dicated around the three data points which are not constrained to 1.0 by this standardization procedure.

Two relationships are immediately apparent in these data. First, the amplitude of anterior tongue pressure, for both sugars, is a direct function of sugar concentration: as concentration doubles, there is a near doubling of tongue pressure. Second, at least at the concentrations we employed, sucrose is a more effective stimulus than is glucose. An idea of how this situation compares with adult responses to varying concentrations of these two sugars can be derived from the two sweetness magnitude estimation functions (*11*) superimposed on Fig. 2. There is a remarkable similarity in slope and in relative height between the psychophysical functions and those implicit in the four data points from the infant tongue pressure responses. Future work, of course, must include more than two concentrations of each sugar to determine more precisely the slope of the concentration function in the newborn.

We feel that two conclusions are justified on the basis of these observations. First, the sensory apparatus responsible for assessing relative sweetness of sugars is essentially as competent in the newborn as it is in the adult. Second, this sensory apparatus is capable of systematically eliciting a precisely graded response in the human newborn. The possibility that this graded response is reflexive in nature is enhanced by the report (*12*) of reflexlike lateral tongue movements from single drop gustatory stimulation in the newborn.

The relationship of these competencies to the problem of palatability and control of food intake in the newborn is one which has not yet been resolved. Simultaneous with our initial report of the relative effectiveness of these four solutions in controlling tongue movements (*9*), an independent investigation (*13*) reported similar results for sugar type and concentration in increasing amount of fluid ingested by newborns. The fact that the slopes relating concentration of sucrose and glucose to amount of fluid ingested were different from those reported here for tongue pressure could reflect the possibility that other factors than sweetness are involved in determining amount of fluid ingested, perhaps including the post-ingestional effects of the fluids. A subsequent study with newborns (*14*) counted number of sucks to sucrose and glucose concentrations presented either before or after water. No systematic effects of sugar type or concentration were found when water was offered first, but sugar type and concentration had a strong effect on number of sucks to subsequently presented water; newborns sucked less to water following stronger or sweeter sugars than following weaker or less sweet sugars. Since water has a sour-bitter taste to adults after adaptation of the taste buds to sugar (*15*), these results could be viewed as a demonstration of a similar phenomenon in the newborn, with stronger or sweeter sugars producing a stronger or more persistent effect on the subsequent water taste.

Thus, the ability of the newborn to utilize the gustatory apparatus to discriminate among ingestible substances has here been clearly demonstrated, and has been shown (*13*) to affect the amount the infant will ingest; however, the precise relationship between the infant's sensory and ingestive competencies has yet to be fully explored. One theoretical approach to this problem has been elaborated elsewhere (*8, 9*).

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10. The exploration of stimuli other than those reported here rendered difficult a full design balanced with respect to order of presentation of the four stimuli. We therefore adopted a less complete strategy in which stronger or sweeter stimuli generally followed (after 2 minutes) weaker or less sweet stimuli; the reverse order was not used because it would allow the interpretation that weaker responses to later-presented weaker stimuli were attributable to adaptation, habituation, satiation, or fatigue, rather than to weaker stimuli. As an additional partial control, we tested four babies with eight consecutive 2-ml samples of 5 percent glucose. The anterior tongue response remained remarkably stable: it did not deviate significantly either up or down from 1.0 across these eight trials. The increments in tongue pressure score reported above may therefore be properly attributed to increasing strength or sweetness of sugar.
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Measurement of an Inhibitory Zone

Abstract. *An inhibitory zone mechanism generates the heterocyst pattern in* Anabaena. *These inhibitory zones can be measured; we find that they extend about five cells on either side of a heterocyst. We can use our observations to predict with reasonable accuracy which cells in the filaments will differentiate into heterocysts.*

Little is known about the biochemical mechanisms that control biological spaced patterns, but a simple and attractive hypothesis is that the spacing is due to the production of an inhibitory compound by the elements of the pattern, which prevents other elements from forming too close to them. This hypothesis has been proposed in many cases where patterns are formed by the intercalation of new elements in a growing tissue, for example, insect bristles (*1*), leaf primordia (*2*), and stomata (*3*). The filamentous blue-green alga *Anabaena* is an ideally simple organism for testing the hypothesis. It has a spaced pattern of differentiated cells (heterocysts) formed by intercalation, and an inhibitory zone mechanism has been conjectured (*4, 5*). We now report that there are zones of a well-defined size around heterocysts within which other heterocysts do not develop, and also that these zones can reasonably be attributed to an inhibitory influence from the heterocyst.

The way that the alga grows and maintains its pattern can best be understood from a time-lapse sequence of part of a growing filament (Fig. 1). In the interval shown, the vegetative cells divide, increasing the distance between the two heterocysts. When this distance has roughly doubled, one of the vegetative cells becomes a proheterocyst (an early heterocyst) and eventually develops to maturity. Vegetative cell divisions are asymmetrical (*6*) and a heterocyst only develops from the smaller daughter of a division. Notice that this asymmetry produces an asynchrony in cell cycle stages along the filaments.

If there are constant inhibitory zones, *n*

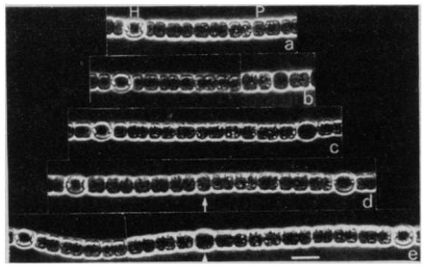

Fig. 1. Maintenance of the heterocyst pattern in *Anabaena*. A sequence of light micrographs of an interval (the set of cells between two differentiated cells, in this case a mature heterocyst H and an initially early proheterocyst P) in a growing *A. catenula* filament. Arrows show the next proheterocyst to develop. Photographs (b) to (e) were taken at 5, 10, 15, and 21 hours respectively after (a) was taken; bar, 10 μm.

cells for example, on either side of a heterocyst, then we expect that the first smaller daughter that arises outside these zones will develop into a heterocyst. In that case there will be a distribution of distances of these newly differentiating cells from their nearest heterocyst neighbors which would have n for a minimum. To test this we have measured the distance, in numbers of cells, from each presumptive heterocyst to the nearest heterocyst (or proheterocyst) at the time when the former arises by cell division (Table 1). The assumption behind this is that the decision to initiate proheterocyst differentiation is taken by a cell early in the cycle. In fact, there is a period of some 6 to 8 hours after division during which no difference can be detected between a vegetative cell and one destined to become a heterocyst; both elongate during this time. After this, a vegetative cell shows the beginnings of a division furrow (the time between divisions in a smaller daughter averages 16 to 18 hours), while a potential heterocyst is recognizable as a stage I proheterocyst (*7*) within about 10 hours from the division. Thus we could suppose that a cell begins to differentiate at any time during the first 8 hours from the division. We chose the moment of division because it serves as a convenient reference point and because we have some biochemical evidence which suggests that important events related to the differentiation process occur at this time (*8*).

The distribution of distances shows that a zone of about five cells exists around each heterocyst and proheterocyst (Table 1, column 2). When we look at smaller daughters that do not develop into heterocysts, we find that, in all cases, there is either a heterocyst or a proheterocyst nearby, or else another smaller daughter which subsequently differentiated (*9*). If we measure distances to the nearest such cell we find that they form an approximate complementary distribution (column 3). There is an overlap at $n = 5$, and a few stragglers, in column 2 at $n = 4$ and in column 3 at $n = 6$. There is no large variability in the width of inhibitory zones, although the developmental fate of a smaller daughter which arises on the edge of the zone, at $n = 5$, is unpredictable.

We have evidence that these zones are maintained by an inhibitory influence from heterocysts (*10*). If we puncture a proheterocyst, another heterocyst can subsequently develop within what had been the inhibitory zone of that cell; that is, within five cells of it. Such an experiment is shown in Fig. 2, where it can be seen that removing the proheterocyst P allows a new proheterocyst to develop within two cells of the new end of the filament. The fact that the presence of the proheterocyst is required to maintain its zone rules out certain alternatives to the inhibition hypothesis, for example, a lineage rule.

We have suggested that the asymmetry and consequent asynchrony of cell division ensures that there will be a first smaller daughter in an interval (*11*). It may happen, however, that two arise almost simultaneously by cell division outside the inhibitory zones of an interval, and that both cells may begin to differentiate before one regresses (*8*). This shows that proheterocyst development is competitive in character, a hypothesis for which we have much additional evidence (*7, 12*). Yet pairs such as these are rare, and a simple inhibitory zone description is, in general, adequate (*13*).

Table 1. Size of inhibitory zones. Individual *A. catenula* filaments, growing on salts medium agar plates (*12*), were observed and photographed at regular intervals (usually 2.5 hours) through several generations, and the fate of each smaller daughter determined. The left-hand column in the table shows the total number of cells lying between the smaller daughter cell at the time of its formation (*11*), and the nearest heterocyst or proheterocyst. The middle column indicates the number of smaller daughters, at each distance, which developed into heterocysts, and the right-hand column the number which continued to divide.

No. of cells to nearest heterocyst or proheterocyst	Fate of smaller daughter	
	Developed	Failed to develop
0	0	77
1	0	29
2	0	106
3	0	65
4	4	34
5	20	12
6	16	1
7	4	0
8	2	0
9	1	0

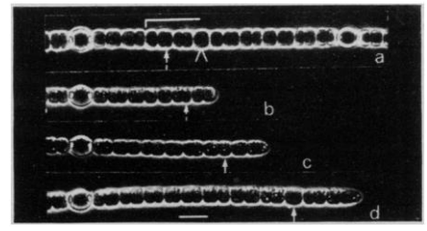

Fig. 2. A stage II proheterocyst (*7*), which would have an inhibitory zone of four to five cells, as marked in (a), is broken (^). A proheterocyst develops from the cell [indicated by the arrows in (a) to (d)] which is inside the original zone. Note that it develops from the first smaller daughter cell outside the inhibitory zone of the mature heterocyst on the left. This operation can only be performed on proheterocysts up to stage IV, since higher stages, having thick outer envelopes, are resistant to puncturing. Photographs (b) to (d) were taken 4, 8, and 14.5 hours respectively after (a) was taken; bar, 10 μm.

Our measurements of the inhibitory zone enable us to predict, with a 75 percent success rate, which cell will differentiate in a given interval. The failures of prediction come mainly from cells which lie on the edge of the zone at $n = 5$, and reflect the inevitable limitations of measurement by cell counting.

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9. There are a few cases in which a potential cell fails to differentiate although apparently outside an inhibitory zone. Such cells, in all cases, are found to lie close to a small daughter which subsequently differentiated. We have therefore assigned a zone to these presumptive heterocysts. Our justification is the assumption that the zone will develop relatively rapidly with respect to the cell cycle time of the organism, which is consistent with our view that differentiation begins shortly after division.
10. Wolk has produced evidence that heterocyst formation is inhibited by other heterocysts (*5*). He made small filament fragments by mechanical breakage. Such fragments will often lack heterocysts and in these cases, he argued, a new heterocyst would be able to develop in the absence of another. Accordingly, he predicted and found an increased frequency of heterocysts after fragmentation. The interpretation of this result is complicated by the fact that the increase may well be attributable to the formation of terminal heterocysts. Indeed, this is what we find in very small filament fragments. These terminal heterocysts do not occur under normal growth conditions, a finding that can be explained in terms of competition (*11*). This implies that there is a departure

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Mutagenicity of Malonaldehyde, a Decomposition Product of Peroxidized Polyunsaturated Fatty Acids

Abstract. *Incubation of histidine requiring auxotrophs of the bacterium* Salmonella typhimurium *with malonaldehyde, a three-carbon dialdehyde, produced an increased number of revertants in specific strains. Mutagenesis was only observed in frameshift mutants with normal excision repair and did not occur in those base-pair substitution mutants tested. The results are consistent with the cross-linking of bacterial DNA by malonaldehyde leading to mutagenesis expressed through the error-prone repair system.*

A possible role for membrane lipid peroxidation in carcinogenesis and mutagenesis has been indirectly suggested by the ability of antioxidants, including vitamin E, to protect against experimental carcinogenesis (*1*), although other studies have failed to demonstrate such an effect (*2*). Similarly, there is evidence both supporting (*3*) and opposing (*4*) the contention that diets high in polyunsaturated fatty acids lead to a higher incidence of cancer. The relationship of lipid peroxidation to carcinogenesis and mutagenesis therefore remains tenuous, particularly in the absence of a clear-cut mechanism by which the breakdown of cell membrane unsaturated fatty acid might damage genetic material.

One potential intermediary in such a process is malonaldehyde, a three-carbon dialdehyde ($OHC\text{-}CH_2\text{-}CHO$) produced during the oxidative decomposition of polyunsaturated fatty acids. Malonaldehyde is formed during the metabolism of certain hydrocarbon carcinogens (*5*), has been reported to initiate skin carcinogenesis in mice (*6*), and will cross-link the amino groups of DNA in solution, presumably through the formation of Schiff bases (*7*). Although this latter process has a low *p*H optimum, the recent observation in this laboratory (*8*) indicating that malonaldehyde cross-links of aminolipid are formed as a consequence of red cell lipid peroxidation suggests that the cross-linking of amino groups by malonaldehyde does occur in vivo in humans with normal vitamin E levels.

The present study utilizes a microbial mutagenesis assay system to evaluate the possibility that this lipid peroxide decomposition product is capable of inducing mutation. Recent studies utilizing similar systems suggest that there is a high positive correlation between carcinogenic and mutagenic activity (*9*). The 12 *Salmonella typhimurium* strains used (kindly provided by Dr. B. N. Ames) are shown in Table 1. The mutations involved in these strains have been fully described elsewhere (*10*). Briefly, the bacteria are all histidine-requiring auxotrophs, each containing a mutation on one of four histidine loci. One set of mutants has the normal polysaccharide coat as well as a normal excision repair system; the second set has a deep rough mutation (rfa) in the cell wall constituents and normal repair; and the last set of mutants has both the rfa mutation and a deletion through the uvrB gene which eliminates normal excision repair. Three of the strains (hisG46, TA1975, TA1535) have been used to detect mutagens causing base-pair substitutions, while the remaining nine strains have been used to detect various kinds of frameshift mutagens.

Mutagenic activity was determined by the standard spot tests and the quantitative soft agar techniques as described by Ames *et al.* (*10*). Malonaldehyde was prepared from malonaldehyde bis-(dimethylacetal) by shaking with Dowex 50 (X2) in the H^+ form.

The mutagenic activity of malonaldehyde on the various tester strains is shown in Table 2. The results indicate that malonaldehyde is mutagenically active, but only on frameshift mutants with normal excision repair: the hisD3052 mutant is the most sensitive strain, followed by the hisC207 strain and the hisC3076 strain. None of the base-pair substitution mutants tested thus far has shown any increase in revertant frequency.

The pattern of mutagenic specificity demonstrated by malonaldehyde is similar to the mutagenic activity of Mitomycin C, a DNA cross-linking agent (*11*). This carcinogenic (*12*), bifunctional antibiotic requires the participation of excision repair processes to show mutagenic activity (*13*). It has been concluded that mutations produced by Mitomycin C are due to errors in repair by the error-prone repair system as a result of excision repair–induced DNA damage (*13*). Malonaldehyde, which is also a cross-linking agent, can alter DNA as indicated by the formation of fluorescent

Table 1. Strains used for mutagen testing.

Histidine mutation in strains				Additional mutations	
G46	C207	C3076	D3052	Repair	Cell wall
hisG46	hisC207	hisC3076	hisD3052	+	+
TA1975	TA1976	TA1977	TA1978	+	rfa
TA1535	TA1536	TA1537	TA1538	uvrB	rfa

Table 2. Mutagenicity of malonaldehyde on tester strains.

Strain	Average number of revertants per plate*				Additional mutations	
	Spot test		Soft-agar test			
	Control	MDA†	Control	MDA†	Repair	Cell wall
hisG46	19	21	8	12	+	+
TA1975	15	19	4	3	+	rfa
TA1535	9	11	13	16	uvrB	rfa
hisC207	22	62	21	72	+	+
TA1976	23	48	18	57	+	rfa
TA1536	2	2	8	8	uvrB	rfa
hisC3076	41	103	47	118	+	+
TA1977	34	74	40	95	+	rfa
TA1537	22	21	87	88	uvrB	rfa
hisD3052	38	285	28	164	+	+
TA1978	45	337	35	161	+	rfa
TA1538	61	78	39	57	uvrB	rfa

*Numbers represent averages from duplicate plates. †Ten millimoles of malonaldehyde in 0.1 ml of distilled water was applied to sterile filter disks (12.7 mm in diameter) placed on the center of seeded petri plates (for spot test) or mixed with cells in 2.5 ml of top agar (soft-agar test). Plates were counted after 2 to 5 days of incubation at 37°C. All numbers presented are counts on day 5.