BIOLOGICAL SCIENCES

Intracellular Concentration of Electrolytes in the Housefly

THE concentrations of blood Na and K in the adult housefly, *Musca domestica*, fed a normal diet of milk and sugar are fairly constant. The usual amounts are Na, 109–140 mequiv./l., 0·11–0·14 M (2·4–3·2 μg/μl.); K, 0·0–4 mequiv./l., 0·0–0·004 M (0·0–0·16 μg/μl.). Blood cells contain very small quantities of these ions.

The total amount of intracellular K in nerve and muscle tissue is easy to determine because it accounts for all K in the head and thorax respectively. I have determined the total amount of intracellular Na in the following way, using the ion exchange method previously described for the determination of blood volume[1]. Fifteen or more beads of *IR*-120(H) mesh 30 were inoculated into the abdomen, and after 1–2 min the resin was removed and ashed. *In vitro* experiments, in which a similar number of beads are equilibrated with up to 5 μl. of blood, have shown that this quantity of resin exchanges with at least 90 per cent of blood Na. No intracellular Na has been found to diffuse into the blood in these circumstances[2]. The fly was then frozen on a slab of cardice and the head, thorax and abdomen were separated and ashed separately. The Na content of the different parts of the body represents the respective intracellular amounts. A male fly weighing 12 mg gave the following results: head, 1·5 μg Na, 4 μg K; thorax, 2·8 μg Na, 19 μg K.

To determine the volume of haemolymph in any of the three parts of the body, a control fly of the same weight and age, reared in the same conditions, was deep frozen and separated into head, thorax and abdomen. These parts were ashed separately and assayed for Na. The results are given in Table 1. The difference in the Na content between control and treated flies is the result of blood Na which has been exchanged by the resin.

Table 1. SODIUM CONTENT (μG) IN FLIES WEIGHING 12 MG

	Resin	Head		Thorax		Abdomen	
Control fly		1·95		5·55		2·65	
Inoculated fly	4·5	1·50		2·90		0·75	
Calculated blood Na		0·45	+	2·65	+	1·9	= 5 μg

The concentration of blood Na averaged 2·9 μg/μl., from which it follows that the volumes of haemolymph in head, thorax and abdomen were 0·15, 0·92 and 0·66 μl. respectively (9, 53 and 38 per cent of the total blood volume of 1·73 μl.). The amount of Na in the resin (4·5 μg as compared with a difference in the Na content of 5 μg) can be used to check the degree of accuracy of the results.

The blood volume of houseflies is related to body weight, and a volume of 0·17 ± 0·02 μl./mg body weight has already been found[1]. The volume of haemolymph (V) in any of the three parts of the body can therefore be determined from the equation

$$V = (0{\cdot}17 \times \text{weight of fly } (W) \times \text{corresponding percentage volume})$$

There are two constants in this equation, which can be simplified to $V = W \times C$. The value of the constant C will be 0·015, 0·09 and 0·064 for head, thorax and abdomen respectively.

Because the total blood volume is related to body weight, it is natural to expect that the blood volume in any part of the body is also related to the weight of that part. This seems to be the case, in view of the striking identity of the percentage blood volume of 9, 53 and 38 per cent for head, thorax and abdomen, obtained by the ion exchange method, and their respective weights of 11·7 ± 0·17 per cent, 47·5 ± 1·4 per cent and 39·9 ± 0·9 per cent.

The moisture content of the head, thorax and abdomen of houseflies 4–6 days old is 60 ± 1 per cent; that of blood is 80 ± 0·5 per cent, specific gravity 1·016 g. These data can be used in calculating the volume of intracellular water ($(H_2O)_i$) in any part of the body. For example, $(H_2O)_i$ in brain = total water in head − moisture content of head haemolymph

$$= [W \times 0{\cdot}1 \times 0{\cdot}65] - [0{\cdot}8 \times W \times 0{\cdot}1 \times 0{\cdot}17] = W \times C_1 \ (\mu\text{l.}) \quad (1)$$

C_1 is constant and equals 0·0515 for housefly heads and five times that value (0·2575) for thorax. I have not attempted to carry out similar calculations for the abdomen because of its structural diversity. The calculations here are based on the fact that heads and thoraces are composed very largely of brain and muscle tissue respectively.

The intracellular concentration of K in nerve and muscle will be

$$(\text{K})_i = \frac{\text{K}}{W \times C_1} \ (\text{g/l.}) \quad (2)$$

K is the total amount (μg) of potassium in the head or thorax, which accounts for all intracellular K because the amount of K in the blood is negligible.

The intracellular concentration of Na is obtained in a similar way except that the amount of blood Na must be subtracted from the total amount.

$$(\text{Na})_i \text{ in the head} = \frac{(\text{Na}) - [W \times 0{\cdot}1 \times 0{\cdot}17 \times 2{\cdot}9]}{W \times C_1}$$

$$\frac{1}{C_1}\left[\frac{\text{Na}}{W} - 0{\cdot}049\right]$$

$$= \frac{\text{Na}}{W \times C_1} - \frac{0{\cdot}049}{C_1}$$

$\frac{0{\cdot}049}{C_1}$ is constant and is equal to 0·951, so the equation can be simplified to

$$(\text{Na})_i = \frac{\text{Na}}{W \times C_1} - 0{\cdot}951 \quad (3)$$

The value of 0·951 is based on an average of 2·9 μg of Na/μl. of blood. If the amount of Na is X μg/μl., however, then its value becomes $0{\cdot}951 \times X/2{\cdot}9$, using equations 2 and 3. The results presented earlier gave the following internal concentrations: Na = 96, 36·9 mequiv./l.; K = 161, 153 mequiv./l. for brain and muscle, respectively. These results are as good as can be expected except that the internal concentration of Na in the brain is high as compared with that of the haemolymph, which may imply that not all brain Na is exchangeable.

I have assumed in these calculations that both internal electrolytes and water are entirely free to exchange. This may be true for the electrolytes, but it is not the case for water because cell proteins will be solvated to an extent partially governed by the surrounding electrolyte concentration[3]. Attempts are now being made to determine indirectly the amount of bound water in excitable tissues by determining either the resting or action potential from which the internal K or Na concentration can be deduced from the Nernst electrode potential equation. If the concentrations of any of the ions are X and X_1, as determined by the Nernst equation and equations 2 and 3 respectively, then the volume of bound water will be $\frac{X^1}{X_1} \times W \times C_1$ (μl.).

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[1] Shatoury, H. H., *Nature*, **211**, 317 (1966).
[2] Shatoury, H. H. (in the press).
[3] Shatoury, H. H., *Nature*, **199**, 1192 (1963).