Raffinose as reserve sugar in *Dysdercus koenigii* – a biosynthesis confirmation by ^{14}C glucose

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Summary. Dysdercus koenigii, studied for its sugar spectrum, showed consistent absence of trehalose in its blood and body muscles. Instead of this known reserve sugar, raffinose has been shown to fulfil this need. The biosynthesis confirmation has been made by ^{14}C glucose.

Except in some earwigs, where it has been reported absent[1,2], trehalose is known to be consistently present in insect bodies as a reserve sugar[3,4]. Contrary to such a generalisation, our study has revealed its absence in yet another insect, *Dysdercus koenigii* F., and confirmed raffinose to function as the substitute reserve in the body of the bug.

The analyses of sugars in haemolymph, body muscles and dietary item were made with the known methods[5,6] which showed raffinose to occur as a common sugar in the bug, and trehalose to be consistently absent. It was also found that through enzymatic hydrolysis[7] galactose breaks off from the raffinose molecule, into whose synthesis it enters along with glucose and fructose (table 1). In case of starved insects also, a gradual fall interrupted by a steep decline in the raffinose level of the haemolymph was observed. After 3 h, a fall from 48 mg/100 ml to 28 mg/100 ml was recorded (figure 1). The level almost reached zero after 9 h of starvation. At this stage, when insects were fed on cotton seeds, raffinose was detected from 3 h onwards (figure 1). Since raffinose exists in the cotton seed, the question of whether or not raffinose acts as a reserve sugar in the insect and can be synthesized in its body, it was necessary to establish this biosynthesis. The biosynthesis was tried through experimental feeding of the insects on a mixture (1%) of glucose (1 ml) containing D (U-^{14}C) glucose (10 µCi/1 ml); galactose (1 ml) and fructose (1 ml). The feeding was continued for 24 h. Radioactivity was measured by the method of Moloo[8] with the help of liquid scintillator (ECI make). By using this ^{14}C activity in various fractions of chromatograms (figure 2) following the feeding of the sugar mixture. The total recovery was 2.24×10^3 cpm, i.e. nearly 83.12%. It is apparent from the table that raffinose has the highest ^{14}C activity (28.6%) amongst all

Table 1. Enzymatic breakdown of raffinose (mg/100 ml) by flight muscle enzymes (A), and haemolymph enzymes (B)

Time h	A	B
0	45	-
4	22	45
8	8.5	21
12	0	16
16	-	12
20	-	9
24	-	8

Table 2. Distribution of ^{14}C activity in various fractions from chromatogramed sugar sample, 24 h after (U-^{14}C) glucose feeding in sugar mixture

Fraction	cpm/min	%
Spotted area (X_1)	416	18.4
Trailed area (a)	585	26.0
Raffinose (Raff)	644	28.6
Blank area (X_2)	49	2.2
Galactose (gal)	123	5.5
Glucose (glu)	199	8.8
Fructose (Fr)	135	6.0
Blank area (X_3)	61	2.7
Blank area (X_4)	40	1.7

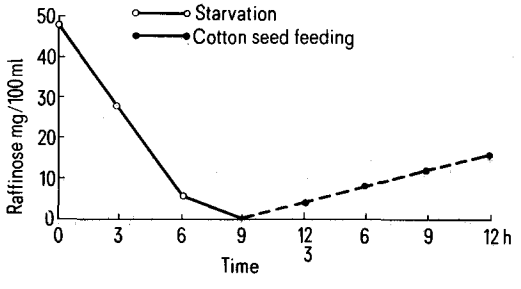

Fig. 1. Raffinose breakdown and assimilation in *D. koenigii.*

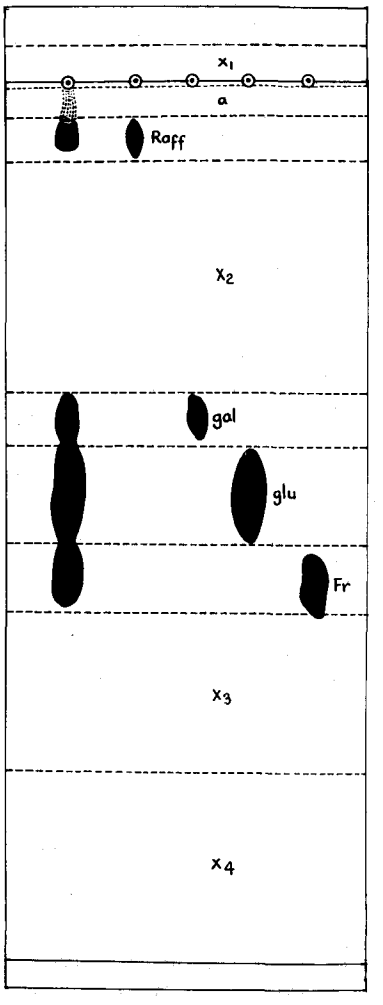

Fig. 2. Chromatogram showing the spots of different sugars (pyridine:ethyl acetate:water = 14:39:9).

other fractions. Though the trailed area (a) also showed a high activity of 585 cpm, it can be attributed to its close connection and proximity with the raffinose spot (figure 2). It is of interest to note that only 8.8% of the total recovery activity was in the glucose part, showing that the bulk of the ^{14}C has got transferred to raffinose, indicative by its increased activity. However, the radioactivity of 5.5 and 6% detected in the galactose and fructose fractions may also be due to the influence of the glucose fraction which lies in close proximity with either of these sugars. The detection of minor activity in the blank areas (X spaces, figure 2) may be due to some breakdown products, which, however, were not assayed.

Since biosynthesis of raffinose has been confirmed to take place in the insect body, the only probable function which it could perform appears, therefore, to be as a reserve sugar in this bug[9].

1 J.P. Leader and J.J. Bedford, Comp. Biochem. Physiol. *43*, 233 (1972).
2 V.V. Raghavan and H.V. Rao, Indian J. exptl Biol. *14*, 194 (1976).
3 K. Myrback, Egebn. Enzymforsch. *10*, 168 (1949).
4 G.R. Wyatt and G.F. Kalf, J. gen. Physiol. *40*, 833 (1957).
5 J.S. Barlow and H.L. House, J. Insect Physiol. *5*, 181 (1960).
6 G.H. Schmidt and H. Brunnet, J. Chromat. *10*, 190 (1963).
7 H.C.S. DeWallery, in: Methods of enzymatic analysis, p. 93. Academic Press, New York 1963.
8 S.K. Moloo, J. Insect Physiol. *22*, 195 (1976).
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Sodium-dependence of sustained force in potassium contracture of cat ventricle

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Summary. Following exposure to low-Na solution, the initial phase of K-contracture in cat ventricle is prominent while the second (sustained) phase is markedly attenuated. Monensin, a Na-specific ionophore, enhances the second phase of K-contracture following exposure to low-Na solution.

The role of Na in the genesis of KCl-contracture in mammalian ventricular muscle is unclear. Although K-contractures are augmented by Na-poor solutions[3,4], some extracellular Na is apparently necessary to permit the appearance of K-contracture[5]. A reduction of extracellular Na or an elevation of intracellular Na facilitates Ca influx via Na:Ca exchange. The contribution of this process to the development and maintenance of K-contracture in ventricular muscle is unclear[5,6]. Additionally, the interpretation of the importance of intracellular Na for K-contracture is complicated by the recent discovery that the bulk of myocardial intracellular Na is sequestered in non-ionic form[7]. The present research was undertaken to reinvestigate the relation of Na to the development and maintenance of contracture during K-depolarization.

Methods. The methods used in this study were similar to those previously published[6]. Small (< 1 mm diameter) cat right ventricular muscles were maintained in control Tyrode's solution (solution I). The composition of all solutions used is shown in the table. Solutions I and II were gassed with 95% O_2, 5% CO_2; pH was 7.4 at 34 °C. Solutions III and IV were gassed with 100% O_2; pH was adjusted to 7.4 at 34 °C with HCl. All solutions contained nadolol (10^{-4} M), a beta-adrenergic blocking agent; this concentration has no effect on isometric contraction of mammalian myocardium[8]. In some experiments, a Na-selective ionophore, monensin[9], was added to solutions III and IV. Monensin (mol. wt 670; Lilly) was dissolved in 50% dimethylsulfoxide and 50% ethanol. The vehicle for monensin did not affect the results. Muscles were stimulated (0.33 Hz) only when exposed to solution I. After each contracture, the muscle was exposed to solution I for 45–60 min before proceeding with the next phase of the experiment.

Results and discussion. Exposure of ventricular muscles to low-Na (1.8 mM) solution (solution III) resulted in small 'sodium-lack' contractures[10] of variable duration (~ 10–15 min). K-contracture (using solution IV) evoked after their spontaneous relaxation, i.e., after 30 min in solution III, were higher than K-contractures (using solution II) evoked following exposure to normal Na (solution I); peak contracture force after solution III averaged 161% of that seen after solution I. The major change in K-contracture evoked following exposure to low-Na solution is a marked change in the time course of contracture. Following exposure to normal Na, contracture force is generally well sustained for the duration of exposure to elevated-K[6,11] (figure A). Following exposure to low-Na solution, contracture force is markedly phasic, declining from a prominent initial peak to a very low level for the duration of K-depolarization (figure B). [Following exposure to zero Na solution, K-contracture is similar to that noted following exposure to 1.8 mM Na[12].] As suggested by Gibbons and Fozzard[3], the phasic nature of the 1st phase of K-contracture following exposure to low Na solution indicates that this initial phase may be attributed to a release of cellular stores of Ca brought about by the rapid depolarization. The 2nd phase is Na-sensitive and probably represents a balance between inwardly directed Ca and the continued uptake or removal of Ca by cellular organelles (see below).

In Na-free solutions, total myocardial tissue Na falls from 60 to less than 5 mmoles/kg tissue water within 20 min[5,13]. This may represent Na 'irreversibly' sequestered in muscle[14] or bound to connective tissue. The free Na activity (a_{Na}) within the sarcoplasm is probably much lower than this, since a_{Na} is only 6 mmoles/kg when total intracellular Na is on the order of 40 mmoles/kg[7]. The free Na in the

Composition of saline solutions[a,b]

	NaCl	KCl	$NaHCO_3$	NaH_2PO_4	Tris[c]	Sucrose
I	129	4	20	1.8	–	–
II	–	133	20	1.8	–	–
III	–	4	–	1.8	5	260
IV	–	133	–	1.8	5	–

[a] All concentrations in mmoles/l. [b] In addition, all solutions contained $CaCl_2$, 2.7; $MgCl_2$, 0.5; and dextrose, 5.5. [c] Tris (hydroxymethyl) aminomethane.