ment. We believe, therefore, that the catch in the trap is a minimum amount. Bottom currents at the time of collection varied from 1 to 28 cm/sec, with an average current velocity of 6 cm/sec.

If the data are accepted with the reservations outlined, one can draw the following tentative conclusions: (i) The main growth of the species *Globigerina bulloides, Globigerina quinqueloba, Globigerinoides ruber,* and *Globoquadrina eggeri* is likely to take place in the upper layers of water, as shown by the relatively dense populations there. Also, the oxygen deficiency of the water at greater depths may be expected to be detrimental to growth and reproduction. (ii) The individuals below 100 m seem to have been brought in with submerged southerly water, judging from the assemblage of species there. Species that were not represented in the upper waters at the time did not contribute to the sediment catch on the bottom. They do not seem to reproduce at a rate comparable to the other species, and their turnover time must be very long under these circumstances. (iii) If the entire population below 100 m can be considered inert on the basis of these arguments, the turnover times for the species found in the surface layers are obtained in the following way. The densities given in Table 1 are converted to standing crops for the upper 100 m of the water column. These productive standing crops for each species are divided by the appropriate fluxes of empty tests, which are also given in Table 1. The resulting turnover times for *Globigerina bulloides, Globoquadrina eggeri, Globigerinoides ruber,* and *Globigerina quinqueloba* are 58, 33, 73, and 27 days, respectively (*8*). For reasons given above, the average life spans of these species should be shorter than the turnover times by a factor of 1 to 2.

These conclusions do not preclude the existence of a longer cycle, including the submergence of mature individuals under adverse environmental conditions. Such cycles are described for copepods (*9*) and may well run parallel to the shorter cycles of high productivity proposed here.

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7. The recovery of only large tests suggests that predation destroys the shells of ingested specimens.
8. Example calculation (*G. bulloides*): [(35 × 9.7) + (10 × 4) + (55 × 2.2)] m × number per m³/8.6 number per m² per day = 58 days.
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10. Supported by the Marine Life Research Group at Scripps Institution of Oceanography and by the National Science Foundation. F. B. Phleger and J. D. Isaacs contributed interest, advice, and encouragement throughout this project. Basic assumptions underlying the calculations were clarified in discuscions with E. W. Fager. R. Schwartzlose was scientist in charge during the cruise and provided the data on bottom currents. D. Brown and E. Duffrin assisted during the technical operations. Information in Fig. 1 was provided by the Data Processing Group, S.I.O.

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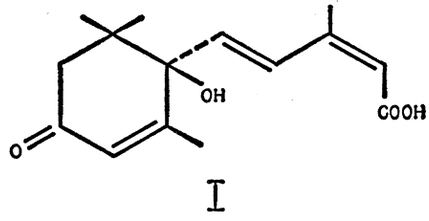
Dormin (Abscisin II), Inhibitor of Plant DNA Synthesis?

Abstract. *Dormin (abscisin II), inhibits growth of* Lemna minor *cultures. At 1 part per million (3.8 × 10⁻⁶M), the culture appears nearly completely dormant but can be revived readily by transferring it to fresh medium free of dormin. The cytokinin benzyladenine, but not auxin or gibberellin, will counteract the dormin effect. Quantitative restoration of normal growth by cytokinin, however, can be achieved only if the dormin concentration does not exceed a critical level. Separation, after phenol-detergent extraction, of nucleic acids on methylated albumin kieselguhr columns showed suppression of nucleic acid synthesis by dormin in all fractions. Inhibition of the synthesis of the DNA fraction seems to precede that of RNA. Cytokinins reverse the process. They promote synthesis of all nucleic acid fractions, but again DNA seems to lead. Further work on the interaction of dormin with growth-promoting hormones might be facilitated by adopting the Monod model of allosteric transition, with, for example, DNA polymerase as the protein, dormin as the inhibitor, and cytokinin or other growth promoters as activators.*

Plant growth and development is regulated by a number of naturally occurring substances of hormonal nature. These chemically well-defined agents include gibberellins, cytokinins, auxins, ethylene, and, most recently, also dormin (*1*). Dormin, an appropriate and physiologically descriptive appellation given by Wareing to the dormancy regulator of sycamore (*2*), was identified and synthesized by Cornforth *et al.* (*3*). Its absolute stereochemical configuration (I) was also determined by



I

Cornforth and associates (*4*). Dormin turned out to be identical to abscisin II from cotton fruit, earlier described by Addicott *et al.* (*5*). Dormin is also identical to the lupin growth inhibitor (*6*) and the peach seed inhibitor (*7*), and seems to occur widely in buds, leaves, tubers, seed, and fruit (*8*). Below we report studies concerned with the mode of action of dormin. This appears to be the inhibition of the synthesis of all nucleic acid fractions as analyzed in the methylated albumin kieselguhr (MAK) column, with the possibility that inhibition of DNA synthesis is an early effect of dormin.

In order to avoid any preconceived notions of its mode of action, part-per-million concentrations of synthetic (±)-dormin from Cornforth's laboratory were applied to all sorts of biological objects: bacteria, fungi, algae, higher plants, insects, and mammalian cell cultures. Only the higher plants responded, and among these, cultures of *Lemna minor* (duckweed) were the most sensitive. Therefore, sterile cultures of *L. minor*, grown under constant fluorescent light (27,500 lu/m²) and constant temperature (22° ± 1°C) were used throughout our tests as experimental material. Growth is vegetative, by budding, and was determined as increase of fresh weight. A dormin concentration as low as 1 part per billion (10^9) ($3.8 \times 10^{-9}M$) causes detectable inhibition (Fig. 1). At 1 part per million (1 ppm) ($3.8 \times 10^{-6}M$), growth inhi-

bition is 95 percent, but normal growth can be restored by simply transferring the cultures to fresh Hoagland solution, free of dormin. After 9 days of culturing, weakly inhibited cultures (100 parts per billion of dormin and lower) have a tendency to resume the normal growth rate, even without being transferred. This was never found with 1 ppm of dormin, which kept the cultures dormant for a month and presumably much longer. Even such old dormant cultures could be brought back to normal growth by transferring them to dormin-free medium.

When benzyladenine (BA) or other purine-type cytokinins were added together with dormin to the medium, the inhibition could be prevented or reduced, depending upon the concentration of the inhibitor (Fig. 1). No such promotive effects on *Lemna* growth were found from additions of gibberellin (GA_3) or auxin (IAA) to the medium, either with or without dormin.

Benzyladenine greatly promotes growth of *Lemna*. The maximum effect is achieved with a concentration of slightly more than 100 parts per billion. After 9 days of growth in such a solution the increase in fresh weight is nearly twice that attained in nutrient solution alone (Table 1). Benzyladenine, if added at this concentration to a culture inhibited by 1 ppm of dormin, promotes an increase in fresh weight. Although this increase is not very great if expressed in absolute terms (Fig. 1 and Table 1), the *ratio* of the growth increase caused by BA under these conditions is practically the same as the one achieved by BA over an untreated control. This suggested to us that the fraction of the system left uninhibited by dormin is still free to respond to cytokinin.

As a working hypothesis we assumed that nucleic acid synthesis might be involved, since we had found previously an increased synthesis of nucleic acids when leaves of dicotyledonous plants had been treated with benzyladenine. At first we were thinking in terms of RNA synthesis. Accordingly, 6-methylpurine (6-MP) was added to the nutrient solution in which *Lemna* was growing. This compound, according to Key *et al.* (*9*), inhibits all DNA-dependent RNA synthesis. Figure 1 shows that 6-MP inhibits growth of *Lemna*. Within the range of concentrations indicated in Fig. 1, the activity of this compound in many ways resembles that of dormin. Higher concentrations are toxic. Thus, 6-MP inhibition can be reversed by transferring the *Lemna* to a fresh medium. The inhibition can also be reversed by cytokinin. However, here again cytokinin is able to restore growth quantitatively to a normal level only when the concentration of 6-MP is below a critical level (Fig. 1). This supports our working hypothesis that the action of dormin involves inhibition of nucleic acid synthesis, and we tested this by measuring the amount of P^{32} incorporation.

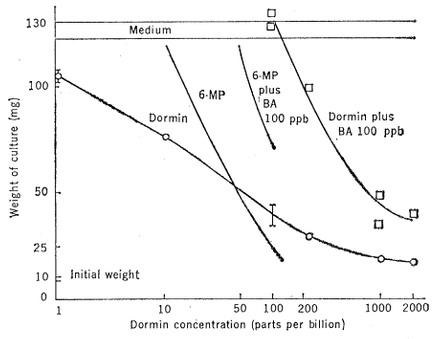


Fig. 1. Effect of dormin alone and in combination with benzyladenine (BA) on fresh weight of *Lemna* cultures after 9 days of culturing. Similar data obtained with the RNA inhibitor 6-methylpurine (6-MP) are drawn in for comparison.

Table 1. Effect of interaction of dormin and benzyladenine, added simultaneously, on growth of *Lemna minor* cultures. Fresh weight (mg) after 9 days of culturing. Initial weight was 8.6 mg.

Benzyladenine (ppm)	Dormin (ppm)	
	0	1
0	130 ± 4.0	20 ± 0.7
0.1	229 ± 6.0	33 ± 1.5
1	252 ± 4.9	87 ± 2.6

Table 2. Relative ratios of incorporation of P^{32} into MAK column fractions (nucleic acid synthesis). Calculation from total counts used for the construction of the graphs of Figs. 2, 3 and 4.

Ratio calculated	Value, in fraction:		
	sRNA	DNA	rRNA
$\left(\frac{\text{Dormin 1 day}}{\text{Medium alone}}\right)-1$	−0.17	−0.38	−0.12
$\left(\frac{\text{Dormin 3 days}}{\text{Dormin 1 day}}\right)-1$	−0.27	0.0	−0.15
$\left(\frac{\text{BA 3 days}}{\text{Medium alone}}\right)-1$	+3.10	+5.03	+2.81
$\left(\frac{\text{Dormin 7}^{d}+\text{BA 3}^{d}}{\text{Medium alone}}\right)-1$	+0.30	+1.40	+0.14

Nucleic acids were extracted by the phenol-detergent method of Key (*9*) and of Cherry (*10*), and separated on MAK columns (*11*). *Lemna* contained the usual fractions (*12*) indicated in Figs. 2, 3, and 4. When the *Lemna* cultures were pulse-labeled with P^{32} for 2 to 16 hours, synthesis of all fractions was depressed, even after only 1 day of exposure to dormin (Fig. 2). After 3 days there was a further depression of all the RNA fractions. One will notice, however, that the maximum depression of the DNA peak occurred after just 1 day of exposure to dormin. This peak, by analogy with peanut cotyledons, may contain 25 percent RNA (*10*). Addition of 100 parts per billion of benzyladenine to the inhibited cultures shows a resumption of synthesis of all fractions (Fig. 3). Especially notable is the striking response of DNA synthesis to cytokinin. The effect of benzyladenine at 100 parts per billion by itself is shown in Fig. 4.

Relative rates of incorporation of radioactive phosphate into the soluble RNA, DNA, and ribosomal RNA fractions are presented in Table 2. These data show that incorporation into the DNA fraction after 1 day of treatment with dormin is considerably more inhibited than incorporation into any of the RNA fractions. This indicates that the inhibition of DNA synthesis is one of the earliest effects of dormin and that the inhibition of synthesis of the RNA fractions occurs later. Table 2 also shows that DNA synthesis is the first to speed up after treatment with cytokinin.

What sort of mechanism can one envision from these results? In the first place, there is little doubt that DNA synthesis is involved in the *Lemna* system, because the specific inhibitor of DNA synthesis, 5-fluorodeoxyuridine (*13*), at $10^{-6}M$ completely inhibited growth of *Lemna*. Normal growth was retained when thymidine ($10^{-5}M$) was added to this inhibitor. In a similar test under conditions where DNA synthesis could not take place, cytokinin showed no growth-promoting effect either.

Then one must ask whether or not dormin acts directly on DNA itself. Materials which combine with DNA directly, such as actinomycin D, happen to be general cell poisons, and affect plants as well as animals and microorganisms. The action of dormin is not of this nature, as it seems to affect higher plants only. Further, the

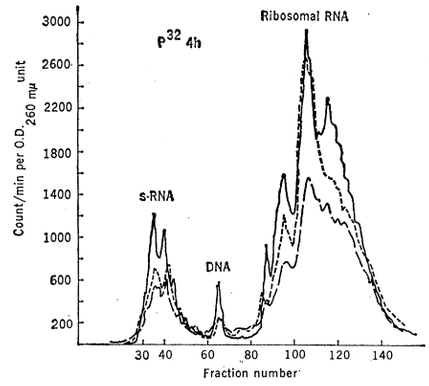


Fig. 2. Effect of dormin on nucleic acid synthesis of *Lemna*. Fractionation of pulse-labeled nucleic acids on MAK columns. Solid line, *Lemna* cultured in Hoagland medium only; dash line, after 1 day in dormin at 1 ppm; broken line, after 3 days of dormin at 1 ppm.

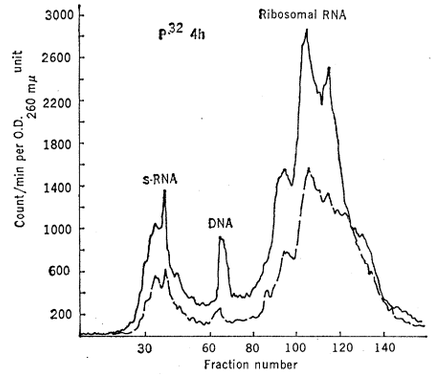


Fig. 3. Restoration of dormin-inhibited nucleic acid synthesis by benzyladenine. Fractionation of pulse-labeled nucleic acids on MAK columns. Solid line, *Lemna* cultured for 7 days in nutrient which contained 1 ppm dormin and to which was then added 100 parts per billion (10^9) of benzyladenine, for 3 more days of culture. Broken line, dormin at 1 ppm for 3 days (drawn here for comparison).

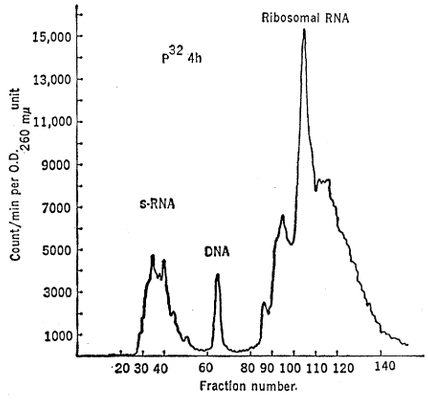


Fig. 4. Nucleic acid fractions separated from *Lemna* treated with 100 parts per billion (10^9) of benzyladenine for 3 days. Note that the scale on the ordinate differs from that of Figs. 2 and 3.

chemical structures of dormin and benzyladenine are so different from each other that its seems unlikely that their antagonism in the *Lemna* system could be due to competitive inhibition of an enzyme system. Such considerations attracted us to Monod's scheme of allosteric enzyme inhibition and activation (*14*, his figure 6) as a working hypothesis. Further work will be necessary to establish whether such an allosteric inhibition of DNA polymerase, for example, is indeed functioning in *Lemna*. Our observations on *Lemna* appear to be compatible with the Monod model. Dormin as the inhibitor would lock some of the enzyme in its *inactive* conformation. This would cause a shift of equilibrium in this direction, leaving only a little of the enzyme in its active conformation, thus leaving only a small residual capacity for DNA synthesis. The structure of dormin would make one suspect that it fixes itself to its site of action by a two-point hydrogen bond attachment. Such a loose bonding would be easily reversible. With dormin detached, the enzyme could then resume its normal state of equilibrium. Now cytokinin could lock the enzyme in its *active* conformation, thereby increasing DNA synthesis.

Because in our *Lemna* system there is an antagonism between dormin and cytokinin one should not think that cytokinin is the only possible antagonist for dormin. Thus, at the MSU/AEC Plant Research Laboratory (*15*), an antagonism involving dormin and gibberellin has been found in the aleurone system of barley. Here dormin inhibits the gibberellin-controlled amylase production, and gibberellin will overcome this inhibition only when the inhibition does not exceed a critical level, as we found in *Lemna* for dormin and cytokinin. It could be, then, that activation can be achieved by a number of promotive hormones, which differ in different systems, and which may function as activators in the sense of Monod's model. These conclusions suggest that it may be profitable to look at other systems such as cell elongation in the lentil epicotyl (*16*) in which it was found that gibberellin activates DNA synthesis.

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Mosquitoes: Female Monogamy Induced by Male Accessory Gland Substance

Abstract. *Male accessory glands were implanted in virgin females of* Aedes aegypti. *When exposed to males, females copulated readily but were not inseminated; they remained sterile for life. Extract from one male could sterilize more than 64 females. The active principle may be a protein or peptide. Intraspecific transplant prevented insemination in 12 species, including* Aedes, Anopheles, *and* Culex; *interspecific transplant gave partial protection.*

Frequent, repeated copulation can be observed in laboratory colonies of numerous species of mosquitoes. It is often assumed that a single female can be inseminated by several males. However, experiments with genetically marked males of *Aedes aegypti* have shown that females of this species are usually inseminated only once (*1*). Copulation may take place many times, but a female mated only once is refractory to subsequent insemination for life. Multiple insemination (*2*) can take place only when several males copulate with a female in a short period of time. In some *Culex* (*3*) and *Anopheles* (*4*), genetic-marker experi-