Table III. Effect of long-chain (Z)-9-alkenes on both sexes (1:1) of houseflies in tests with pseudofly-flypaper strips[a]

Test material[b]	No. of traps	Total number of flies captured	Ratio (male/female)	Times increase of captures (%)
Standards	100	4248	2.7	
Controls	16	1312	3.0	1.8
I	4	467	5.7	2.4
II	4	679	3.3	2.5
III	4	442	2.7	2.4
IV	4	398	3.5	2.5
V	4	462	3.4	1.7
VI	4	657	3.9	2.7

[a] Tests on 55000 houseflies (total) at 22 to 24 °C in 14 six-hour test series using 10 flypaper strips, each, with 300 black spots (5 mm ∅). [b] 2.5 ml hexane, for standards (10 test series) or 125 mg (Z)-9-alkenes dissolved in 2.5 ml hexane, for tests (4 test series) and 2.5 ml hexane, for control strips run simultaneously, were applied to each trap and the solvent was allowed to evaporate.

to 5.7 times higher than that of females, though in the test room the starting ratios of the sexes was 1:1. In the presence of long-chain (Z)-9-alkenes, both traps impregnated with these compounds and those not impregnated caught more flies compared to standards run in the absence of the unsaturated hydrocarbons. The efficiency of the pseudofly-flypaper strips loaded with test material V, which contained the smallest proportion of biologically active (Z)-9-alkenes[3,4] was increased by a factor of 1.7, whereas that of the controls, run simultaneously, was increased by a factor of 1.8. The percentages of captures of all other traps impregnated with (Z)-9-alkenes were increased 2.4 to 2.7 times.

Discussion. The results obtained with houseflies show that a wide spectrum of long-chain (Z)-9-alkenes, in varying combinations, exhibit, both qualitatively and quantitatively, the same behavioral responses, depending upon the different visual signals in the tests using pseudofly-Petri dishes and pseudofly-flypaper strips.

Recently, (Z)-9-tricosene has been characterized as a short range sex pheromone[13]. In the present study (Z)-9-tricosene did not give clear evidence for short range orientation of male houseflies to the source of odor in the modified pseudofly tests using Petri dishes, although it is well known that houseflies pay attention to nearly any odor at a short distance[8]. However, in the presence of long-chain (Z)-9-alkenes the optical stimuli of sex attraction by untreated pseudoflies (controls) exerted an enhanced releasing effect, compared to those in standard tests run in the absence of pheromones.

The conventional tests with pseudofly-Petri dishes also showed unequivocally, that in the presence of long-chain (Z)-9-alkenes the optical stimuli of sex attraction by male partner flies were enhanced, compared to those in the standard tests which were run in the absence of pheromones. Similar observations have been made using benzene extracts of female houseflies[10].

Similarly, in the tests using pseudofly-flypaper strips, the visual stimuli for aggregation provided by the untreated strips (controls) were potentiated in the presence of high dosages of long-chain (Z)-9-alkenes compared to standards run in the absence of pheromones.

On the basis of all these findings, it is concluded that the threshold for optical cues stimulating houseflies to sex attraction or aggregation is decreased in the presence of long-chain (Z)-9-alkenes in the test systems. Thus, long-chain (Z)-9-alkenes can be considered 'psychedelics' to houseflies with regard to visually stimulated sex attraction and aggregation. The state of expanded sensoric susceptibility is a characteristic effect of psychedelic drugs.

Sex attraction of male houseflies by moving pseudoflies is not changed in the presence of long-chain (Z)-9-alkenes[9], because the releasing effect of such fast moving dummies is greatly increased compared to static female fly models[7]. In tests using plexiglas olfactometers[1,3,4] or other types of traps[5,6] for the assay of pheromones on houseflies, visual stimuli may have been involved to varying extents.

The results obtained in tests using pseudofly-flypapers show poor captures compared to the total number of flies present, although both olfactory and visual stimuli were provided. Therefore, the use of long-chain (Z)-9-alkenes or other olfactory stimuli[8] seems to be of minor value for control of the housefly.

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Sodium Pumps and Galactose Transfer in the Short-Circuited Small Intestine

J. F. UDE and R. J. C. BARRY

Department of Physiology, University of Ibadan, Ibadan (Nigeria), and Department of Physiology, University of Sheffield, Western Bank, Sheffield S10 2TN (England), 10 June 1975.

Summary. In a short-circuited preparation of rat jejunum, there are two sodium pumps, one electrogenic and the other neutral. When energy sources are limited, the total sodium transfer is limited. In the presence of a non-metabolized actively transferred hexose, the electrogenic pump is preferentially used. The neutral sodium pump is only able to function when additional energy is available.

The absorption of Na and certain hexoses by the small intestine are closely linked[1–8]. The situation in the rat jejunum in vitro is complicated by there being two distinct Na pumps, one which is electrogenic and the other which is non-electrogenic[7,9]. Na transferred by the electrogenic mechanism gives rise to a transmural potential difference and is linked in part to active hexose transfer, while that transferred by the non-electrogenic mechanism is linked to hexose metabolism and causes an increase in fluid absorption. The capacity of the jejunum to transfer Na and the relationship between these two types of Na pump has been investigated using a short-circuiting technique.

Materials and methods. White male rats (220–250 g) were anaesthetised with i.p. pentobarbitone sodium (Nembutal). The small intestine was removed and

Table I. Short-circuit current, fluid and Na transfers with different hexoses

Hexose added [a]	Fluid transfer (ml)	Short-circuit current (mA)	(μmole) [c]	Net Na transfer (μmole)
Mannitol (19) [b] (control)	0.13 ± 0.01	1.13 ± 0.06	21.1 ± 1.1	48.9 ± 1.9
Glucose (12)	0.40 ± 0.04	3.11 ± 0.08	58.1 ± 1.5	75.9 ± 2.6
Galactose (35)	0.19 ± 0.01	2.67 ± 0.04	49.9 ± 0.70	49.9 ± 1.6
α-Methyl glucoside (6)	0.21 ± 0.03	2.60 ± 0.08	48.7 ± 1.4	52.1 ± 4.8
3-*O*-Methyl glucose (6)	0.16 ± 0.02	2.56 ± 0.15	47.8 ± 2.8	53.2 ± 4.1
Fructose (6)	0.24 ± 0.02	1.35 ± 0.20	25.2 ± 3.4	61.8 ± 3.3

[a] 28 m*M* hexose or mannitol initially present in both mucosal and serosal fluids. [b] The number of individual experiments. [c] Short-circuit current expressed as monovalent cation equivalent for 30 min period. Values expressed as the mean ± standard error of the mean of absolute values obtained from 8 cm sacs incubated for 30 min.

everted as described by BARRY, MATTHEWS and SMYTH[10]. An 8 cm sac of midjejunum was attached to a cannula and positioned in the apparatus illustrated in Figure 1. The intestine was incubated in Krebs Bicarbonate saline to which different concentrations of galactose and mannitol had been added to make a total osmotic contribution of 28 m*M*. The tissue was short-circuited by a method similar to that used by BARRY, SMYTH and WRIGHT[7], correction being made for solution resistance as described by CLARKSON and TOOLE[11]. The intestine was short-circuited throughout the 30 min incubation period, the current being adjusted at 1 min intervals. A mean short-circuit current was calculated for the 30 min period and expressed as both mA and μmole of monovalent cation transfer.

Net mucosal transfers were estimated directly as the total net uptake into the combined intestinal wall and serosal fluid. Fluid transfers were calculated from the increase in the weight of the intestine. Na concentration was estimated by flame photometry and galactose by SOMOGYI'S[12] modification of the NELSON method[13]. In calculating transfers, allowance was made for solutes present in non-incubated sacs of intestine.

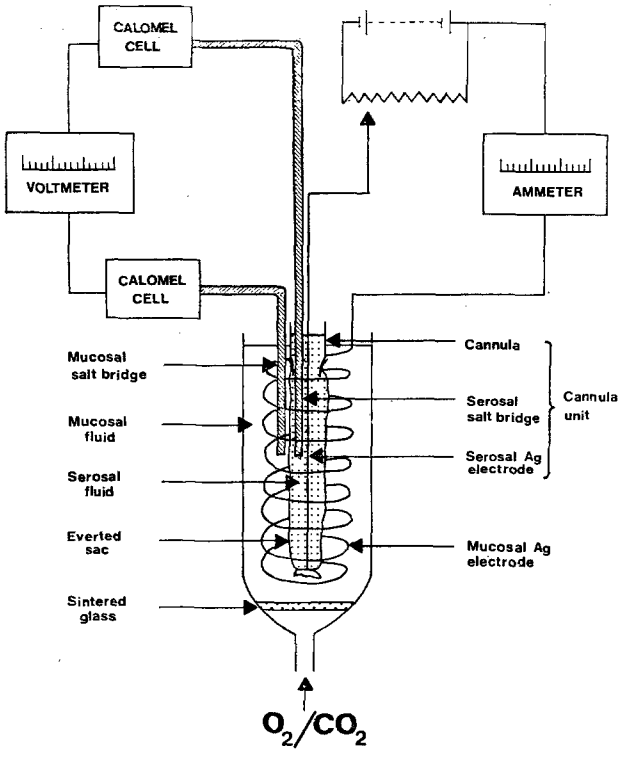

Fig. 1. Short-circuit apparatus.

Results and discussion. When sacs were short-circuited in the presence of 28 m*M* galactose, α-methyl glucoside or 3-*O*-methyl glucose, the net Na transfers were not significantly different from those obtained in the absence of added sugar (Table I), mannitol being added to maintain the osmolarity of the incubating fluid constant. The net Na transfers also agreed well with the short-circuit current when expressed as monovalent cation equivalent. The short-circuit current is widely regarded as being a measure of electrogenic ion movement and these results are in accord with the widely held view that Na is the major ion implicated. However, when incubated with glucose or fructose not only were there marked increases in both Na transfer ($p < 0.001$ and $p < 0.01$) and fluid transfers ($p < 0.001$ and $p < 0.001$), but the net Na transfer was also considerably greater than the short-circuit current. Whilst fluid and Na transfers were greatest with glucose, the discrepancy between short-circuit current and net Na transfer was greatest with fructose. In the mannitol control, net Na transfer also exceeded short-circuit current. These results differ from those of BARRY et al.[7] who found an equivalence between short-circuit current and net Na transfer with glucose and mannitol and a short-circuit current greater than net Na transfer with galactose and α-methyl glucoside. However, their method depended on measuring transfers from the serosal surface of the isolated intestine and calculating steady state transfers for a 30 min period from those

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Table II. Short-circuit current, Na and galactose transfers at different initial galactose concentrations

Galactose concentration[a] (mM)	Short-circuit current[b] (mA)	(μmole)	Net Na transfer (μmole)	Galactose transfer (μmole)
0 (19)	1.26 ± 0.06	23.5 ± 1.4	51.4 ± 2.9	—
1.0 (6)	1.47 ± 0.07	26.2 ± 1.3	50.1 ± 3.4	7.7 ± 0.6
1.5 (6)	1.49 ± 0.09	27.6 ± 1.9	49.0 ± 1.6	7.0 ± 0.7
2.0 (6)	1.85 ± 0.09	34.5 ± 1.6	53.3 ± 3.4	11.5 ± 1.0
5.0 (8)	2.14 ± 0.07	39.9 ± 1.3	50.2 ± 2.5	22.6 ± 2.2
28.0 (35)	2.67 ± 0.04	49.9 ± 0.7	49.9 ± 1.6	36.0 ± 1.4

[a] Mannitol was added with galactose so that the total added osmolarity was 28 mM at each galactose concentrations. [b] Other details and expression of results as in Table I.

obtained in experiments over 15 and 45 min. It appears that Na transfers calculated from serosal transfers are less than those obtained from measuring mucosal uptake over 30 min directly.

Different hexoses are handled in different ways by the rat jejunum[14]. Galactose, α-methyl glucoside, 3-*O*-methyl glucose and glucose are actively transferred and all these gave rise to an anticipated increase in the short-circuit current. Of the sugars used in these experiments, only glucose and fructose are metabolized significantly by the rat jejunum. Thus only when a metabolizable sugar is present is the net Na transfer increased above the control level and this does not correlate with an increased short-circuit current. It may be inferred that the total capacity of this preparation to transfer Na is related to the availability of energy-providing substrates and in the absence of such externally supplied substances the transfer of Na is limited by the endogenous energy resources of the tissue.

The discrepancy between Na transfer and short-circuit current under certain conditions may result from the operation of a neutral Na pump. It would appear that this pump can utilize endogenous substrates if they are available, i.e. such as when no actively transferred hexose is present, but is stimulated by the addition of a metabolizable substrate such as glucose.

The relationship between the electrogenic and the neutral Na pumps was investigated further by incubating the intestine with different concentrations of galactose (Table II). The total net Na transfer remained constant at all galactose concentrations as in the absence of galactose. However, with increasing concentration of galactose, the net galactose transfer increased and there was a corresponding increase in short-circuit current. The relative contributions of the two Na pumps in the absence of galactose are similar (Figure 2). But as the galactose concentration increases, the increase in electrogenic Na transfer is reflected by a decrease in non-electrogenic transfer until at an initial galactose concentration of 28 mM all the Na is transferred by the electrogenic pathways. As the hexose linked Na transfer increases, Na is diverted from the neutral pump and the electrogenic pump becomes predominant. It is possible that the results could be explained on the basis of a single Na pump, which behaves as a neutral pump in the absence of actively transferred sugar, but in its presence shows a decreased coupling with increasing concentrations of sugar. Whilst not excluding this possibility, the simplest model at present would seem to be that of two separate pumps whose summed activity would depend on the availability of energy from the cell's metabolism. In either case, this does not indicate the nature of the coupling ion, whether an anion moving in the same direction as Na or a cation in the opposite direction. It is hoped to present evidence concerning the nature of the coupling in a later communication.

In conclusion, the results obtained in the in vitro short-circuited rat jejunum, under conditions of limited energy sources, suggest that when an actively transferred hexose is present the electrogenic pump is preferentially used and the neutral Na pump is only able to function when additional energy is available.

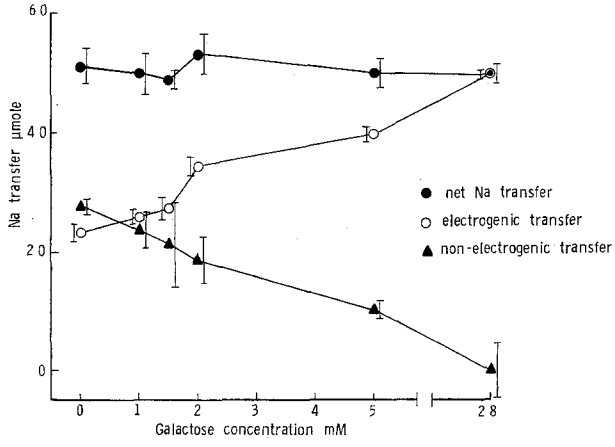

Fig. 2. Na transfers, non-electrogenic and electrogenic, at different initial galactose concentrations.

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