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The pH and ionic composition of the sub-embryonic fluid of the Japanese quail (*Coturnix c. japonica*)

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Latter & Baggott (2002) proposed a pivotal role for carbonic anhydrase in the production of sub-embryonic fluid (SEF) by the blastoderm of the Japanese quail. Located on the endothelial cell membrane this enzyme would ensure SEF hydrogen ions entered cells in exchange for bicarbonate ions; a process powered by a Na/K ATPase on the same membrane. However, the current theory of acid-base chemistry regards $[H^+]$ as a dependent variable (Stewart, 1981): changes in pH of a fluid can only occur by alteration of strong ion concentrations ($[Na^+]$, $[K^+]$, $[Cl^-]$). The objective of this study was to determine whether manipulation of SEF strong ion composition would alter SEF $[H^+]$ in the direction predicted by theory.

Eggs were incubated at 37.6°C for 54h, explanted into culture vessels and further incubated for 48h (*in vitro*). At this time albumen Na, K and Cl concentrations were measured. SEF was then sampled, pH measured at 37.6°C, and the ionic composition (Na, K, Cl,) and total CO_2 assessed. In addition, some eggs were incubated at 37.6°C for 102h and SEF pH and composition measured (*in ovo*). The concentrations of unmeasured organic anions (A^-), HCO_3^-, CO_3^{2-} and P_{CO2} were estimated by solving 6 simultaneous equations relating these and measured parameters using Maple 9.5 (Maplesoft). At time of explantation the albumen of cultured embryos were also subject to the following treatments: (*1*) explantation control where albumen was exchanged between embryo pairs (albumen Na 0.080M, K 0.060M, Cl 0.060M) (n=6);(*2*) low K albumen (0.016M) produced by replacing most albumen with 0.075M Na and Cl, balance sucrose, (osmolality 240 mOsmole/kg) (n=9); (*3*) high Cl albumen (0.110M) produced by replacing most albumen with 0.075M Na, 0.045M K and 0.120M Cl (osmolality 240 mOsmole/kg) (n=7). For both (*2*) and (*3*) the changes in SEF K and Cl concentrations of albumen were predicted to increase $[H^+]$ of SEF to maintain electrical neutrality.

Cultured embryos did not differ ($P>0.05$) in SEF pH (Figure) or P_{CO2} from those *in ovo* (n=52 *in vitro* 8.8 Torr; n=9 *in ovo* 9.1 Torr). Similarly, SEF ionic concentrations did not differ (*in vitro* Na 0.102M, K 0.018M, Cl 0.063M, HCO_3^- 0.018M, A^- 0.047M; *in ovo* Na 0.104M, K 0.016M, Cl 0.064M, HCO_3 0.015M, A^- 0.041M). Likewise, albumen exchange (*1*) had no effect ($P>0.05$) on pH or ionic composition of SEF. Low K albumen significantly ($P<0.05$) reduced SEF K to 0.009M and A^- by 21%, whilst increasing Cl by 29%; SEF Na and HCO_3^- did not change. SEF was acidified with a substantial proportional reduction in A^- (Figure). High Cl albumen also acidified SEF (Figure) with a significant ($P<0.05$) increase in Cl (44%) and a decrease in A^- (35%); again SEF Na and HCO_3^- did not change. High Cl albumen did not alter SEF cations apart from the increase in $[H^+]$; the biggest response was a reduction in the proportion of A^- (Figure). As predicted, changes to SEF strong ion concentrations decreased pH in treatments (*2*) and (*3*) and the changes in SEF Cl suggest a passive distribution. Also, changes in A^- suggest an essential role for organic anions in acid-base chemistry of SEF. It was notable that both Na and HCO_3^- were unaffected by the treatments emphasising the importance of these two ions in fluid production by the blastoderm.

References

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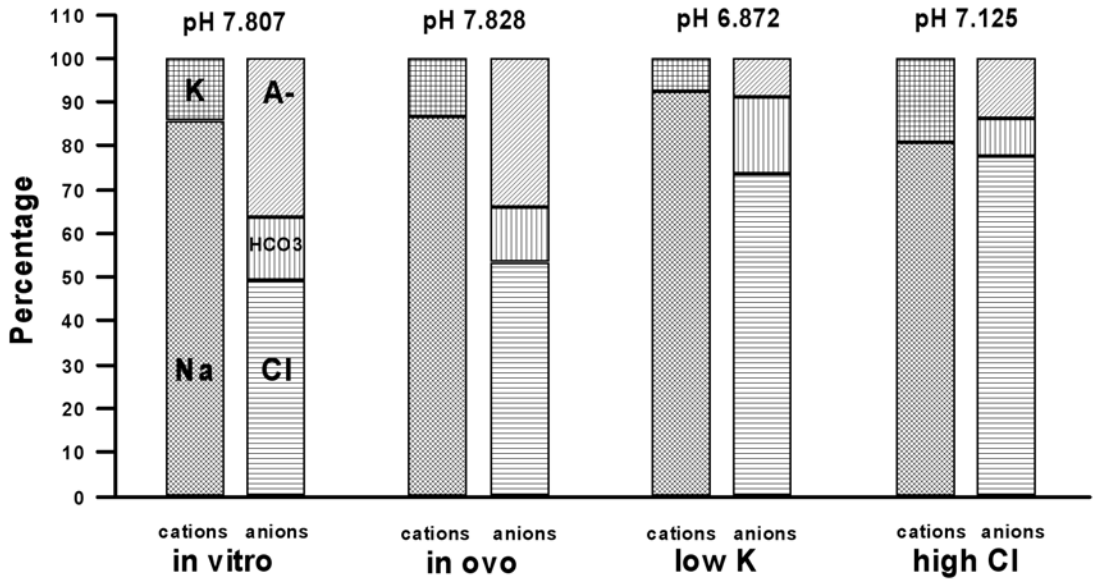

Figure. Gamblegrams for SEF from Japanese quail embryos after 102h of incubation. The *in vitro* group was cultured for 48h with native albumen; low K group cultured 48h with 0.075M Na and Cl, balance sucrose; high Cl group cultured 48h with 0.075M Na, 0.045M K, 0.120M Cl; *in ovo* group SEF from eggs incubated for 102h.