verse as soil and enteric bacteria. (ii) Growth in very dilute environments may be accompanied by a limitation of available nutrients. The diversion of a significant fraction of the total cell mass toward the synthesis of periplasmic oligosaccharides under such conditions must be presumed to have adaptive value. (iii) It is estimated that 10 to 15 enzymes are required for the synthesis of the MDO of *E. coli*. At least some, and probably all, of these enzymes are synthesized constitutively, even by cells growing in media of high osmolarity (*27*).

With these considerations in mind, it is paradoxical that the *mdoA* mutation, which blocks the synthesis of MDO at an early stage in the assembly of the carbohydrate chains, is not associated with any distinct phenotype (*28*). It is now recognized, however, that there may be considerable redundancy in many processes localized in the cell-envelope of *E. coli*, as exemplified by the multiple transport systems for potassium, for iron, and for sugars. The function of any one of these multiple systems is difficult to detect until alternative systems have been genetically deleted. Perhaps the function of the periplasmic oligosaccharides will be seen more clearly if it becomes possible to recognize and eliminate alternative mechanisms for adaptation to low osmolarity.

The membrane-derived oligosaccharides of *E. coli* appear to be well suited to function in the maintenance of the osmolarity of the periplasm at a value close to that of the cytoplasm, as originally indicated by the study of Stock *et al.* (*7*). With molecular weights of about 2500, MDO are large enough to be retained by the pores of the outer membrane but not so large as proteins, which are less active osmotically per unit weight. The multiple substitution of MDO with anionic residues further enhances their osmotic effect through the binding of counterions and contributes to the Donnan potential across the outer membrane. The neutral cyclic (1 → 2)-β-D-glucans of *A. tumefaciens* may contribute to the osmolarity of the periplasmic compartment of this bacterium, as their size and periplasmic concentrations are comparable to that of the MDO of *E. coli*. Furthermore, the amounts of anionic oligosaccharides within *A. tumefaciens* were also greatly increased when cells were grown in a medium of low osmolarity (Table 1).

The fundamental mechanisms by which cells recognize and respond to changes in the osmolarity of their extracellular environment are unknown. Detailed study of effectors that modulate the activity of enzymes catalyzing the initial steps of periplasmic oligosaccharide biosynthesis may provide insight into this important problem.

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Heat Shock Elicits Production of Sexual Inducer in *Volvox*

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In the green alga *Volvox carteri*, heat shock had an unusual and adaptive effect mediated by induced production of a well-defined effector molecule. Females of this species normally reproduce asexually in the absence of a potent sexual inducer produced by mature sexual males, but they generated egg-bearing sexual daughters after a brief exposure to elevated temperatures. This response involved an "autoinduction" of sexuality, in which heat-shocked somatic cells made and released the sexual inducer, which then redirected development of the reproductive cells. Males, including a sterile mutant incapable of producing inducer in the usual manner, also produced the inducer in response to heat shock. The phenomenon probably is of significance in the wild, where *Volvox* reproduces asexually in temporary ponds in spring but becomes sexual and produces dormant, overwintering zygotes before the ponds dry up in the summer heat.

HEAT SHOCK, WHICH HAS SIMILAR effects on gene expression and development of thermotolerance in a wide range of organisms (*1*), has an additional, adaptively significant effect on gene expression in *Volvox carteri* f. *nagariensis*. The sexual inducer of this species, an approximately 30-kD glycoprotein (*2*), is one of the most potent biological effector molecules known. Males developing sexually, either in response to inducer made by another male (*3*) or because of a *sex*^{c}, or "constitutive sexuality," mutation (*4–6*), make and accumulate inducer in their sperm packets (*7*) and release it as sperm are released (*5, 7*). It is effective at $6 \times 10^{-17}M$, and one sexual male releases enough inducer to convert all the related males and females in a volume of 1000 liters from asexual to sexual reproduction (*8*). Because inducer is not made by sexual females (*3*) or the somatic cells of sexual males (*7*), it appeared that expression of the gene involved in inducer production was tightly linked to sperm development. However, the results reported here demonstrate that this is not the case: heat shock caused both asexual females and asexual

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Table 1. Sexual-induction titers of culture filtrates. All strains were obtained from the University of Texas Culture Collection of Algae and maintained in SVM (*13*). For production of a culture filtrate to be assayed for inducer, adult spheroids containing inverted but unexpanded juveniles were placed in SVM (density, five spheroids per milliliter) early in the illumination period and subjected to the standard heat shock. Eighteen hours later medium was collected by filtration. The bioassay was performed by making duplicate serial dilutions of a filtrate, adding test organisms (adults with unexpanded juveniles) at a density of one per 2.5 ml, culturing them for 72 hours, and then examining the (approximately 1000) progeny in each tube for sexuality (*2*). Undiluted filtrates from heat-shocked cultures gave 100 percent sexual progeny, those from control cultures gave none; the titer of a filtrate is the reciprocal of the dilution which gave closest to 50 percent sexual progeny; NSP indicates that no sexual progeny were seen at any concentration of filtrate. The antibody to inducer used in experiment 1 was provided in the form of an ammonium sulfate–precipitated rabbit immunoglobulin fraction. Normal immunoglobulin G (IgG) was prepared from serum of a nonimmunized rabbit. Immunoadsorbents were prepared by treating aliquots of a washed protein A preparation (IgGSorb, The Enzyme Center) with equivalent concentrations of the two immunoglobulins for 1 hour at 37°C and washing extensively with phosphate-buffered saline and SVM. Aliquots of culture filtrate were then treated with the appropriate immunoadsorbent for 1 hour at 37°C, clarified, and bioassayed. Somatic cell and juvenile fractions used in experiment 2 were isolated on the day preceding heat shock (*9*) and used at densities equivalent to those of intact spheroids.

Heat-shocked strain	Experimental variables	Titer
	Experiment 1	
HK 10	Standard female, filtrate untreated	64
	Filtrate adsorbed with normal IgG	64
	Filtrate adsorbed with antiinducer	NSP
	Experiment 2	
HK 10	Intact maternal spheroids as source	32
	Isolated maternal somatic cells as source	64
	Isolated juveniles as source	16
	Experiment 3	
69-lb	Standard male	128
Poona	Wild-type male from India	128
Poona-sterile	Noninducible variant of Poona male	64

males to make inducer and release it immediately into the medium, resulting in "autoinduction" of sexuality.

In a previous study (*9*), we used a 1-hour heat shock (42.5°C) as a transcription-mediated control event to aid in the demonstration that the comprehensive protein synthetic changes elicited in female *V. carteri* by the dark-to-light transition used to synchronize development are mediated at the translational level. Serendipitously we discovered that the progeny of such heat-shocked females exhibited a low but significant frequency of sexual development.

In that initial study, the organisms were heat-shocked in the first hour of the illumination period, when the females who subsequently gave rise to sexual daughters were themselves free-living juveniles (having been mechanically released from their maternal spheroids the preceding day) and were just entering their most rapid phase of cytodifferentiation. In the present study we found that the frequency with which sexual progeny are produced can be increased by leaving the juveniles within the maternal spheroids, delaying the initiation of heat shock by 3 hours (Fig. 1A) (*10*), and increasing its duration to 2 hours (Fig. 1B). A still further increase was observed when the final portion of the heat shock was at 45°C (which was lethal in the absence of preconditioning at 42.5°C). Intact maternal spheroids containing juvenile asexual daughters, when subjected to 42.5°C for 100 minutes followed by 45°C for 20 minutes (beginning 3 hours into the illumination period), routinely yielded 100 percent sexual granddaughters (Fig. 1C). This constituted the "standard heat shock" used in the experiments described below.

Males had patterns of responsiveness to heat shock similar to those of females (Fig. 1C), and when heat shock–induced males and females were combined, they mated with high efficiency and generated zygotes indistinguishable from those produced by conventionally induced cultures. When assayed for the sexual inducer by the standard procedure (*2*), filtrates from heat-shocked asexual female cultures were positive: they induced sexual development in the test spheroids (Table 1). However, the sex-inducing properties of filtrates were abolished by treatment with antibody to the inducer made by sexual males (experiment 1 in Table 1) (*11*). Maternal somatic cells generated higher inducer titers in response to heat shock than did the juveniles derived from the same spheroids (experiment 2 in Table 1), although the latter contained about 16 times as many cells (*3*). From all these results we conclude that, in response to heat shock, asexual females—most notably their fully differentiated somatic cells—make and release to the medium a substance that cross-reacts immunologically with and hence is probably identical to the inducer produced by the sperm cells of sexual males.

Asexual males (including a "sterile" strain incapable of responding to inducer produced by wild-type males and hence incapable of making inducer by the "conventional" route) also released inducer in response to heat shock (experiment 4, in Table 1). Although the inducer titers of heat-shocked asexual male cultures are far lower than those typical of mature sexual males (*2, 8*), they are adequate to divert many individuals of both sexes into the sexual pathway of development and thereby elicit sufficient additional inducer production in the next generation (by the conventional pathway) to convert the entire population to sexuality.

When medium was withdrawn from cultures of asexual females at intervals during and after the heat shock and bioassayed, inducing activity was detectable by the end of the heat shock, and reached near maximal levels by 1 hour after return to normal temperature (Fig. 1D). This is extremely different from the time course of inducer production and release by conventionally induced males, for which the lag between induction and inducer release is on the order of 3 days (*7*). Although production of sexual daughters in the next generation in response to heat shock exhibited a distinct temporal optimum (Fig. 1B), production and release of inducer by the heat-shocked cultures did not. Filtrates from cultures that were heat-shocked at later times had extremely similar inducer titers in the bioassay (Table 2); probably the reason that the next generation is not completely sexual in such cases is because inducer is not present long enough in advance of the next round of embryogenesis to achieve full induction (*6, 8*). The failure of heat-shocked males to yield 100

Table 2. Inducer titer as a function of time of heat-shock initiation. Test filtrates were generated and assayed as described in the legend to Table 1, except that the time of heat-shock initiation was varied as indicated.

Time of heat-shock initiation	Resulting titer of filtrate
1000	2
1100	16
1200	32
1300*	64
1400	64
1500	32
1600	64
1700	64
1800	32
1900	32

*"Standard" hour.

percent sexual progeny in the next generation appears to have a similar basis.

Three pieces of evidence (Table 3) indicate that development of sexual progeny from heat-shocked individuals can be attributed entirely to autoinduction by the inducer released into the medium and not to activation of inducer-independent sexual development (*5*) by the heat shock. First, the number of progeny that developed sexually was markedly reduced if the heat-shocked individuals were merely washed with fresh medium, as is also the case with cultures exposed to exogenous inducer (*5, 8*). Second, sexuality was reduced to zero if washing was combined with exposure to concanavalin A (Con A), which blocks the action of the inducer (*8*). Third, adding anti-inducer antibody to the culture medium blocked development of sexuality in response to heat shock.

Other stresses that induce the synthesis of heat shock proteins in other organisms—an amino acid analog (methionine sulfoximine), a heavy metal (cadmium), a sulfhydryl reagent (arsenite), and hypertonicity (with added sucrose) (*1*)—were all tested at sublethal levels on spheroids at the same stage as those used for the heat-shock studies; none elicited any sexuality. Therefore, the activation of inducer production appears to be a heat-shock–specific response not a generalized stress response.

Our observations on induction of sexuality by heat shock may have been foreshadowed by the findings of Powers (*12*) more than 75 years ago. Attributing his several-year failure to locate populations of *Volvox* containing sexual individuals to his having searched in "too large bodies of water, *e.g.*, ponds one to two feet deep," Powers stated that "in the full blaze of Nebraska sunlight, *Volvox* is able to appear, multiply and riot in sexual reproduction in pools of rainwater of scarcely a fortnight's duration" (*12*, p. 141). His most useful collection—one including all sexual phases of both *V. carteri* and *Volvox spermatosphaera*—was one made for him by a colleague "in a broad but shallow pond. . . . The water of the pond was said to be decidedly warm to the touch when the collection was made" (*12*, p. 141). In the shallow, temporary ponds where *Volvox* abounds in the asexual phase in late spring, but typically can only be found as dormant, desiccation-resistant zygospores in midsummer and fall, temperatures such as those used in our study are not unusual. For any organism, the enhanced thermal resistance provided by the ubiquitous "heat-shock proteins" (*1*) has obvious adaptive significance. But equally obvious is the advantage to be gained by organisms living in vernal waters (waters that are destined to dry up soon

Table 3. Sexual development as a function of culture treatment. Test cultures were established by placing maternal spheroids in SVM (with or without additions as noted) at a density of one per 2.5 ml; they were then subjected to the standard heat shock and cultured for an additional 72 hours, at which time the progeny were examined. In experiment 2, aliquots of the normal IgG and anti-inducer IgG described in the legend to Table 1 were diluted ten times, dialyzed exhaustively against SVM, and used in a fivefold dilution series as test medium.

Treatment of culture during or after heat shock	Sexual progeny (%)
Experiment 1	
No heat shock	0
Standard heat shock	100
Washed 2 and 18 hours after heat shock	15
Heat-shocked in Con A (2.5 μg/ml) and washed 18 hours later	0
Experiment 2	
No heat shock	0
Standard heat shock	100
Normal IgG in medium, undiluted to 1:6250 dilution	100
Anti-inducer in medium, undiluted to 1:1250 dilution	0
Anti-inducer in medium, 1:6250 dilution	90

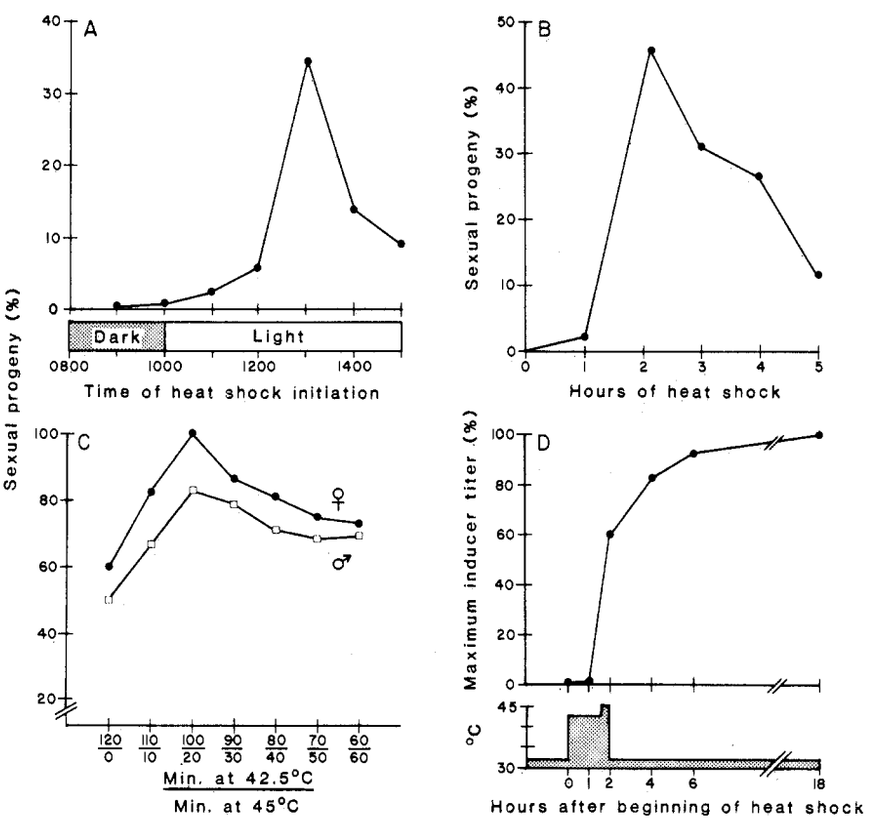

Fig. 1. Effects of temporal parameters on the production of sexual progeny (A to C) or sexual inducer (D) by heat-shocked asexual female spheroids of strain HK 10 of *V. carteri* that had been maintained at 32°C in standard *Volvox* medium (SVM) (*13*). (A) Production of sexual progeny as a function of time of initiation of heat shock. (Inset: illumination conditions prevailing in this portion of the light-dark cycle used to synchronize development.) Maternal spheroids, containing unexpanded juveniles, were cultured at a density of one per milliliter and exposed to 42.5°C for 90 minutes beginning at the indicated times; 72 hours later all tubes were examined to determine the proportion of first-generation progeny that had developed sexually. (B) Production of sexual progeny as a function of duration of exposure to 42.5°C. All aspects of the experimental design were as in (A), except that heat shock was begun at 1300 hours in every case and continued for the time indicated. (C) Production of sexual progeny as a function of the portion of a 2-hour heat shock (1300 to 1500 hours) that was at 42.5°C and the portion that was at 45°C. (D) Time course of appearance of sexual-inducing activity in medium of a heat-shocked culture. Beginning at 1300 hours, a culture with five spheroids per milliliter was subjected to the temperature regimen shown; replicate aliquots of medium were removed at intervals, filtered, serially diluted, and assayed as described in Table 1. Resulting titers are plotted as percentages of the maximum titer observed (18 hours after the beginning of the heat shock).

after they heat up) by adding sexual induction, and the consequent production of drought-resistant zygospores, to their heat-shock repertoire.

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Detection of Smoking-Related Covalent DNA Adducts in Human Placenta

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The presence of covalent DNA chemical addition products (adducts) in human term placentas was investigated by recently developed immunologic and ^{32}P-postlabeling assays. DNA from placental specimens of smokers showed a small but not statistically significant increase in adduct levels when tested by antibodies to DNA modified with a benzo[*a*]pyrene dihydrodiol epoxide (BPDE-I), the ultimate carcinogenic derivative of benzo[*a*]pyrene. The postlabeling assay detected several modified nucleotides, one of which (adduct 1) strongly related to maternal smoking during pregnancy. This adduct was present in placental tissue from 16 of 17 smokers, but only 3 of 14 nonsmokers. Among smokers, levels of adduct 1 in general were only weakly related to questionnaire and biochemical measures of the intensity of smoking exposures, which suggests modulation by individual susceptibility factors. The adduct seemed to be derived from an aromatic carcinogen, but it may not result from several of the most intensely studied polycyclic aromatic hydrocarbons or aromatic amines in tobacco smoke. The data show the association of cigarette smoking with covalent damage to human DNA in vivo.

CIGARETTE SMOKING IS THE MAJOR single known cause of cancer mortality in the United States, and tobacco's contribution to all cancer deaths is estimated to be 30 percent (*1, 2*). Tobacco smoke contains numerous substances that cause cancer in animal bioassays, but it is not clear which of the several thousand components of this complex mixture are responsible for human carcinogenesis (*1, 2*). In animals and humans, chemical carcinogenesis is a multistage process consisting of mechanistically discrete events that may occur over a considerable portion of the lifespan of an individual (*3*). The initial event (initiation) is thought to involve DNA damage, as evidenced by the ability of the majority of chemical carcinogens to give rise to covalent chemical addition products (adducts) in the DNA of experimental animals (*4*), but little is known about the nature of DNA adducts formed in vivo in humans. In this study the presence of DNA adducts formed in placentas of women who smoked during pregnancy was investigated with complementary immunologic (*5*) and postlabeling (*6*) assays.

Smoking induces placental mono-oxygenase activity, alters placental morphology, and seems to increase the risk of malignant and benign gestational trophoblastic disease (*1, 7*). In addition to responding to smoking, placental tissue was appropriate for these studies because a large amount of fresh tissue was available from healthy subjects, which facilitated replicate analyses of each specimen by multiple assays. Women who donated placental specimens also provided multiple blood specimens and interview data concerning smoking and other exposures. These samples and data allowed a study of relations among measures of smoking exposure and the formation of adducts; they also provide materials for further studies of the effect of metabolism or DNA repair on these relations as well as for correlations with clinical end points such as birth weight.

Healthy pregnant volunteers receiving care from the obstetrical service of the University of North Carolina Memorial Hospital in Chapel Hill were selected on the basis of their smoking history. Informed consent and a maternal blood specimen were obtained during a clinic visit at about the 32nd week of pregnancy. A second specimen of maternal blood, a sample of cord blood, and the placenta were obtained at delivery. Blood specimens were analyzed for three biochemical markers of smoking exposure—cotinine, thiocyanate, and carboxyhemoglobin (*8*). Subjects were interviewed within a few days after delivery. Smoking data were collected separately for each trimester and the last 2 weeks of pregnancy; 17 of the 31 subjects reported active smoking during pregnancy and were classified as smokers. Shortly after delivery, placentas were frozen at −80°C. Laboratory personnel conducting the assays had no knowledge of the exposure status of the subjects. DNA was analyzed for adducts by a competitive enzyme-linked immunosorbant assay (ELISA) with antibodies to DNA modified with 7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydro-benzo-[*a*]-pyrene (BPDE-I) (*5*), and by the ^{32}P-postlabeling assay (Fig. 1).

The antibodies used in the ELISA cross-react with several other polycyclic aromatic hydrocarbons containing a diol-epoxide region similar to BPDE-I (*9*), so that inhibition of antibody binding may have resulted from adducts other than BPDE-I derivatives. In the ELISA, human placental DNA competitively inhibited antibody binding to an extent equivalent to BPDE-I adduct levels ranging from nondetectable to 5.8 fmol

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