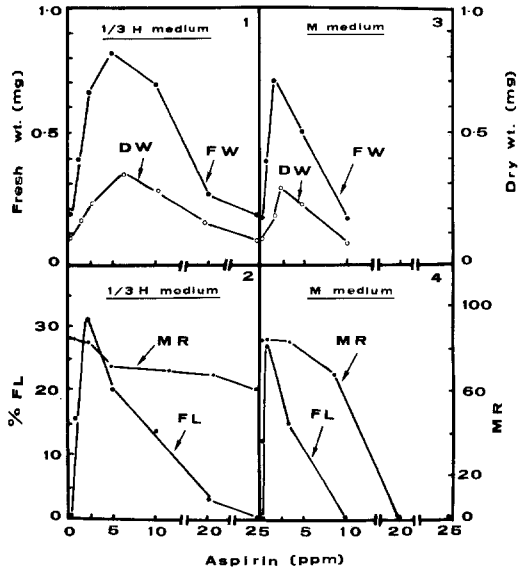
the size of the fronds. The fresh and dry weights of the plants also increased considerably in 1/3 strength HUTNER's medium containing 5 ppm of aspirin and in M-medium supplemented with 1 ppm of aspirin. The former increased by 300% in 1/3 HUTNER's medium and 250% in M-medium; the latter increased by 200% in 1/3 HUTNER's medium and 150% in M-medium (Figures 1 and 3).



Figs. 1–4. Effects of different concentrations of aspirin on fresh and dry weights of *Lemna gibba* G3 cultivated on 1/3 strength HUTNER's medium (Figure 1) and M-medium (Figure 3). Note the multiplication rate (MR) and flowering (Fl) in the presence of various concentrations of aspirin in 1/3 HUTNER's medium (Figure 2) and M-medium (Figure 4).

The most interesting effect of aspirin, however, was on the initiation of flowering under long-day conditions. In 1/3 strength HUTNER's medium, *Lemna gibba* G3 plants continued to grow and multiply but did not flower. When these plants were subcultured on a similar medium supplemented with aspirin (5 ppm) the flowering was discernible 4-5 days after inoculation. The maximum percentage of flowering plants, i.e. 33% was obtained in a medium containing 2.5 ppm of aspirin while 16%, 20%, 15% and 4% flowering plants were recorded at 1, 5, 10 and 20 ppm respectively (Figure 2). No flowers were produced in 25 ppm aspirin. At higher concentrations (50, 100 and 250 ppm), aspirin proved toxic and plants failed to grow. Aspirin's effects were similar on plants grown in M-medium, but occurred at slightly lower concentrations. Significant flowering took place even at 0.1 ppm while the maximum was attained at 1 ppm (Figure 4). In this nutrient medium 20 and 25 ppm of aspirin proved toxic. The plants did not multiply at these concentrations and died 2 days after inoculation.

This is the only report where aspirin has been demonstrated to induce flowering. Since aspirin is considered to be a copper-chelating drug, we believe that the metal which influences flowering in *L. gibba* G3 is most likely copper. These conclusions are in general agreement with our earlier observations[3,6]. However, the mechanism by which copper regulates flowering remains to be investigated. This and other related questions are under study and results will be reported subsequently.

Zusammenfassung. Die Zugabe von Aspirin in geringen Konzentrationen (ppm-Bereich) zur Nährlösung der Wasserlins (*Lemna gibba* G3) beeinflusst das Wachstum sowie die Vermehrung und induziert die Blütenbildung.

P. R. BHALLA and P. S. SABHARWAL

Department of Botany, University of Kentucky, Lexington (Kentucky 40506, USA), 12 December 1974.

Molecular Weight Estimation of two Carboxylic Ester Hydrolases of *Escherichia coli*

Two principal esterase bands designated as A and B in order of decreasing mobility and distinguishable by the extent of their hydrolyzing activity have been separated from cellular extracts of *Escherichia coli* by horizontal slab electrophoresis in polyacrylamide-agarose gel[1].

The aim of this work was to estimate the molecular weight (M.W.) of these esterases (carboxylic ester hydrolase, E.C.3.1.1.) in strains differing by esterase electrophoretic pattern by molecular sieving effect in polyacrylamide disc-electrophoresis.

Materials and methods. Of 25 strains previously examined for esterase pattern K_{12}, HB_{14}, HB_{10} and HB_{18} were selected for further study. The bacteria were grown on minimal salt medium supplemented with glycerol and were harvested during the logarithmic phase. The cellular extracts were prepared by sonic treatment. Details have been previously described[1].

The molecular weights were investigated by disc-electrophoresis according to the method described by HEDRICK and SMITH[2] who have shown that when the acrylamide concentration of gel changes, the electrophoretic mobility of a globular protein varies as a function of its molecular weight.

The buffers and gel solutions were the same as those described by DAVIS[3]. Gel columns were formed in glass tubes 80 mm long with an internal diameter of 4 mm. For small pore gel, the *bis* acrylamide/acrylamide weight ratio was kept constant at 1/30 and the concentrations of acrylamide varied from 6% (w/v) to 11%. Each run contained the same small pore gel concentration. The following reference proteins were used for the establishment of the calibration curve. Bovine serum albumin (Pentex), M.W.: monomer, 67,000; dimer, 134,000; trimer, 201,000[4]; Ovalbumin (Worthington), M.W.: monomer, 43,500; dimer, 87,000[5]; β-lactoglobulin (Serva), M.W.: 35,000[5].

The sample gel solutions were composed of 0.15 ml of large pore solution and 5 µl of sample containing 250 µg of protein for crude extracts of *E. coli*, 5 µg for monomer and dimer of bovine serum albumin, 100 µg for trimer of bovine serum albumin, 5 µg for monomer of ovalbumin, 200 µg for dimer of ovalbumin, 10 µg for β-lactoglobulin.

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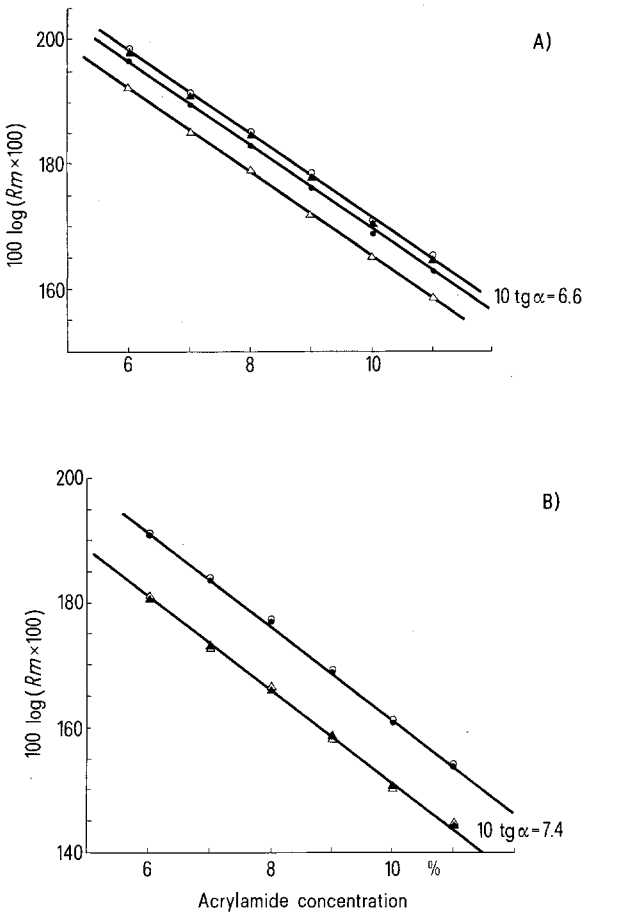

Fig. 1. Plot of the log. of the esterase mobility versus gel concentration. ○, strain K_{12}; ●, strain HB_{14}; △, strain HB_{18}; ▲, strain HB_{10}. A), esterase bands A; the lines of esterase bands of K_{12} and HB_{10} are practically superposed. B), esterase bands B; upper: superposed lines of esterase bands B_1 of K_{12} and HB_{14}; lower; superposed lines of esterase bands B_2 of HB_{10} and HB_{18}. The negative slopes are noted on the figure.

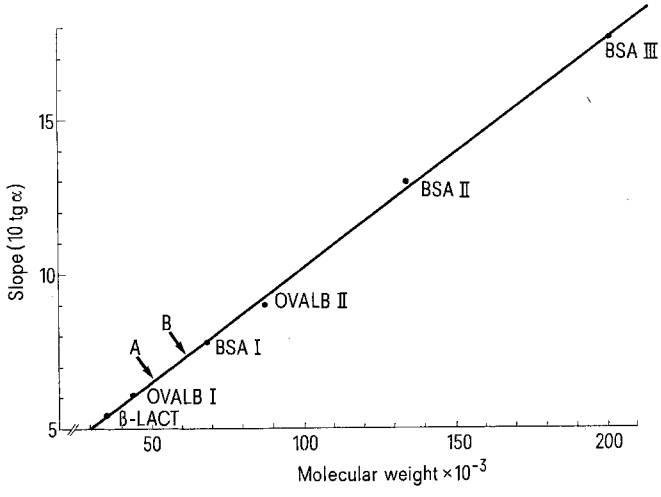

Fig. 2. Plot of the negative slopes versus the molecular weight of reference proteins. BSA I, II and III: bovine serum albumin monomer, dimer and trimer. OVALB I and II: ovalbumin monomer and dimer. β-LACT: β-lactoglobulin. A: esterase band A. B: esterase band B.

The bromophenol blue was the tracking dye. Electrophoresis was performed at room temperature in a model 1200 apparatus from Canalco. The current was adjusted to about 2 mA per tube until the dye front had migrated into the small pore gel, and then a constant current of 5 mA per tube was maintained until the dye front had migrated to 40 mm. After electrophoresis, the dye front was marked by insertion of metallic wire through the gel. Every effort was made to insure that all experimental conditions were constant and reproducible.

The esterases were stained with mixture containing 30 mg of indoxyl acetate (Sigma) in 3 ml of acetone and 150 ml of 0.15 *M* phosphate buffer pH 7. After 60 min of incubation at room temperature with agitation, the gels were rinsed overnight in water. The reference proteins were visualized with Amidoblack 10 b.

The relative mobility (R_m) of esterases or reference proteins is the ratio (%) between the distance of protein band and the distance of dye front. For each concentration of acrylamide, the R_m value of esterases and reference proteins was the average obtained from 4–6 runs. To obtain molecular weight estimates for esterases, the relative mobilities in gels of different acrylamide concentrations were compared with the mobilities of proteins of known molecular weight[2,5–8].

Results and discussion. Table I shows the esterase mobilities obtained by disc-electrophoresis using 7% (w/v) acrylamide. The esterase band A of HB_{18} migrated as the esterase band B or K_{12}. The esterase bands B presented 2 types of mobility: B_1 (fast mobility) for K_{12} and HB_{14}; B_2 (slow mobility) for HB_{10} and HB_{18}. These results confirm the data previously obtained by horizontal slab electrophoresis[1].

Figure 1 (A) and (B) indicates the effect of variations of acrylamide concentrations on the esterase mobilities. The plots of 100 log ($R_m \times 100$) versus gel concentration resulted in straight lines. As pointed out by HEDRICK and SMITH[2] parallel lines reveal occurrence of proteins of identical size but different charge, and non parallel lines reveal existence of proteins of different size; the negative slope (10 tg α) of the lines is related to molecular size. Thus, the parallel or superposed lines from the Figure 1 show that there is not a significant difference in molecular size between esterase band A of HB_{18} and those of other strains. The same is true for esterase bands B_1 and B_2. As is noted on the Figure, the negative slope of the bands B is larger; the molecular size of esterase bands B is then greater than that of esterase bands A.

Table I. R_m values obtained by disc-electrophoresis using 7% acrylamide

Carboxylic ester hydrolase bands	Escherichia coli strains			
	K_{12}	HB_{14}	HB_{10}	HB_{18}
A	82	78	81	71
B	71	69	54	53

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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.

PH. GOULLET[9,10]

Laboratoire de Microbiologie, Faculté de Médecine Xavier-Bichat, Université de Paris VII, 21, rue de l'Ecole de Médecine, F-75270 Paris-Cedex 06 (France), 14 October 1974.

[9] With technical assistance of Madame DANIELLE BILLOT.
[10] Service de Microbiologie, Hôpital Beaujon, 100, Boulevard du Général Leclerc, F-92110 Clichy, France.

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

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