

A Cube Sugar Technique for Laboratory Rearing of Adult Mosquitoes

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Abstract: Although the soaked cotton pad method seems to be the most commonly used technique for providing the sugar solution for adult mosquitoes, the cotton pad must be exchanged with a fresh one at an appropriate interval. For this reason, we examined the cube sugar technique to save us the trouble of handling mosquitoes in the laboratory for rearing several mosquitoes, *Culex pipiens* complex, *Aedes togoi* and *Armigeres subalbatus*. The results showed that this technique seems to be very useful to rear the adult mosquitoes, though the blood feeding rate in cube sugar group was slightly lower than that in 1-5% sugar solution groups. We are maintaining several strains of *Cx. pipiens* complex successfully by using this technique over 6 years.

Key words: Cube sugar technique, Mosquito rearing, *Culex pipiens* complex, *Aedes togoi*, *Armigeres subalbatus*

INTRODUCTION

Although female mosquitoes usually must take a blood meal for ovarian development, adults of both sexes require carbohydrate foods in addition. In laboratories, carbohydrates are generally supplied as a sugar solution in concentration of 2-20%. Other forms of sugar, such as honey, various fruit juices, raisins, apple slices and bananas, have also been used. The soaked cotton pad method seems to be a very convenient technique of providing the sugar solution for adult mosquitoes. The cotton pad, however, must be

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renewed at an appropriate interval, because sugar solution is easily deteriorated so the mosquitoes receive harmful influence. The renewal is also necessary for other forms of sugar.

Eliason (1963) used a technique of feeding mosquitoes on solid sugar, and Polk (1978) maintained a strain of *Culex pipiens quinquefasciatus* for about 2 years by using sugar cubes.

Because the cube sugar technique seemed to save us the trouble of handling mosquitoes in the laboratory, we applied this method and compared the longevity, survival rate, blood feeding rate and fertility with the sugar solution soaked cotton pad method.

MATERIALS and METHODS

Two experiments of different water supply systems were made. In the first experiment, 3 species of mosquitoes, *Culex pipiens pallens*, *Aedes togoi* and *Armigeres subalbatus*, were used. The survival rate or longevity, blood feeding rate, and fertility of mosquitoes reared by cube sugar were compared with those reared by sugar solution. A cube sugar on a petri dish and a cotton pad soaked with fresh water in a conical flask were put separately in each adult rearing cage. The cotton pad soaked with sugar solution was hanged from the ceiling of the cage and exchanged for fresh pad every other day. Two hundred mosquitoes of each species, 100 females and 100 males that emerged within 24 hours, were reared in a cage of 20×20×30cm. Each cage was placed on a vinyl chloride plate of 230×320×2mm in size. The observation of longevity was continued till the death of all mosquitoes. The longevity was compared between mosquitoes of cube sugar group and 2% sugar solution group. To compare blood feeding rates and fertilities, sugar solution of 4 different concentrations (10, 5, 2 and 1%) were used besides cube sugar. A mouse for blood feeding was confined tightly in a 16 mesh wire netting cage, and exposed to mosquitoes for one night in a rearing cage. The experiment was conducted in an insectarium with temperature of about 25°C, relative humidity of about 70%, and long photoperiod of 16 hours.

In the second experiment, 2 subspecies of *Cx. pipiens* complex, *Cx. p. pallens* and *Cx. p. quinquefasciatus*, were used, and the survival rates of adults of both sexes were observed during 35 days from emergence, and all females were allowed to feed on a mouse through the ceiling cloth of cage for one night 3 weeks after emergence. A mouse was confined tightly in a small 16 mesh wire netting cage, and kept overnight on a rearing cage. Longevity and blood feeding were compared between 2 types of sugar, cube sugar and 2% sugar solution. In the former, fresh water in a polyethylene cap of 90ml and a cube sugar on the lid of the cup were separately put in one cage. Other conditions of the second experiment were same as the first one.

RESULTS

The longevities or daily survival rates after adult emergence in 3 mosquito species are shown in Figs. 1, 2 and 3, respectively. As shown in Fig. 1, though the survival rate of females of *Cx. p. pallens* given 2% sugar solution was maintained over 80%, the rate decreased rapidly after that, and all mosquitoes died by about 120 days after emergence. In cube sugar group, the survival rate of females were almost 100% till about 60 days after emergence, but the rate began to decrease rapidly and all mosquitoes died by about 120 days. Survival curves of male *Cx. p. pallens* in cube sugar and 2% sugar solution groups were similar each other, and the males died earlier than the females.

Fig. 2 shows the survival period in *Ae. togoi*. Males and females of this species died earlier than *Cx. p. pallens* in both groups of 2 sugar types. However, some survived

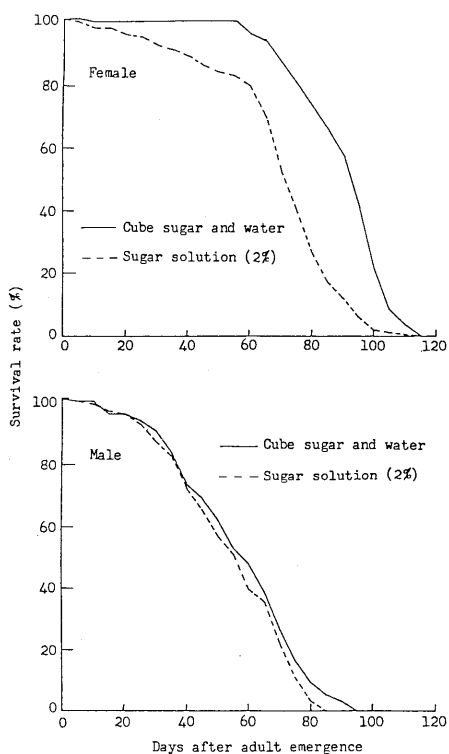


Fig. 1. Comparison of 2 types of sugar, cube sugar and sugar solution, as a carbohydrate source affecting longevity of adult *Culex pipiens pallens*. In case of cube sugar, fresh water in a conical flask was also supplied through an absorbent cotton pad in the cage.

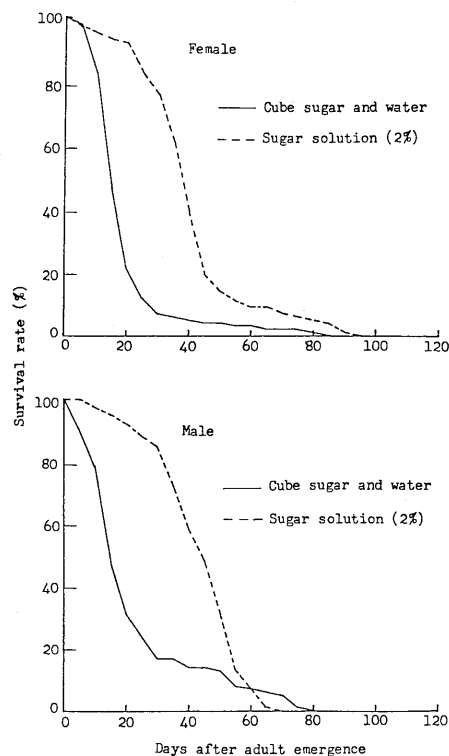


Fig. 2. Comparison of 2 types of sugar, cube sugar and sugar solution, as a carbohydrate source affecting longevity of adult *Aedes togoi*. In case of cube sugar, fresh water in a conical flask was also supplied through an absorbent cotton pad in the cage.

for about 80 days after emergence, and the survival rate in 2% sugar solution group was higher than that in cube sugar group.

The survival rates in *Ar. subalbatus* are shown in Fig. 3. Males and females showed high survival rates within about 30 days, and then the rates decreased rapidly. The survival period of the species with 2% sugar solution was longer than that with cube sugar and water.

Table 1 shows the survival rates, on the 10th day from emergence, of the 3 species of mosquitoes, *Cx. p. pallens*, *Ae. togoi* and *Ar. subalbatus*, given 2 types of sugar. In all species, survival rates in cube sugar group were almost the same as, or slightly lower (*Ae. togoi*) than, those in 2% sugar solution group.

Blood feeding rates of the 3 mosquito species on the 10th day from emergence, when they were reared with cube sugar and sugar solutions of 4 different concentrations, are comparatively shown in Table 2. The highest blood feeding rate was found in one or 2% sugar solution group, and the rate in every species decreased as the concentration of

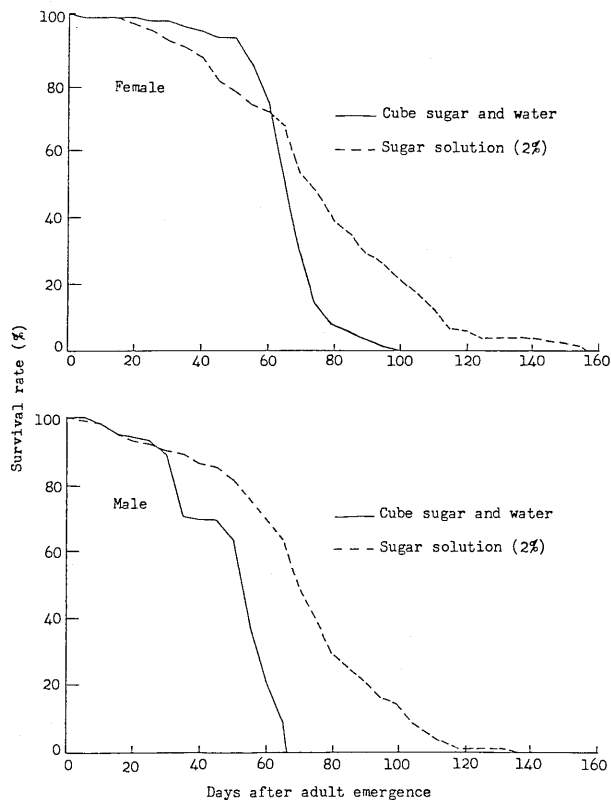


Fig. 3. Comparison of 2 types of sugar, cube sugar and sugar solution, as a carbohydrate source affecting longevity of adult *Armigeres subalbatus*. In case of cube sugar, fresh water in a conical flask was also supplied through an absorbent cotton pad in the cage.

Table 1. Comparison of 2 types of sugar, cube sugar and sugar solution, as a carbohydrate source affecting survival rate (%) on the 10th day from emergence in 3 mosquito species

Type of sugar	<i>Cx. p. pallens</i>		<i>Aedes togoi</i>		<i>Armigeres subalbatus</i>	
	Female	Male	Female	Male	Female	Male
Cube sugar and water	99	99	83	78	99	98
2% sugar solution	97	98	95	97	99	98

Newly emerged 200 mosquitoes (100 females and 100 males) were reared in one cage in each species.

Table 2. Comparison of cube sugar and sugar solutions as a carbohydrate source affecting blood feeding rate on the 10th day from emergence in 3 mosquito species

Form of sugar	<i>Cx. p. pallens</i>			<i>Aedes togoi</i>			<i>Armigeres subalbatus</i>		
	No. of females		Blood feeding rate (%)	No. of females		Blood feeding rate (%)	No. of females		Blood feeding rate (%)
	allowed to feed	fed		allowed to feed	fed		allowed to feed	fed	
Cube sugar	119	68	57.1	57	43	75.4	49	13	26.5
10% solution	59	26	44.1	60	51	85.0	61	4	6.6
5% solution	57	42	73.7	58	56	96.6	65	31	47.7
2% solution	59	57	96.6	56	56	100.0	64	60	93.8
1% solution	63	62	98.4	50	50	100.0	59	55	93.2

Table 3. Comparison of cube sugar and sugar solutions as a carbohydrate source affecting fertility of 3 mosquito species

Form of sugar	<i>Cx. p. pallens</i>			<i>Aedes togoi</i>			<i>Armigeres subalbatus</i>		
	No. of females examined	Fertility		No. of females examined	Fertility		No. of females examined	Fertility	
		Total	Mean		Total	Mean		Total	Mean
Cube sugar	13	2,850	219	43	6,948	162	13	2,018	155
10% solution	26	7,068	272	51	6,662	131	4	308	77
5% solution	42	11,005	262	56	8,385	150	31	2,524	81
2% solution	57	13,858	243	56	11,357	203	60	7,811	130
1% solution	62	17,418	281	50	9,775	196	55	8,732	159

sugar solution became high. Although the rate in cube sugar group was slightly lower than that in 1–5% sugar solution groups, 26.5–75.4% of females took blood.

The fertility, as expressed by the number of eggs deposited, of the fed females mentioned above in each species is shown in Table 3. Range of mean numbers of eggs in cube sugar and 4 sugar solution groups were 219–281 in *Cx. p. pallens*, 131–203 in *Ae. togoi*, and 77–159 in *Ar. subalbatus*, respectively. The results showed that the fertility was enough to get the next generation, though they were somewhat different among females given cube sugar and sugar solutions.

The results of the second experiment with *Cx. p. pallens* and *Cx. p. quinquefasciatus* are shown in Figs. 4 and 5, and Table 4. In females of *Cx. p. pallens* (Fig. 4), survival rates on the 35th day from emergence were 74% in cube sugar group and 67% in 2% sugar solution group. In males of this species, the rates were 56% and 43%, respectively and lower than those in females. In *Cx. p. quinquefasciatus*, the survival rates in females on the 35th day were 66% in cube sugar group and 39% in 2% sugar solution group, and those in males were 80% and 73%, respectively (Fig. 5). All female mosquitoes were allowed to feed on mice on the 21st day from emergence. The blood feeding rates in females given 2 types of sugar in each species are tabulated comparatively in Table 4. The rates in cube sugar females were nearly 50% in both species, though slightly lower than those in 2% sugar solution females.

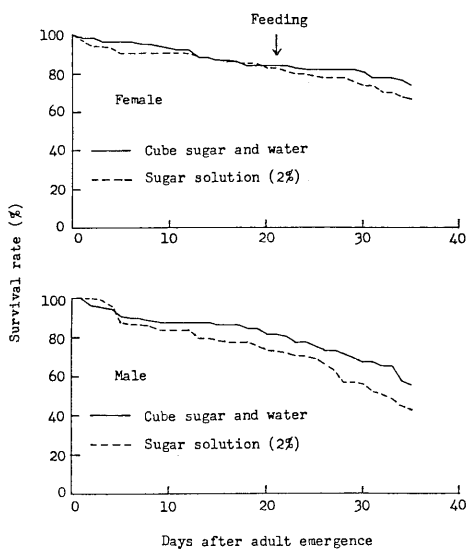


Fig. 4. Comparison of 2 types of sugar, cube sugar and sugar solution, as a carbohydrate source affecting longevity of adult *Cx. p. pallens*. In case of cube sugar, fresh water in a plastic cup was also supplied in the cage.

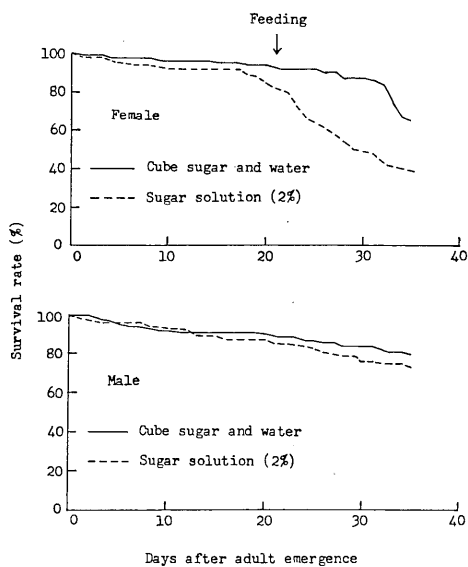


Fig. 5. Comparison of 2 types of sugar, cube sugar and sugar solution, as a carbohydrate source affecting longevity of adult *Cx. p. quinquefasciatus*. In case of cube sugar, fresh water in a plastic cup was also supplied in the cage.

Table 4. Comparison of 2 types of sugar, cube sugar and sugar solution, affecting blood feeding rate after 3 weeks of adult emergence in *Cx. p. pallens* and *Cx. p. quinquefasciatus*

Type of sugar	<i>Cx. p. pallens</i>			<i>Cx. p. quinquefasciatus</i>		
	No. of mosquitoes		Blood feeding rate (%)	No. of mosquitoes		Blood feeding rate (%)
	allowed to feed	fed		allowed to feed	fed	
Cube sugar and water	84	40	47.6	92	43	46.7
2% sugar solution	83	50	60.2	82	45	54.9

DISCUSSION

Eliason (1963) reported that 8 species of mosquitoes belonging to *Culex*, *Culiseta*, *Anopheles* and *Aedes* could feed on crystalized sugar. However, it was not indicated whether this finding could be put to practical usage in maintaining laboratory colonies. It was also reported by Polk (1978) not only that the adults of *Culex pipiens quinquefasciatus* prefer sugar cubes as a carbohydrate source, but that they live 3–4 times longer than those fed on raisins. He maintained the colony of the mosquito successfully by using sugar cubes for about 2 years. In spite of the usefulness of cube sugar as a carbohydrate food for adult mosquitoes as mentioned above, the cube sugar technique has not been used so commonly up to the present.

The results of our first experiment showed that the cube sugar is as good as 2% sugar solution for survival in *Cx. p. pallens*. In *Ae. togoi* and *Ar. subalbatus*, though cube sugar was a little inferior to 2% sugar solution for survival, it was shown that they live long enough to produce the next generation. In addition to the survival rates, the blood feeding rates and the fertilities in 3 species were high enough to suggest that the cube sugar technique is good for rearing adult mosquitoes. The results of the second experiment also showed that the cube sugar is slightly better than 2% sugar solution for survival, and is similar each other in blood feeding in *Cx. p. pallens* and *Cx. p. quinquefasciatus*.

The observations of different water supply systems showed that the polyethylene cup system is better than cotton pad system, because the former system will save us the trouble of handling mosquitoes.

The suitable relative humidity of the insectarium for keeping mosquitoes may be about 70% with the range from 50 to 80%. The humidity higher than about 85% should be avoided, because the cube sugar in the rearing cage will be melt by humid air.

The suitable number of sugar cubes in a cage is 2 or 3 for about 500–1,000 adult mosquitoes. Usually, autogenous mosquitoes, like *Cx. p. molestus*, will take more sugar than the others. The surface of the sugar cube contaminated by mosquitoes must be removed by using the edge of a knife. The sugar cubes with refreshed surface can be used again though they become smaller than the original one.

It is concluded from the experiments described above that the cube sugar technique is recommendable to save us trouble in routine mosquito rearing. This technique will be applicable at least to *Culex*, *Aedes* and *Armigeres*. We are actually maintaining several strains of *Cx. pipiens* complex successfully by using this technique for more than 6 years.

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角砂糖による蚊成虫の一飼育法

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多系統の蚊を累代飼育する場合, なるべく手間のかからない飼育法の開発が望まれる. 蚊成虫の飼育には通常, 砂糖水を脱脂綿に含ませて栄養として与えているが, その場合, 1~数日置きに新しいものと交換するか, 新しい砂糖水を補充しなければならない. われわれは市販の角砂糖と汲み置き水道水とを別々の容器に入れて与え, 成虫を飼育することに成功した. 飼育に使用するケージは大きさが 200×200×300mm の針金枠にテロンゴース製の袋をかぶせたもので, このケージを 230×320×2mm の塩化ビニール板の上に乗せて使用する. このケージ 1個で飼育できる成虫数は約 1,000 個体以内である. ケージの内部には水道水を満たした 90 ml のプラスチックコップと角砂糖 2~3個 (蚊の個体数による) を載せた同コップの蓋をそう入する. 成虫はコップの水を飲み, 角砂糖をだ液で液かして摂取することにより, 2~3週間放置しても健康な状態で生存し, その後もマウスから吸血し, 産卵する. 蚊の生存率, 吸血率, 産卵率, 及び卵の孵化率は羽化して約 1週間後から多少低下するが, 次世代を得るのに支障はない. 飼育室内は温度約 25℃, 湿度約 70%, 薄明・薄暮を含む長日照明の状態に保つことが望ましい. 湿度が高すぎると角砂糖が溶け出し, また低すぎるとコップの水が早くなるので共に避けなければならない. 出張などで10日間以上放置する場合には, 角砂糖を更新し, 水の量を多くする. 角砂糖の表面が甚だしく汚れた場合には, 表面をナイフの縁などで削り落して新しい面を出すことにより再使用できる. われわれはこの方法で, アカイエカ群の多系統の蚊を 6年以上にわたり累代飼育している.