1968 #34

PRELIMINARY STUDIES ON WATER EXCHANGE IN FRESH WATER LEECHES (Hirudinae)

Bodil Schmidt-Nielsen and H. D. Pagel, Case Western Reserve University, Cleveland, Ohio

Leeches can live for long periods in fresh water without food (up to 1-1/2 years). To maintain electrolyte and water balance they must take up electrolytes actively and excrete excess water. Krogh (1939) showed that the salt depleted horse leech Haemopsis sanguisuga actively takes up Na^+ as well as Cl^- ions from dilute media.

The excretion of water has not been investigated. The excretory organs consist of 10-17 pairs of nephridia located in the middle third of the body, one pair per body segment. The first six and the last pair of nephridia are not connected with the nephridiostome and presumably end blindly. The middle nephridia have several ciliated funnels imbedded in a pulsating blood sinus. After many convolutions the nephridium runs to the vesicle or bladder which opens to the nephridiopore on the ventro-lateral side.

To determine the filtration rate in the nephridia, leeches (1g body weight) were injected with tritiated inulin, 12.5 μC/g body weight and the rate of appearance of inulin in the surrounding bath was measured. The filtration rate was found to vary from 2-5 μl/g leech/hr. To determine the turnover rate for H_2O in fresh water, leeches were labeled with tritiated water by leaving them in tritiated water overnight (5 μC/ml H_2O). Each leech was then placed in a bath of 10 ml unlabeled pond water which was shaken almost constantly. 10 μl of the bathing fluid was removed every 2 minutes for the first 1.5 hr, then every 4 minutes for the next 1.5 hr. The rate constant for water turnover was determined from the exponential decrease between the count at time ∞ (the asymptote) and the count at time (t). The mean turnover rate for 10 leeches was found to be 336 ± 22% hr^{-1}. (The turnover rate was also determined in isosmotic saline and was found to be 597 ± 42% hr^{-1}. This higher rate is probably due to the unphysiological conditions.) The percentage of body water in the leeches was 84.4 ± 0.4%. Thus, the unidirectional water fluxes ϕ_{in} and ϕ_{out} per gram leech were 2.84 ml hr^{-1}.

Then: $\phi_{in} = P_d A C_{w_{out}}$ and $\phi_{out} = P_d A C_{w_{in}}$

Where $C_{w_{out}}$ and $C_{w_{in}}$ represent the concentration of water on the outside and inside of the leech respectively. P_d is the diffusion permeability of the leech, A is the area, and C_w is the concentration of water 55.6 M/l. If we assume only diffusional water flux into the leech and no bulk flow with an osmotic gradient, and if we assume the area A to be constant per gram body weight we can estimate an approximate minimum net-influx through the cuticle (ϕ_{net}):

$$\phi_{net} = P_d A (C_{w_{out}} - C_{w_{in}})$$

$$C_{w_{out}} - C_{w_{in}} \approx \Delta C_{os}$$

Thus, with an osmolality of 10 mOs in the pond water and 230 mOs in the blood of the leech ΔC = 0.220 Os.

Therefore: $\phi_{net} = \frac{2.84}{55.6} \times 0.220$ ml/hr = 11.2 μl hr^{-1} per gram leech.

This value exceeds the filtration rate by a factor of about 3. Therefore if these preliminary results are valid more fluid is excreted than that which is filtered. This is quite possible since many nephridia have no nephridiostome and presumably must function by tubular secretion.

Techniques for micropuncture studies in vivo of the nephridia were developed. Analysis of nephridial fluid will be made in later experiments.

Supported by NIH, Grant No. AM-09975-03.

1968 #35

THE MISSING MILLIEQUIVALENT–H^+ BALANCE DURING METABOLIC ALKALOSIS IN THE DOGFISH, Squalus acanthias

Jan Smith, H. V. Murdaugh, and Eugene D. Robin, Department of Medicine, University of Pittsburgh, Pittsburgh, Pa.

Previous studies have shown that following a bolus intravascular injection of $NaHCO_3$ in Squalus acanthias there is a rapid increase in blood pH and HCO_3^- concentration followed by a rapid progressive return to normal values over several hours (Bull. MDIBL 4-4:75, 1962). This return to normal is not accompanied by changes in urinary pH nor is it quantitatively accounted for by intracellular buffering. The present studies were designed to define the quantitative kinetic processes responsible for the return to normal acid-base values and to attempt to define the anatomical sites of buffering and HCO_3^- excretion. Six animals were studied. The rate of restoration of normal acid-base values was estimated by sequential measurements of arterial pH, pCO_2 and HCO_3^- concentrations. Gill net H^+ excretion and renal net H^+ excretion were measured as previously described.

Restoration toward normal values occurs at a rate consistent with approximately 1.0 to 1.5 mEq/Kg/hour HCO_3^- loss. Urinary pH remained unchanged and preliminary studies of urinary titratable acid and ammonia excretion do not account for the observed HCO_3^- loss. Direct penetration of coelomic fluid by HCO_3^- occurred, but buffering in this compartment accounted for less than 0.25 mEq. Preliminary studies of gill HCO_3^- excretion do not indicate substantial changes during control versus HCO_3^- loading periods. Measurements of renal and gill TMA and TMAO are now in process.

Although gill excretion is possible, the precise locus of this regulation has not been established and approximately 1 mEq of HCO_3^-/hour is still missing.

1968 #36

THE DEVELOPMENT OF APOLAR EMBRYOS OF Fucus vesiculosus L. IN SUCROSE-SEAWATER

John G. Torrey and Harry L. Phillips, Jr., Harvard University, Cambridge, Mass.

Using cytological methods developed over the past several years, it has been possible to prepare squash preparations of Fucus embryos in which accurate nuclear counts can be made of developmental stages up to about ten days following fertilization. These data provide a quan-