the method of PARK and JOHNSON[13].

Metabolic studies with $_{\alpha}$-$_{\alpha}$'-U-^{14}C-trehalose. Freshly emerged adults were fasted for 24 h. Approximately 250 to 300 adults were kept in a mosquito cage (6″×6″). A sterile cotton pad was moistened with 1 ml of 1% U-^{14}C trehalose (Radiochemical Centre, Amersham, specific activity 0.05 mCi/27.78 m*M*). The mosquitoes were allowed to feed on the trehalose-cotton pad for 1 h. The cotton pad was then removed and one half of the mosquitoes were collected immediately after removal of the pad and the other half 3 h later. Both the groups were processed for lipids[14]. The neutral and phospholipids were separated on TLC and quantitated by methods described elsewhere[9]. The radioactivity in the lipid fractions were counted in Nuclear Enterprise 8312 Liquid Scintillation Counter using PPO (0.4%), POPOP (0.05%) in toluene.

Table I. Total free sugars, trehalose and glucose levels of *Culex pipiens fatigans*

Developmental stage	Carbohydrates (mg/g)		
	Total free sugar	Trehalose	Glucose
Egg	2.70	0.63	Traces
4th Instar	2.24	1.07	Traces
Pupae	2.58	1.26	0.70
Adults	4.30	1.01	1.00

Table II. Percent change in carbohydrate levels of insects after agitation for 30 min

Developmental stage	Trehalose	Glucose
4th Instar	27 (−)	60 (+)
Adults	0	56 (−)

(+), Increase; (−), Decrease.

Table III. Incorporation on $_{\alpha,\alpha}$'-U-^{14}C-trehalose into major lipids of *Culex pipiens fatigans*

Lipid	Radioactivity (% of total lipids)	
	0 h	3 h
Triglyceride	47.1	28.2
Phosphatidyl-ethanolamine	14.9	27.6
Phosphatidyl-choline	10.9	18.9

Results and discussion. The total free sugars, trehalose and glucose levels of different developmental stages of *Culex* have been given in Table I. As seen from the Table, eggs contained lowest amounts of trehalose (0.63 mg/g) which increased in other stages to about 1.1 mg/g of the insects. Thus, trehalose comprized 23 to 49% of the total free sugars of different stages of the insect. Glucose was present only in traces in eggs and fasted larvae which progressively increased in content in the next two developmental stages reaching maximum levels in the adults. Adults also contained highest amounts of total free sugars.

The variation in trehalose and glucose of the insects subjected to continuous agitation for 30 min is presented in Table II. There was significant reduction (27%) in trehalose levels in 4th instar larvae on agitation suggesting utilization of the carbohydrate. There was a concomitant rise (60%) in the glucose content of the larvae. The trehalose and glucose content did not change in the pupae on agitation. In adults there was no change in the trehalose but there was marked reduction in the glucose content, suggesting glucose as the main fuel for flight in this stage of the insect. In *Aedes* the preferred substrate for energy during flight was similarly reported to be glucose[15]. Indeed, the carbohydrate involved in providing energy under stress seems to depend on the species and developmental stage of the insect[7,16–18].

The incorporation of label from U-^{14}C-trehalose into major lipids of adults is shown in Table III. Triglycerides, which are the major neutral lipids of the insect[9], were highly labelled followed by the major phospholipids phosphatidyl ethanolamine (PE) and phosphatidyl choline (PC). Although PE and PC constitute 57 and 20% of the total phospholipids respectively of adults, the labelling in PC was almost similar to that of PE. There was considerable reduction (20%) in the label in triglycerides in 3 h after termination of the feedings suggesting rapid utilization of triglycerides. On the other hand, the label in the phospholipids increased significantly.

VAN HANDEL[19] reported synthesis of triglycerides from sugars in mosquitoes. The data of the present investigation suggest a precursor role for trehalose in the biosynthesis of lipids in *Culex*. The results also suggest increased synthesis of PC when compared to PE from trehalose and rapid turnover of triglycerides of the adults[20].

Summary. Trehalose was found to occur in the mosquito, *Culex pipiens fatigans*, to the extent of 23 to 49% of total free sugars in different developmental stages of the insect. Induction of stress to the insects led to significant reduction in the trehalose of larvae and glucose of adults. The label from $_{\alpha,\alpha}$'-U-^{14}C-trehalose readily incorporated into lipids of the adults in which triglycerides seemed to undergo rapid turnover.

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