At 1 mg/liter GA slightly enhanced the speed with which plantlets appeared, but did not improve the final percentage of stamens producing embryos. It did not allow younger stages (such as stage 1) to produce more embryos (Table 1). At 1 mg/liter, GA caused an abnormal elongation of the hypocotyl and production of spindly, chlorotic plantlets.

Abscisic acid (ABA) from 10^{-7} to $10^{-5}M$ did not reduce the percentage of stamens producing plantlets, but it delayed their development markedly. In the presence of $10^{-6}M$ or more of ABA, the embryos formed were shorter and thicker than the controls and remained in an ungerminated condition for at least 2 months (see above the three criteria for germination) by which time the controls had developed into plantlets with green leaves, reaching 5 cm in height.

In some experiments, addition of L-glutamine or of L-asparagine at 1 to 3×10^{-3} M stimulated the production of plantlets from excised stamens. L-arginine, at the same concentrations, was completely inhibitory. The purine and pyrimidine constituents of nucleic acids gave variable results, adenine (at 1 to $3 \times 10^{-4}M$) being generally inhibitory. Addition of all the bases together ($10^{-4}M$ of each) did not improve the percentage of stamens producing plantlets.

The fact that the male prothallus can proliferate and form embryos explains certain abnormalities reported in the literature. Thus haploid plants which had only the characters of the male parent have been obtained in crossing *N. digluta* by *N. tabacum* (*4*) or *N. tabacum macrophylla* by *N. langsdorffii* (*5*). In these cases, androgenesis probably occurred in the embryo sac. This natural tendency may be increased to such a degree by the present method that it is a practical way to produce haploid tobacco plants at will (*6*).

J. P. NITSCH
C. NITSCH

Laboratoire de Physiologie Pluricellulaire, Centre National de la Recherche Scientifique, 91, Gif-sur-Yvette, France

References and Notes

1. Medium H, consisting of: (i) Mineral salts (mg/liter)—KNO_3, (950), NH_4NO_3, (720); $MgSO_4 \cdot 7\ H_2O$ (185); $CaCl_2$ (166); KH_2PO_4 (68); $MnSO_4 \cdot 4\ H_2O$ (25); H_3BO_3 (10); $ZnSO_4 \cdot 7\ H_2O$ (10); $Na_2MoO_4 \cdot 2\ H_2O$ (0.25); $CuSO_4 \cdot 5\ H_2O$ (0.025). In addition, 5 ml/liter of a solution of 7.45 g of disodium ethylenediaminetetraacetate and .557 g of $FeSO_4 \cdot 7\ H_2O$ in 1 liter of distilled water was added. (ii) Organic addenda (mg/liter)—*myo*-inositol (100); glycine (2); nicotinic acid (5); pyridoxine HCl (0.5); thiamine HCl (0.5); folic acid (0.5); and biotin (0.05). (iii) Sucrose (20 g/liter). (iv) Difco Bacto-agar (8 g/liter). Indole-3-acetic acid (IAA) at 0.1 mg/liter is beneficial but not indispensable. The *p*H is adjusted to 5.5 with HCl or NaOH before the agar is added. The medium was sterilized in an autoclave for 15 minutes at 20 pounds per square inch.
2. J. P. Bourgin and J. P. Nitsch, *Ann. Physiol. Vég.* **9**, 377 (1967); J. P. Nitsch, C. Nitsch, S. Hamon, *Comp. Rend. Soc. Biol.* **162**, 369 (1968).
3. W. Halperin and D. F. Wetherell, *Nature* **205**, 519 (1965).
4. R. E. Clausen and W. E. Lammerts, *Amer. Natur.* **63**, 279 (1929).
5. D. Kostoff, *Z. Zellforsch. Mikroskop. Anat. Abt. Histochem.* **9**, 650 (1929).
6. While this paper was being processed for publication, it came to our attention that K. Nakata and M. Tanaka [*Jap. J. Genet.* **43**, 65 (1968)] had also obtained embryos from cultured anthers of *N. tabacum*. Their highest reported yield of 6 percent falls short of the yield of embryo-producing anthers (45 percent) reported here.
7. The authors are indebted to the Station de Recherches pour l'Amélioration des Planets de Grande Culture, Gembloux, Belgium, and to the Institut Expérimental du Tabac, Bergerac, France, for seeds. We also thank M. Mousseron for a sample of *cis*-abscisic acid. Photographs taken by B. Norreel; the microscopic preparations of Fig. 3 made by S. Hamon.

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Algae: Amounts of DNA and Organic Carbon in Single Cells

Abstract. *An analysis of ten different unicellular algae, varying in size and containing from 10 to 6000 picograms of carbon per cell, indicates that the amount of DNA per cell is in direct proportion to cell size. The content of DNA is equal to approximately 1 to 3 percent of the cellular organic carbon.*

There has been speculation on the evolutionary significance of the amount of DNA in diverse plant and animal cells (*1–3*). Most data on DNA as a function of cell size deal with bacteria or with vertebrates or higher plants. Commoner (*4*) has postulated that DNA plays important physiological roles in addition to its role as the template for genetic information, and that DNA content of a cell should be proportional to cell size. To test the hypothesis that DNA is directly proportional to cell size, it is essential to use cells which are closely related phylogenetically and which are similar in physiology and nutrition. These criteria are met by eukaryotic, unicellular algae that are growing photoautotrophically. The smallest cells used in this investigation (*Monochrysis lutheri* and *Navicula pelliculosa*) contained approximately 10 pg of organic carbon and 0.1 pg of DNA per cell, and the largest cells (*Gonyaulax polyedra*) contained 6000 pg of carbon and 200 pg of DNA per cell. The DNA content per cell therefore is nearly directly proportional to cell size as determined by total organic carbon content. Because these cells could be expected to require about the same amount of DNA-template information, the large variations in DNA per cell (up to 2000 times) indicate that DNA does much more than merely convey genetic information in the cell.

The ten species of unicellular algae used were unialgal, bacteria-free cultures grown as described (*5, 6*). Samples of the algal suspensions were filtered through HA (0.45-μ pore size) Millipore filters, and the cellular contents of DNA were determined by a fluorometric measurement with 3,5-diaminobenzoic acid dihydrochloride. This procedure is based on that of Kissane and Robbins (*7*), with modifications for laboratory cultures of phytoplankton (*8*). Samples of algal suspensions were also filtered through glass fiber filters and the total cellular organic carbon was determined by measurement of CO_2 by infrared gas analysis after complete combustion of the sample by wet oxidation (*9*). Cell counts were determined with a Coulter model A particle counter.

There is nearly a direct proportionality between cell size and content of DNA in these species (Fig. 1). These values represent total cellular DNA and thus include nuclear DNA as well as any extranuclear DNA. It is unlikely, however, that the observed correlation between DNA content and cell size can be attributed solely to extranuclear DNA. The amount of DNA in mitochondria and chloroplasts generally accounts for only a few percent of the total cellular DNA. In *Euglena gracilis*, for example, the DNA of the mitochondria and the plastids together amounts to less than 5 percent of the nuclear DNA, as judged by microdensitometer readings of ultraviolet-absorption photographs of DNA separated on cesium chloride density gradients (*10, 11*). Studies with chloroplast-containing flagellates and with their colorless counterparts also show that the amount of DNA contained in the chloroplasts is minor compared to the amount in the nucleus (*10*). Therefore the correlation between cell size and DNA (Fig. 1) is caused predominately by varying amounts of nuclear DNA. Microscopic examination of

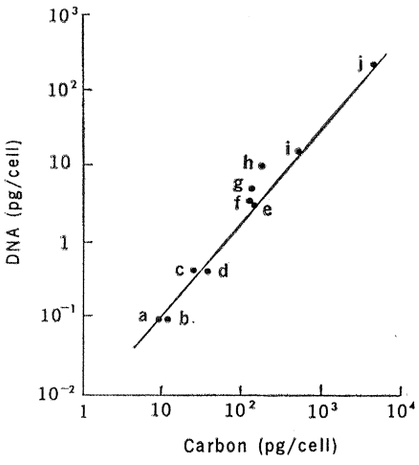

Fig. 1. Content of DNA unicellular algae as a function of the total organic carbon. *(a) Navicula pelliculosa; (b) Monochrysis lutheri; (c) Skeletonema costatum; (d) Dunaliella tertiolecta; (e) Amphidinium carteri; (f) Syracosphaera elongata; (g) Thalassiosira fluviatilis; (h) Cachonina niei; (i) Ditylum brightwellii; (j) Gonyaulax polyedra.*

Navicula pelliculosa and Cachonina niei indicates that the volume of the nucleus also increases proportionally with cell size (*6, 12*).

The observed correlation between cell size and DNA content in algae (Fig. 1) apparently holds for a variety of diverse plant and animal cells. Thus, data for bacteria (*13*), yeasts (*14*), protozoans (*15*), flagellates (*16*), sponge cells, and vertebrate cells (*1*) show that DNA accounts for about 0.2 to 5.0 percent of the cellular dry weight, or about 0.4 to 10 percent of the cellular organic carbon. Each cell of the amoeba *Chaos chaos* (approximately 400,000 pg of carbon per cell) contains 1400 pg of DNA, whereas somatic cells of man contain only about 6 pg of DNA (*2, 15*).

The amount of DNA in a cell cannot be equated either with phylogenetic position or biochemical and physiological complexity of the organism. There is, however, a parallelism between DNA content and cell size. It seems likely therefore that the amount of DNA required per cell does not directly reflect the amount of template information required by the cell, but rather it reflects rate-limiting reactions between the nucleus and the cytoplasm. A similar conclusion (*1*) has been suggested previously by comparison of DNA contents of bacteria and vertebrate cells. Thus, Mirsky and Ris (*1*) have stated that "the variations in DNA content per cell in vertebrates would hardly seem to be due simply to differences in the number of genes," while Brawerman and Shapiro (*3*) have written "a larger cytoplasm would require a larger number of metabolic units, such as enzymes, to perform a certain function, and if the genetic factors that control the formation of these units operate at a relatively slow rate, more of these factors would be required." Such a redundancy of certain DNA segments has been described by Britten and Kohne, who report that the genomes of some organisms contain hundreds of thousands of copies of certain DNA sequences (*17*). A correlation between DNA content and metabolic activity is afforded by Brown and others who have shown by specific hybridization techniques that the oocyte of the amphibian *Xenopus laevis* contains an enormously increased number of genes for 28*S* and 18*S* RNA (*18*). This increase in ribosomal genes correlates with the period of massive ribosome synthesis during oogenesis, after which the extra ribosomal DNA copies are somehow destroyed. It has been postulated (*4*) that DNA has two main roles: (i) It conveys genetic information through its action as a template for protein synthesis and (ii) it regulates cellular metabolism through the control of concentrations of free nucleotides by sequestration reactions (*4*).

The proportionality between DNA and cell carbon can be used for biomass determinations in ecological studies. Some estimate of the total living crop of organisms, both autotrophic and heterotrophic forms, is often desired, and analyses for total organic carbon or protein are not feasible because of organic detrital material. The usefulness of DNA measurements for estimation of biomass may be limited to some extent by the rate of hydrolysis of cellular nucleic acids upon death of the cell. However, DNA has been used as a biomass indicator in ocean samples (*8*).

OSMUND HOLM-HANSEN
University of California, Institute of Marine Resources, P.O. Box 109, La Jolla 92037

References and Notes

1. A. E. Mirsky and H. Ris, *J. Gen. Physiol.* **34**, 451 (1951).
2. R. Vendrely, in *The Nucleic Acids*, E. Chargaff and J. N. Davidson, Eds. (Academic Press, New York, 1955), vol. 2, pp. 155–180.
3. G. Brawerman and H. S. Shapiro, in *Comparative Biochemistry*, M. Florkin and H. S. Mason, Eds. (Academic Press, New York, 1962), vol. 4, part B, pp. 107–183.
4. B. Commoner, *Amer. Sci.* **52**, 365 (1964).
5. O. Holm-Hansen and C. R. Booth, *Limnol. Oceanogr.* **11**, 510 (1966).
6. J. Coombs, P. J. Halicki, O. Holm-Hansen, B. E. Volcani, *Exp. Cell Res.* **47**, 315 (1967).
7. J. M. Kissane and E. Robins, *J. Biol. Chem.* **233**, 184 (1958).
8. O. Holm-Hansen, W. H. Sutcliffe, Jr., J. Sharp, *Limnol. Oceanogr.* **13**, 507 (1968).
9. O. Holm-Hansen, J. Coombs, B. E. Volcani, P. M. Williams, *Anal. Biochem.* **19**, 561 (1967); D. W. Menzel and R. F. Vaccaro, *Limnol. Oceanogr.* **9**, 138 (1964).
10. J. A. Schiff and H. T. Epstein, in *Reproduction: Molecular Subcellular, and Cellular*, M. Locke, Ed. (Academic Press, New York, 1965), p. 131.
11. M. Edelman, H. T. Epstein, J. A. Schiff, *J. Mol. Biol.* **17**, 463 (1966).
12. A. R. Loeblich, III, *Proc. Biol. Soc. Wash.* **81**, 91 (1968).
13. H. D. Barner and S. S. Cohen, *J. Bacteriol.* **72**, 115 (1956); S. E. Luria, in *The Bacteria*, I. C. Gunsalus and R. Y. Stanier, Eds. (Academic Press, New York, 1960), vol. 1, pp. 1–34; M. Schaechter, O. Maaløe, N. O. Kjeldgaard, *J. Gen. Microbiol.* **19**, 592 (1958).
14. M. Ogur, S. Minckler, G. Lindegren, C. C. Lindegren, *Arch. Biochem. Biophys.* **40**, 175 (1952).
15. C. T. Friz, *Comp. Biochem. Physiol.* **26**, 81 (1968).
16. J. R. Cook, *J. Cell. Physiol.* **71**, 177 (1968); L. N. Edmunds, Jr., *Science* **145**, 266 (1964); G. F. Leedale, *Euglenoid Flagellates* (Prentice-Hall, Englewood Cliffs, N.J., 1967), p. 23; J. J. Blum and G. M. Padilla, *Exp. Cell Res.* **28**, 512 (1962); G. M. Padilla and T. W. James, *ibid.* **20**, 401 (1960).
17. R. J. Britten and D. E. Kohne, *Science* **161**, 529 (1968).
18. D. D. Brown, C. S. Weber, J. H. Sinclair, *Carnegie Inst. Wash. Year B.* **66**, 580 (1967).
19. Supported by AEC contract AT(11-1) GEN 10, P.A. 20. I thank G. Prevost for technical assistance.

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Intellectual Deficit Associated with Transplacentally Induced Microcephaly in the Rat

Abstract. *Fischer rats injected with methylazoxymethanol late in pregnancy produce young with considerably reduced cerebral hemispheres. They appear normal otherwise. As adults these animals make many more errors in the Hebb-Williams maze than do control animals.*

Microcephaly can be chemically induced in the rat by injection of pregnant females with methylazoxymethanol, a potent carcinogen which is the aglycone of a naturally occurring glucoside (cycasin). The effect is remarkably uniform, and has been replicated in more than one strain of rats. Such microcephalic animals have been maintained for over a year and showed no gross deficiencies in physiology or behavior (*1*).

It would appear, however, that a significant reduction of brain mass should affect adaptive capacity. Therefore we sought to establish whether such transplacentally induced microcephaly is associated with impairment of intellectual function in the adult animal.

The experimental subjects (12 male and 12 female rats) were offspring of six Fischer females that had each been injected intraperitoneally with a total of