Table II. Influence of artificial transfer of spermatophore on the duration of survival, egg production and oviposition of the cricket *P. guttiventris* during starvation

Treatment	No.	Duration of survival (days) (Mean ± SE)	No. of eggs produced (Mean ± SE)	Oviposition (%) (Mean ± SE)
Starved virgin females	20	6.1 ± 0.46^{a}	141.9 ± 18.3^{c}	0.0
Fed virgin females	18	—	194.4 ± 14.5^{d}	5.69 ± 4.8^{g}
Starved females receiving spermatophores artificially	20	8.2 ± 0.57^{b}	85.0 ± 20.9^{e}	11.72 ± 6.8
Fed females receiving spermatophores artificially	20	—	174.9 ± 33.0^{f}	15.99 ± 6.7^{h}

Comparison of means (t-test): $^{a-b}$, $p < 0.01$; $^{c-d}$ and $^{c-e}$, $p < 0.05$; $^{d-f}$ and $^{g-h}$, $p > 0.05$, NS.

Results of this experiment, presented in Table II, interestingly showed increased duration of survival and decreased egg production in starved females that had received spermatophores as against starved virgin females. These results further confirmed the earlier observation that females receiving spermatophores, under normal feeding conditions, did not show any difference in egg production or oviposition from those of virgin females.

Hence, it is apparent that such a transfer of spermatophore to the females, though lacking the component for triggering oviposition, could still increase survival duration during starvation. It is, therefore, obvious that 2 separate components are involved in expression of the dual role of mating, which in itself is unique and noted by us for the first time, in *P. guttiventris*. However, the exact nature of these components, at present, can only be speculated and hence is of interest for further studies[8].

Zusammenfassung. Bei der Kopulation der Grille *Plebeiogryllus guttiventris* werden mindestens 2 unabhängige Stimuli wirksam, welche Oviposition und Eiproduktion beeinflussen. Weder künstliche Spermatophorenübertragung noch Kopulation mit kastrierten Männchen stimuliert die Oviposition; letztere bewirkt aber erhöhte Eiproduktion. Künstliche Übertragung von Spermatophoren in hungernde, virgine Weibchen reduziert die Eiproduktion und bewirkt eine Lebensverlängerung.

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Induction of Flowering in *Lemna gibba* G3 by Aspirin

Several billion tablets of aspirin are used all over the world every year to relieve headaches and other pains, reduce fever and deal with a wide variety of ailments. The prevailing hypothesis is that in these disorders, the amount of copper in blood rises to two or more times the normal level. Simple removal of excess copper from blood does not relieve the pain or reduce the fever. Aspirin probably acts as a chelate, repairing this biochemical 'lesion' by picking up copper from the blood and returning it to the cells from which it was lost[1]. Studies by HILLMAN[2] and in our laboratory[3] demonstrated that copper somehow influences the photoperiodic sensitivity of a duckweed, *Lemna gibba* G3[2,3]. More recently, we have been able to induce flowering in *L. gibba* G3 by adding aspirin to the nutrient medium.

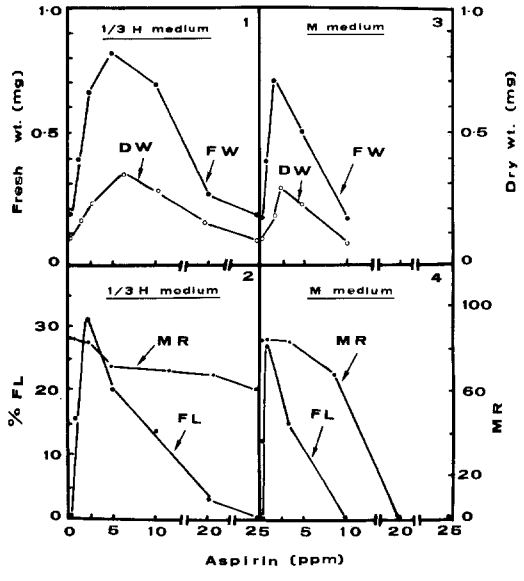
The plants of *Lemna gibba* G3 were aseptically cultured on M-medium[4] and 1/3 strength HUTNER's medium[5]. The methods for raising aseptic cultures, light and temperature conditions, determination of multiplication rate (MR) and evaluation of flowering were the same as described in our earlier papers[3,6]. Aspirin, manufactured by the Bayer Company, Division of Sterling Drug, Inc. New York, N.Y. 10016, was utilized in the present investigation.

The plants grew in 1/3 HUTNER's and M media. Incorporation of aspirin in both media influenced the MR of the fronds. In 1/3 strength Hutner's medium, the MR slightly declined at concentrations of 0.1, 1.0, 2.5 and 5 ppm but at higher concentrations (10, 20 and 25 ppm) no further decrease was observed. In M-medium, MR was not influenced by the presence of low amounts of aspirin (0.1, 1.0 and 5.0 ppm) but at 10 ppm and 20 ppm no growth occurred. The plants became yellow and died within 2 days after inoculation.

Besides its effects on MR, aspirin had pronounced influences on the growth and development of individual fronds. The effects were detectable within 4 days; magnitude of the effects varied with the concentration of aspirin. Plants were larger in 1/3 strength HUTNER's medium containing 0.1, 1.0, 2.5 and 5 ppm of aspirin. In M-medium an increase in the size of the fronds was apparent at 0.1, 1.0 and 2.5 ppm of aspirin. Higher concentrations (10, 20 and 25 ppm in HUTNER's medium, and 5 and 10 ppm in M-medium) resulted in a decrease in

[1] J. SCHUBERT, Scient. Am. *213*, 40 (1965).
[2] W. H. HILLMAN, Am. J. Bot. *49*, 892 (1962).
[3] A. H. PIETERSE, P. R. BHALLA and P. S. SABHARWAL, Pl. Cell Physiol. *11*, 463 (1970).
[4] W. H. HILLMAN, Am. J. Bot. *48*, 413 (1961).
[5] S. H. HUTNER, in *Growth and Differentiation in Plants* (Ed. W. E. LOOMIS; Iowa State College Press, Ames, Iowa 1953), p. 441.
[6] A. H. PIETERSE, P. R. BHALLA and P. S. SABHARWAL, Pl. Cell Physiol. *11*, 675 (1970).

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.

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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.

[1] PH. GOULLET, J. gen. Microbiol. *77*, 27 (1973).
[2] J. L. HEDRICK and A. J. SMITH, Arch. Biochem. Biophys. *126*, 155 (1968).
[3] B. J. DAVIS, Ann. N.Y. Acad. Sci. *121*, 404 (1964).
[4] J. ZWAAN, Analyt. Biochem. *21*, 155 (1967).