Obesity, the most striking clinical manifestation of MSG treatment, has been produced experimentally in mice treated with two other chemical compounds, gold thioglucose (GTG) (*6*) and bipiperidyl mustard (*7*). In each case, however, animals were treated in adulthood, lesions were reported in the ventromedial nucleus ("satiety center") of the hypothalamus, and treated animals were considered hyperphagic. In that hypothalamic lesions in MSG-treated animals routinely spared ventromedial nuclei and these animals were consistently hypophagic by comparison with littermate controls, a mechanism other than appetite disturbance must be considered. Whether a regulatory mechanism affecting fat metabolism in the mouse can be localized to the arcuate nucleus, or other brain areas selectively destroyed by MSG treatment, requires further study.

The assumption that MSG is an entirely innocuous substance for human consumption has been questioned recently in view of its role in the Chinese restaurant syndrome (*8*). The finding that neuronal necrosis can be induced in the immature mouse brain by 0.5 mg/g of MSG raises the more specific question whether there is any risk to the developing human nervous system by maternal use of MSG during pregnancy. The primate placenta maintains amino acids in consistently higher concentrations in the fetal circulation than are found in the maternal circulation, the ratio for glutamic acid being greater than 2:1 (*9*). In fact, when high doses of phenylalanine were given to a pregnant rhesus monkey, the ratio of mother to fetus for this amino acid remained unchanged so that exceedingly high fetal blood levels resulted (*9*). The possibility that brain lesions could occur in the developing primate embryo in response to increased glutamic acid concentrations in the maternal circulation, therefore, warrants investigation.

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10. Supported in part by PHS grants NB-04816, MH-07081, MH-13002, and MH-38894. I thank Drs. E. Robins, A. I. Cohen, M. Constant, and D. Kipnis for advice, and Miss S. Freeman for the original observation that glutamate-treated mice appeared abnormally fat.

11 March 1969

Deoxyribonucleic Acid Methylase Activity in Pea Seedlings

Abstract. *Deoxyribonucleic acid methylase activity has been detected in a preparation of disrupted nuclei prepared from pea seedlings. S-Adenosyl-L-methionine acted as a donor of methyl groups, and the product of the reaction was identified as 5-methylcytosine. The reaction had a sharp temperature optimum at about 30°C and was unusual in that the DNA methylase was able to methylate DNA in the crude extract.*

5-Methylcytosine is a minor component of the DNA of many organisms (*1*), but it is a major component of the DNA's of higher plants (*2*). In bacterial DNA's only about 1 percent and in animal DNA's only about 5 percent of the cytosines are methylated (*1*), but in plant DNA's between 20 and 30 percent of the cytosine is methylated. In bacteria and in animal tissues, 5-methylcytosine is the product of highly specific enzymes, called DNA methylases, which transfer methyl groups from *S*-adenosyl-L-methionine to specific sites in DNA of high molecular weight (*3*). Despite the superabundance of 5-methylcytosine in plant DNA's, nothing has been known about its biosynthesis. To our knowledge this is the first report of DNA-methylase activity in a higher plant, pea seedlings.

The assay for DNA-methylase activity of pea seedlings was identical with that developed for detection of DNA-methylase activity in extracts of mammalian tissues (*4*). This assay measures incorporation of radioactive methyl groups from ^{3}H-methyl-*S*-adenosyl-L-methionine into material which survives deproteinization by the Marmur procedure (*5*), is precipitable in cold 5 percent perchloric acid, and is not degraded by 0.5*M* NaOH at 60°C. This assay removes protein and RNA that is methylated by other enzymes present in the crude nuclear extracts. In plants deproteinization has the additional virtue of removing some green material that interferes with scintillation counting of the product (Table 1). Most of the radioactivity remaining in acid-precipitable material after this procedure appeared to be in DNA, as it was rendered acid-soluble by treatment with pancreatic deoxyribonuclease (Table 1). The product of the reaction was further characterized by hydrolysis in 90 percent formic acid at 180°C for 30 minutes to generate the free bases,

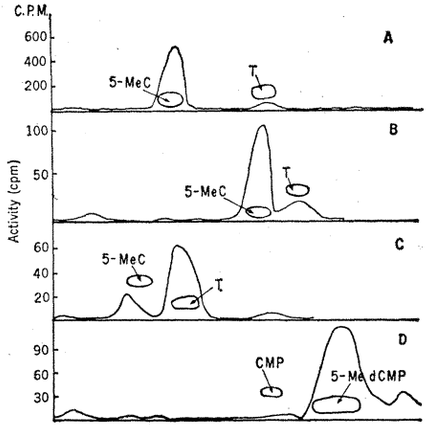

Fig. 1. Chromatography of the methylated product. The reaction mixture and conditions of incubation were the same as those described under Table 1. Product measured by the standard assay procedure described in the text. (A) The product was hydrolyzed at 180°C for 45 minutes with 0.3 ml of 90 percent formic acid and chromatographed in butanol:H_2O:NH_3 for 16 hours. (B) A second sample of the formic acid hydrolyzate was chromatographed in isopropanol:HCl for 20 hours. (C) A third sample of the formic acid hydrolyzate was treated with HNO_2 for 4 hours at 20°C and chromatographed in isopropanol:HCl for 20 hours. (D) The final alkali stabile sediment was washed with ether, dried, and then hydrolyzed with 20 μg of pancreatic deoxyribonuclease (Worthington) in 2.5 ml of tris (*p*H 7.5) 0.01*M* containing 0.005*M* $MgCl_2$ for 3 hours at 37°C. Then 0.3 ml of 0.5*M* tris (*p*H 8.5) was added with 210 μg of venom phosphodiesterase (*Crotalus adamanteus*) (Worthington), and the incubation was continued for 3 more hours. The material was dried, resuspended in H_2O, applied to Whatman No. 3 paper, and chromatographed for 16 hours in borate buffer.

the hydrolyzate then being chromatographed on Whatman No. 3 paper in both butanol:NH_3:H_2O (65:1:35) and in isopropanol:HCl:H_2O (65:17.5:17.5). Most of the radioactivity cochromatographed with 5-methylcytosine in both systems; however, between 5 and 10 percent of the radioactivity chromatographed with thymine (Fig. 1). The formic acid hydrolyzate was further characterized by treatment with HNO_2 at room temperature for 4 hours. This treatment, which causes deamination of 5-methylcytosine to thymine, caused the radioactivity associated with the 5-methylcytosine spot to disappear and to appear in the thymine spot, as expected if the reaction product were 5-methylcytosine (Fig. 1). The product was characterized as the deoxyribonucleotide of 5-methylcytosine after hydrolysis with deoxyribonuclease and phosphodiesterase and subsequent paper chromatography in a borate buffer system, which separates ribonucleotides from deoxyribonucleotides (*5*). Most of the radioactivity migrated as the deoxyribonucleotide of 5-methylcytosine (Fig. 1).

Table 1. Effect of deoxyribonuclease, ribonuclease, NaOH hydrolysis, and deproteinization upon the assay of DNA methylase activity.*

Sample	Methyl incorporated (pmole per mg of protein)
Experiment 1	
Complete assay†	13.7
Minus *E. coli* DNA	10.7
Complete assay plus 50 μg deoxyribonuclease	2.1
Experiment 2	
Complete assay†	8.7
Assay minus deproteinization	4.2‡
Assay minus NaOH hydrolysis	20.0
Assay minus NaOH hydrolysis plus 10 μg ribonuclease	14.5

* Pea seedlings (Pea Thomas Laxton from Burpee Seed Company) were grown for us in vermiculite under greenhouse conditions by Dr. C. A. Price (Department of Plant Biology, Rutgers). When the seedlings were between 8 and 10 days old, the leaves were harvested, and nuclei were prepared from about 70 g of leaves (*8*). The nuclei were further purified by centrifugation in 2.4*M* sucrose at 41,000*g* for 80 minutes. The pellet was resuspended in 20 ml of 0.01*M* tris-buffer, *p*H 7.8, and frozen and thawed three times to disrupt the nuclei. The resulting preparation of broken nuclei was light green and had a protein concentration of about 1 mg/ml. † The reaction mixture contained, in a final volume of 0.2 ml, 10 nmole of tris, *p*H 7.8, 0.5 μc of ^{3}H-methyl-*S*-adenosyl-L-methionine, 40 μg of *E. coli* DNA, and 150 μg of pea chromatin fraction protein. After 60 minutes of incubation at 27°C, the tubes were chilled in ice and 1.8 ml of water added. The reaction mixtures were then deproteinized according to the method of Marmur (*5*): 0.2 ml of 25 percent sodium dodecyl sulfate was added and the tubes were heated for 10 minutes at 60°C. After cooling at room temperature, 0.45 ml of 5*M* $NaClO_4$ was added and samples were deproteinized once with 2.5 ml of chloroform : isoamyl alcohol (24 : 1). The supernatant was removed, 0.2 ml of salmon testis DNA was added as carrier, and the DNA was precipitated by the addition of 0.2 ml of 5 percent trichloroacetic acid. The precipitate was centrifuged, washed twice with 2 ml of 5 percent trichloroacetic acid, dissolved in 1 ml of 0.5*M* NaOH, heated at 60°C for 10 minutes, and reprecipitated with 5 percent trichloroacetic acid. This procedure was repeated once again. The final sediment was hydrolyzed in 0.2 ml of 5 percent perchloric acid at 90°C for 10 minutes. The hydrolyzate was transferred to 10 ml of 1,4-bis [2-(5-phenyloxazolyl)] benzene (POPOP) : 2,5-diphenylazole (POP) : toluene ethanol scintillation fluid and counted in a Packard Tri-Carb scintillation counter. ‡ Not corrected for quenching. The corrected value would be 16.8 pmole per mg of protein.

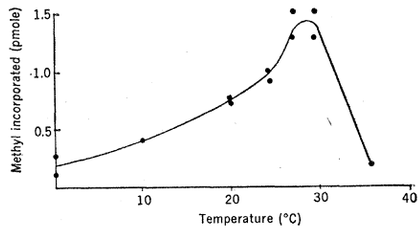

Fig. 2. Effect of temperature upon rate of methylation; 165 μg of pea nuclear extract was used per tube. The duration of incubation was 60 minutes. Otherwise the conditions of incubation and assay were identical with those described under Table 1.

Most of the pea seedling DNA-methylase activity was bound to insoluble material. After centrifugation at 10,000*g* for 10 minutes, only about 7 percent of the activity of the preparation remained in the supernatant. The temperature optimum was between 28° and 31°C; little activity was seen at 37°C (Fig. 2). At 28°C the incorporation of methyl groups into DNA proceeded at a nearly linear rate for about 1 hour, and the product of the reaction appeared to be stable for up to 4 hours. The enzyme had no apparent requirement for either Mg^{2+} or 2-mercaptoethanol and was inhibited by these compounds at concentrations of $5 \times 10^{-3}M$ and $1 \times 10^{-3}M$, respectively. Ethylenediaminetetraacetic acid (5×10^{-3}) had little or no effect.

The observed activity was probably not caused by bacterial contamination of the crude extract or by the presence of intact cells in the extract because (i) upon microscopic observation, no whole cells were seen; and (ii) the reaction was stimulated by the addition of *Escherichia coli* DNA. In addition no activity was seen at 37°C, and no 6-methylaminopurine was found in the product of the reaction. The latter two observations argue strongly against the possibility of bacterial contamination, because the bacterial enzymes are quite active at 37°C and almost all bacteria contain 6-methylaminopurine in their DNA (*1*).

In apparent contrast to the bacterial DNA methylases, the pea seedling enzyme incorporated methyl groups into the DNA in the nuclear extract—the addition of heterologous DNA (from *E. coli*) stimulated incorporation by only 30 to 60 percent (Table 1). The activity of the DNA methylase from pea seedling nuclei was, however, somewhat lower than that of comparable crude cell extracts from bacteria. For example, specific activity of the pea seedling preparation was only about 6 percent that of a cell extract from *Corynebacterium diphtheriae* as measured by Gold and Hurwitz (*3*). Because self-methylation of DNA by the pea seedling preparation is so small in absolute quantity, the question of whether this amount of self-methylation truly represents a difference between the plant enzyme and the known bacterial enzymes awaits purification of the pea seedling enzyme before it can be reliably resolved. Another DNA methylase from a higher organism, rat spleen DNA methylase, which, as a crude extract, also methylated the DNA present in nuclear extracts (*4*), was still able to methylate rat spleen nuclear DNA after partial purification (*6*).

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9. Work undertaken during the tenure of an Eleanor Roosevelt Cancer Fellowship of the International Union Against Cancer awarded to F.K. and supported by American Cancer Society grant P-333C and U.S. Public Health Service grant CA10665-01. We thank E. Kalouskova and C. Fordham for technical assistance.

6 January 1969; revised 13 March 1969