Table II. Common and distinctive characteristics of carboxylic ester hydrolase bands A and B

Carboxylic ester hydrolase bands	Acetyl esters hydrolyzed[a]	Butyryl esters hydrolyzed[a]	Heat inactivation (60 °C)[a]	DFP inhibition (10^{-4} *M*)[a]	Approximate M. W.
A	+	—	—	—	52,000
B	+	+	+	—	63,000

+, Esterase activity; —, no activity. [a] Previous results obtained from 25 strains of *E. coli* by zymogram procedure in acrylamide-agarose gel[1].

Moreover, the esterase bands A of K_{12} and HB_{10} have negative charges greater than HB_{14} and HB_{18}; the esterase bands B of K_{12} and HB_{14} have negative charges greater than HB_{10} and HB_{18}. The comparison of the slopes of the esterase lines with those obtained using reference proteins allowed an approximate estimate of molecular weights (Figure 2). The values were about 52,000 daltons (± 5%) for esterase band A and 63,000 daltons (± 5%) for esterase band B.

In conclusion, the results obtained by molecular sieving effect in polyacrylamide gel electrophoresis demonstrate that the carboxylic ester hydrolases A and B of *E. coli* are distinct in molecular weight. The variations in esterase mobility among the strains appear to be the consequence of differences in molecular net charge. The two molecular weight patterns supplement the characteristics obtained previously (Table II).

Résumé. Des électrophorèses à diverses concentrations d'acrylamide montrent que les carboxyliques esters hydrolases A et B d'*E. coli* possèdent des poids moléculaires distincts: 52,000 et 63,000 daltons. Les variations de mobilité observées selon les souches proviennent essentiellement de différences dans les charges électriques.

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Calcium and pH Homeostasis in the Snail (*Helix pomatia*): Effects of CO_2 and $CaCl_2$ Infusion

When snails (*Helix aspersa*[1] and *H. pomatia*[2]) are exposed to 5–10% CO_2, the concentrations of bicarbonate and calcium in the haemolymph rise in approximately 2:1 ratio and reach new steady levels in 2–6 h, with negligible change in sodium, potassium and magnesium. With the importance of ionic balance to cellular function in mind, we have studied the resulting relationship between pH and free calcium. The results indicate an unexpectedly slow rise in carbon dioxide tension and also an apparent homeostasis of the ionic product $[Ca^{++}] \cdot [CO_3^{=}]$ at values well above the solubility products for calcium carbonate.

Materials and methods. Hydrated, fasted snails (*H. pomatia*) were exposed to 5–10% CO_2 in O_2 or infused with 150 m*M* $CaCl_2$. Haemolymph was drawn from cannulae tied into the optic tentacles[2] for the determination of pH[1] and total calcium[2]. Concentrations of free calcium were calculated on the assumption that 1 m*M* of the total was bound to haemocyanin (Figure 2) and that the only other bound calcium was that of the ion pair $CaHCO_3^+$. In the experiments on the effects of carbon dioxide, it was assumed, for the calculation of concentrations of $CaHCO_3^+$ and also $CO_3^=$, that the total bicarbonate concentration, including free HCO_3^-, $CaHCO_3^+$ and $MgHCO_3^+$, was initially a typical 30 m*M* and that it rose with total calcium in 2:1 ratio. The dissociation constants of $CaHCO_3^+$ and $MgHCO_3^+$ were both taken[3] as 160 m*M* and the concentration of magnesium was taken as 10 m*M*. In the infusion experiments the concentrations of ionized bicarbonate were calculated from the pH of samples equilibrated at 20 °C with 2% CO_2[2]; the carbon dioxide tensions in vivo were calculated from this and the in vivo pH.

Results and discussion. Figure 1 shows, as a representative example, the time course of the changes in total and free calcium and in $CaHCO_3^+$ and pH in the haemolymph of a snail exposed to 8.7% CO_2. Figure 2a shows the relationship between pH and ionized calcium for 7 snails equilibrating with 5–10% CO_2. During these changes, the pH mostly falls as the concentration of calcium rises and, since the level of bicarbonate rises along with that of calcium, it follows that the tension of carbon dioxide increases with similar time course – rather than, say, stabilizing in 10–20 min as in Man. The delay of several hours involved in the attainment of the new steady state is therefore largely due to slow entry of carbon dioxide (most of which becomes bicarbonate) rather than to an inherent slowness in bicarbonate generation. At normal rates of metabolism[5], most of the accumulating carbon dioxide could in any case be metabolic.

The averaged initial and final values of pH and ionized calcium for the same 7 snails are shown in Figure 2b. The continuous curve corresponds to a constant ionic product $[Ca^{++}] \cdot [CO_3^{=}]$, of 3.6 m*M*[2], chosen to be the average pertaining in the snails while still in air. The near-constancy of the ionic product in each snail suggests that

[1] R. F. BURTON, Comp. Biochem. Physiol. *37*, 193 (1970).
[2] R. F. BURTON, Comp. Biochem. Physiol., in press.
[3] I. GREENWALD, J. biol. Chem. *141*, 789 (1941).
[5] H. WESEMEIER, Z. vergl. Physiol. *43*, 1 (1960).

Mean values (± standard deviations) of total calcium, ionized bicarbonate, pH and PCO_2 in the haemolymph of 4 snails just before and just after infusion with 1 ml of 150 m*M* $CaCl_2$, and also 3.4–6.3 h later (final samples)

	Before infusion	After infusion	Final sample
Total calcium (m*M*)	10.5 ± 3.4	20.7 ± 1.8	14.7 ± 1.5
Ionized bicarbonate (m*M*)	26.0 ± 4.9	20.8 ± 4.2	16.5 ± 2.6
pH	7.77 ± 0.05	7.65 ± 0.06	7.60 ± 0.02
PCO_2 (mm Hg)	14.1 ± 2.0	14.3 ± 1.1	13.1 ± 1.8

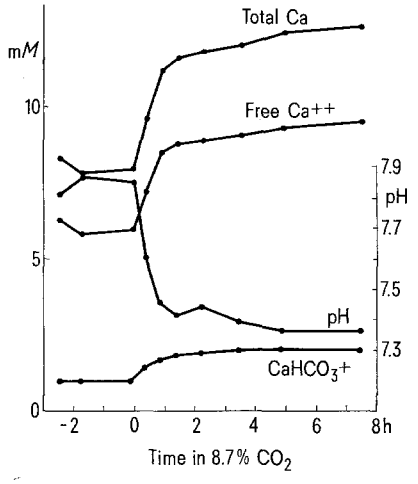

Fig. 1. Changes in total and free calcium and in $CaHCO_3^+$ and pH in haemolymph of a hydrated snail placed in 8.7% CO_2 in O_2 at time zero.

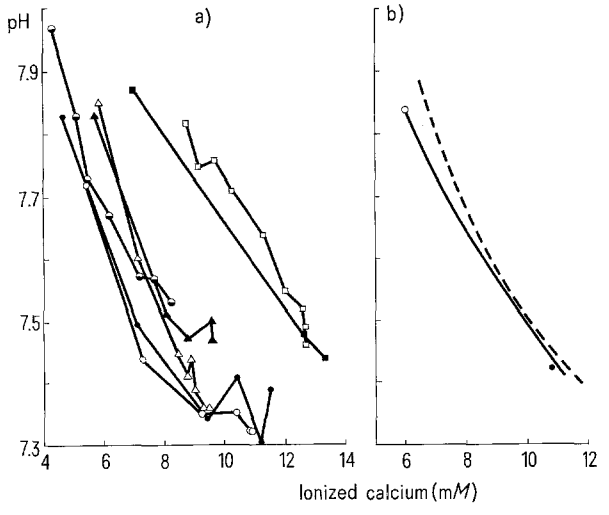

Fig. 2 (a). Relationships between pH and ionized calcium for 7 snails equilibrating with 5–10% CO_2 in O_2. (b) Average initial (○) and final (●) values of pH and ionized calcium for the same snails. Initial concentrations of total calcium averaged 8.3 ± *s.d.* 2.3 m*M*. The continuous curve represents the relationship between pH and $[Ca^{++}]$ corresponding to a constant value of $[Ca^{++}] \cdot [CO_3^=]$. This is 3.6 m*M*[2] if pK_2' for carbonic acid is taken as 9.50. The broken curve represents the relationship between pH and $[Ca^{++}]$ that is needed if the amount of calcium bound by haemocyanin[4] in a 2% solution (containing also 10 m*M* magnesium) is to remain constant at 1 m*M*.

the haemolymph could be in equilibrium with solid calcium carbonate, but in fact it is highly supersaturated with respect to both the common forms, aragonite and calcite, and can remain so because of the presence of phosphate and perhaps other crystal poisons that prevent nucleation. (The solubility product for calcite at 20 °C and the ionic strength of snail haemolymph is approximately 0.1 m*M*[2], while that of aragonite is about 60% higher[6]). There is so much calcium carbonate about the body that there presumably exists some body fluid that is in equilibrium with it. If bicarbonate and calcium ions could readily move from the haemolymph into this fluid and there precipitate, then the concentrations in the haemolymph would quickly fall. This could be prevented by an opposing active transport of one or more of the following ions: Ca^{++}, H^+, OH^-, HCO_3^- and $CO_3^=$. To explain the constancy of $[Ca^{++}] \cdot [CO_3^=]$ in the haemolymph, one need now only postulate that the mechanism of ion transport operates to maintain a constant electrochemical potential gradient for the transported ion(s) – as can the sodium pump in rat diaphragm[7] – and that the other ions are in equilibrium.

Calcium and hydrogen ions have opposite effects on the membrane potential of a particular neurone in the brain of *H. pomatia* and CHRISTOFFERSEN[8] has suggested that these effects might provide a stabilizing mechanism in acidosis. Figure 2b shows that ionized calcium rises on average by 11.5 m*M* per pH unit and this compares well with the value that is needed to prevent changes in resting potential; to judge from CHRISTOFFERSEN'S Figures 1c and 6b, this is 6–13 m*M* per pH unit.

Four snails were each infused through their cannulae, without apparent ill effect, with 1 ml of 150 m*M* $CaCl_2$ over 9 min. Haemolymph was sampled just before this and again 11–16 min after the infusion and 2–4 more times over the next 3.4–6.3 h. Haemolymph bicarbonate was diluted by the infusate and, since PCO_2 was little changed, the pH fell (Table). Bicarbonate concentrations then fell further to new steady values, while calcium concentrations fell without stabilizing. As previously found[2], bicarbonate (total) and calcium left the haemolymph in less than 2:1 ratio (mean ratio = 0.93:1). Values of $[Ca^{++}] \cdot [CO_3^=]$ rose as a result of the infusions, but always eventually fell below pre-infusion values, being lower on average then by 31 ± s.d. 16%. If the same mechanism operates here, in reverse, as in respiratory acidosis, it seems that calcium must also leave the haemolymph by another mechanism not involving bicarbonate.

Résumé. Dans l'hémolymphe des escargots (*Helix pomatia*) traités avec le CO_2 le calcium ionique augmente et le pH diminue de telle façon que le produit $[Ca^{++}] \cdot [CO_3^=]$ ainsi que l'association du calcium avec l'hémocyanine restent à peu près constant. Le temps requis pour atteindre une situation stable est reglé par l'entrée lente du CO_2. Après injection de $CaCl_2$, $[Ca^{++}]$ et $[HCO_3^-]$ diminuent, ainsi que le produit $[Ca^{++}] \cdot [CO_3^=]$.

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[6] J. M. EDMOND and J. M. T. M. GIESKES, Geochim. cosmochim. Acta *34*, 1261 (1970).
[7] H. A. FOZZARD and D. M. KIPNIS, Science *156*, 1257 (1967).
[8] G. R. J. CHRISTOFFERSEN, Comp. Biochem. Physiol. *46A*, 371 (1973).
[4] R. F. BURTON, Comp. Biochem. Physiol. *41A*, 555 (1972).