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WATER LOSS FROM *PERIPATUS ACACIOI* MARCUS & MARCUS (ONYCHOPHORA) UNDER CONDITIONS OF EXPERIMENTAL IMMOBILISATION

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PERIPATUS ACACIOI
ONYCHOPHORE
TECHNIQUES D'ANESTHÉSIE
PERTES D'EAU

RÉSUMÉ. - Pour anesthésier l'onychophore *Peripatus acacioi* à des fins expérimentales diverses, nous avons utilisé le gaz carbonique, l'éther éthylique ou une basse température, au moyen de dispositifs prévus pour diminuer la perte d'eau par évaporation. Dans des conditions ambiantes standard de 19 °C et de 48 % HR, l'immobilisation par le froid a fourni les meilleurs résultats, la perte d'eau par minute correspondant à 0,40 % du poids initial des animaux, soit 30 % moindre que celle relevée avec les deux autres techniques. Les prélèvements d'hémolymphe sont facilités par une anesthésie légère à l'éther éthylique tandis qu'une narcose profonde par ce même produit, en diminuant le tonus musculaire, réduit les risques d'hémorragie. L'anesthésie par le gaz carbonique, qui provoque l'émission spontanée d'urine par les pores néphridiens, en favorise le prélèvement.

PERIPATUS ACACIOI
ONYCHOPHORA
METHODS OF ANAESTHESIA
WATER LOSS

ABSTRACT. - Carbon dioxide, ethyl ether and low temperature, used in conjunction with devices designed to reduce evaporative water loss, were employed to anaesthetise the onychophoran *Peripatus acacioi,* for various experimental purposes. Under standard environmental conditions of 19 °C and 48 % RH, cold-induced anaesthesia gave best results as water loss is reduced to 0.40 % of initial weight/minute, i.e. 30 % less than that recorded with the other techniques. Collection of haemolymph samples is facilited by light, ethyl ether-induced narcosis while deep narcosis, by reducing muscle tone, reduces risk of haemorrhage. Carbon dioxide-induced anaesthesia provokes spontaneous leakage of urine through the nephridiopores, facilitating sampling.

INTRODUCTION

Onychophorans are unable to control water loss by evaporation when maintained under ordinary laboratory conditions (Manton and Heatley 1937) or in experimentally induced, low relative humidity (Manton and Ramsay, 1937). Water evaporates from the animal at a constant rate and for a given temperature, the lower the relative humidity, the higher the rate of water loss (Dodds and Ewer, 1952). Water loss is therefore proportional to the saturation deficit of the air. Control of evaporation is thus an important factor to be considered during experiments examining water balance in onychophorans.

Preliminary data obtained on water loss under anaesthesia and during experimental handling of the onychophoran *Peripatus acacioi* led to the present investigation. Three techniques for immobilisation and their effects on *P. acacioi* are described.

MATERIALS AND METHODS

Peripatus (Macroperipatus) acacioi Marcus and Marcus, 1955, originating from stock collected in the Tripuí Valley (Lavallard *et al.*, 1975) near Ouro Prêto, State of Minas Gerais, Brazil, were raised in our laboratory according to techniques proposed by Lavallard and Campiglia (1975).

Three methods of anaesthesia were used depending on the duration of immobilisation required. Individual reactions to anaesthesia are also considered.

1. Carbon dioxide-induced anaesthesia

A small apparatus constructed from the lower half of a 500 ml Mariotte flask was used to deliver CO_2. The lateral flask aperture was sealed with a rubber stopper. An air stone, connected by a polyethylene tube running through the rubber stopper to a CO_2 reservoir, was placed on the flask bottom and covered with water. The flask mouth was covered with a regularly perforated stainless steel plate. CO_2 gas, flowing at 40-45 ml/min, was controlled by means of a manometer coupled to the reservoir. For delivery of the anaesthetic, a single animal was placed on the external surface of the plate and constrained with thin rubber bands. A Petri dish lined with wet cotton wool was then placed over the animal thus forming a humid chamber in which the onychophoran could be subjected to a variable mixture of CO_2, air and water vapour.

2. Ethyl ether-induced anaesthesia

The above system was also employed for ethyl ether anaesthesia, the polyethylene tubing being connected however, to a further, intact Mariotte flask containing cotton wool soaked in the ether. An air pump, coupled to this flask by a polyethylene tube attached to the lateral aperture provided a regulated air flow driving the ether, air and water vapour mixture to the chamber. By carefully controlling the flow and length of exposure to the gaseous mixture, gradual and lasting anaesthesia could be achieved.

3. Cold-induced anaesthesia

Lowering the body temperature of an onychophoran to 1-3 °C induces complete behavioural arrest. Cooling was provided by a Peltier principle, thermoelectric module (32 × 45 mm) coupled to a stereoscopic microscope stage and driven by an 8 A current from a 3 V generator. The animal, covered with wet cotton wool, was placed over the thermoelectric module regulated at 10 °C. The temperature was then slowly reduced to 2 or 1 °C.

Regardless of technique, from 1 to 3 minutes after complete arrest was attained, the wet cotton wool covering the animal was removed and the onychophoran then maintained for 5 minutes under standard environmental conditions (19 °C, 48 % relative humidity, i.e. under a saturation deficit 8.6 mm Hg). Water loss was determined by weight loss. Since onychophorans expel slime and saliva during the immobilisation process, they were placed on pre-weighed Parafilm sheets to determine weight loss due to the elimination of either secretion.

Initial weight W_1, weight after anaesthesia W_2, total weight loss $\Delta W = W_1 - W_2$ and loss due to slime and saliva S, were determined. Total water loss due to evaporation E was calculated from the equation $E = \Delta W - S$. All values are expressed in milligrammes. Values for E (mean ± SD) are given as milligrammes/minute and as a percentage of W_1/minute. Significant differences ($P \leqslant 0.05$) between means were calculated according to Student's t-test.

RESULTS

1. Carbon dioxide-induced anaesthesia

Table I summarises weight loss data for 10 animals of differing size after anaesthesia with CO_2. The mean weight loss due to evaporation was 1.37 ± 0.24 mg/min, equivalent to 0.60 ± 0.13 % of W_1/min. No correlation exists between initial weight (W_1) and the relative amount (%) of water lost by evaporation over the weight range used. Weight loss due to elimination of slime and/or saliva was detected in three animals but S was greater than E in only two, corresponding to 5.71 % of W_1 for the greater value.

Table I. – Weight loss (in mg) in *Peripatus acacioi* after anaesthesia by CO_2 and exposure to 19 °C and 48 % RH for 5 minutes. Definitions for Tables 1-3 : W_1, initial weight; W_2, final weight; $\Delta W = W_1 - W_2$; S, weight of slime and saliva; E, total water loss by evaporation; E/min, water loss by evaporation expressed in mg/min; E %/min, water loss by evaporation per minute, expressed as a percentage of W_1. Numbers below the last columns at right indicate means ± standard deviation.

W_1	W_2	ΔW	S	E	E/min	E%/min
134.6	122.1	12.5	6.6	5.9	1.18	0.88
182.1	176.4	5.7	–	5.7	1.14	0.63
212.1	207.0	5.1	–	5.1	1.02	0.48
237.6	228.2	9.4	0.9	8.5	1.70	0.71
244.2	237.0	7.2	–	7.2	1.44	0.59
246.5	240.1	6.4	–	6.4	1.28	0.52
257.0	250.5	6.5	–	6.5	1.30	0.51
266.1	242.1	24.0	15.2	8.8	1.76	0.66
280.3	272.9	7.4	–	7.4	1.48	0.53
295.1	288.2	6.9	–	6.9	1.38	0.47
					1.37	0.60
					±0.24	±0.13

2. Ethyl ether-induced anaesthesia

Weight loss in 10 animals subjected to ether vapour is displayed in Table II. The mean decrease in weight due to water loss by evaporation was 1.42 ± 0.21 mg/min or 0.62 ± 0.08 % of W_1/min. This value is not significantly different from that obtained when CO_2 was

used. In 50 % of the animals, S was a variable attaining high values in some cases; the highest S value was 9.03 % of W_1. As also noted in the CO_2-anaesthetised group, there was no correlation between initial weight and relative weight loss due to evaporation.

Table II. – Weight loss (in mg) in *Peripatus acacioi* after anaesthesia by ethyl ether and exposure to 19 °C and 48 % RH for 5 minutes.

W_1	W_2	ΔW	S	E	E/min	E%/min
186.6	180.5	6.1	–	6.1	1.22	0.65
191.8	182.0	9.8	4.2	5.6	1.12	0.58
210.0	202.4	7.6	–	7.6	1.52	0.72
221.2	201.8	19.4	11.6	7.8	1.56	0.71
228.8	212.8	16.0	8.5	7.5	1.50	0.66
231.0	219.8	11.2	3.7	7.5	1.50	0.65
235.1	229.4	5.7	–	5.7	1.14	0.48
255.8	249.2	6.5	–	6.5	1.30	0.51
264.0	256.0	8.0	–	8.0	1.60	0.61
274.4	246.5	27.9	19.3	8.6	1.72	0.63
					1.42	0.62
					±0.21	±0.08

3. Cold-induced anaesthesia

The value of S could not be determined for this group owing to water condensation on the Parafilm sheet introducing an uncontrollable weighing error. However, this error was reduced and may be negligible as the progressive decrease in temperature of the ventral surface of onychophoran caused tight apposition of the lips, sealing the mouth and impeding the elimination of saliva. Similarly, the animals do not eject slime under such conditions.

Table III displays evaporative weight loss under anaesthesia by cold. As also noted for CO_2 – and ethyl ether-induced anaesthesia, there is no correlation between W_1 and the relative weight loss due to evaporation under cold-induced anaesthesia. The mean evaporative weight loss in the present group was 0.67 ± 0.20 mg/min, equivalent to 0.40 ± 0.11 % of W_1/min. This value is significantly different from the results for the two preceding groups ($P < 0.001$). The lower evaporation noted in this group was probably due to a localised drop in the air saturation deficit induced by the low temperature.

Table III. – Weight loss (in mg) in *Peripatus acacioi* after cold immobilisation and exposure to 19 °C and 48 % RH for 5 minutes.

W_1	W_2	$\Delta W = E$	E/min	E%/min
117.9	115.4	2.5	0.50	0.42
154.6	150.5	4.1	0.82	0.53
157.8	156.2	1.6	0.32	0.20
158.1	155.6	2.5	0.64	0.40
166.1	162.9	3.2	0.64	0.40
176.2	171.5	4.7	0.94	0.53
179.5	175.4	4.1	0.82	0.46
185.9	183.4	2.2	0.44	0.24
196.3	192.1	4.2	0.84	0.43
201.9	198.2	3.7	0.74	0.37
			0.67	0.40
			±0.20	±0.11

DISCUSSION

Obtaining haemolymph and urine samples for analysis of ionic composition and determination of the inulin space in onychophorans necessitate immobilisation and anaesthesia (Campiglia, 1976, 1981). Owing to the absence of regulatory mechanisms preventing water loss in these animals, experimental procedures minimising evaporation during immobilisation are necessary. Of course a certain amount of water is always dissipated under such conditions. Anaesthesia by cold interferes least with water balance although total water loss is dependent on the duration of anaesthesia. Although only 5 minutes exposure to CO_2 or ether is necessary to reduce onychophoran body weight by 3 %, recovery, in a water saturated environment requires some 7 hours (Campiglia, 1981). Water loss of about 2 %, such as occurs during anaesthesia by cold is compensated for in about 5 hours under conditions of water saturation. These values define the minimal acceptable interval between the collection of successive haemolymph and urine samples.

Elimination of slime and saliva may contribute significantly to weight loss. This inconvenience is particularly relevant when ethyl ether-induced anaesthesia is used. Hence, the animals should be placed on removable, pre-weighed surfaces, e.g. Parafilm sheets or aluminium foil, allowing accurate weighing of eliminated material. With cold-induced immobilisation, slime ejection is avoided.

In the present study, the onychophorans weighed between 135 and 295 mg. No correlation was found between rate of water loss and weight within these limits. This result possibly indicates that water loss occurs across a surface which increases proportionally to volume, probably the tracheae as suggested by Manton and Ramsay (1937). Alternatively, the extent of such loss might depend on other parameters as yet unknown.

These above experiments were also repeated under common laboratory conditions, i.e. a saturation deficit of 7.9 mm Hg (20 °C and 55 % RH). The mean rates of water loss were: CO_2-induced anaesthesia, 0.38 ± 0.15 % W_1/min; ethyl ether, 0.35 ± 0.06 % W_1/min; cold, 0.27 ± 0.07 % W_1/min. These values are respectively 1.59, 1.77 and 1.48 times lower than those

for the previously described experiments. Water loss is therefore proportional to the saturation deficit of the air.

In experiments requiring manipulation of onychophorans, both the dehydrating effect of anaesthesia and also the intrinsic effects of the anaesthetic itself should be considered. The advantage of ethyl ether-induced anaesthesia is that its depth can be easily regulated by simply controlling the duration of application. One or two minutes exposure produces a brief, light narcosis from which the animals recover in a few minutes. Body wall tonus remains unaltered and haemolymph pressure therefore stays within normal limits. A mechanical stimulus provokes a peristaltic wave that favours haemolymph removal, i.e. a small incision in the dorsal vessel produces an intermittent haemolymph flow dependent on cardiac contractions and/or the peristaltic wave. When ethyl ether anaesthesia is prolonged to 3 or 4 minutes, deep narcosis is induced from which the onychophorans only very slowly recover, usually in not less than two hours. The body wall becomes flaccid due to a decrease in muscle tonus and haemolymph pressure consequently decreases (Campiglia and Lavallard, 1978) thus reducing the risk of haemorrhage.

Anaesthesia by CO_2 has been shown to provoke urine outflow through the excretory pores in onychophorans (Manton and Heatley, 1937; Lavallard and Campiglia, 1974). The animals appear stiff, the result of an increase in body wall muscle tonus. As a consequence, haemolymph pressure increases and its sampling is favoured.

CO_2-induced anaesthesia is usually less detrimental than that produced by ethyl ether. When properly dosed and considering the problem of excessive water loss, it may be employed successively at short intervals as the animals recover very quickly when the gas flow is interrupted. Rapid recovery is particularly well suited for experiments of short duration.

The factors underlying rate of water loss are complex; however, the saturation deficit of the air and the humidity gradient between the evaporating surface and the air play an important rôle. The experimental conditions necessary to induce anaesthesia by cold produced a decreased saturation deficit of the air near the animal, resulting in a weaker humidity gradient which would reduce water loss. Another factor possibly reducing evaporation rate could be reduced water loss from the respiratory pathways due to a decrease in metabolism as a result of the lower body temperature.

Anaesthesia by cold is additionally convenient because complete immobilisation occurs within a few seconds and provokes neither increase nor decrease in muscle tonus. Thus, neither external form nor haemolymph pressure change. The absence of peristaltic body waves is favourable for the injection of solutions as the risk of back-flow and subsequent fluid loss is decreased. After cooling is discontinued, anaesthesia rapidly disappears, without side effects, permitting successive periods of anaesthesia at short intervals. Although this is also possible with CO_2-induced anaesthesia, cold anaesthesia is more recommendable in view of the resultant lower water loss. The disavantage of cold anaesthesia is that the animal's ventral surface must be cooled directly below the nerve chord thus limiting manipulation to dorsal surface.

To maintain water balance during anaesthesia, an onychophoran should be kept in conditions presenting a slight water vapour saturation deficit and the body should, whenever possible, be partially surrounded by moist cotton wool.

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