presence within of microtubular cristae characteristic of protozoan mitochondria and similar to the cristae seen in the mitochondria of the control group (Fig. 2B). Furthermore the light microscope reveals only a single vacuole in uninucleate parasites, the merozoite, and young trophozoite, whereas larger dividing forms have several vacuoles; this finding corresponds well with electron-microscope observation of the normal parasite, showing the merozoite and young trophozoite with a single mitochondrion whereas schizonts have several. The drug had no detectable effect on the ultrastructure of the mitochondrion of the host cell (Fig. 2A). These observations support the view that the swelling of the mitochondria, seen in the parasites treated with primaquine, is not due to any special artifact of fixation but rather to the effects of the drug.

Certain agents are known to cause active swelling of mitochondria (*3*). Our observations indicate that primaquine probably acts similarly except that it is somewhat specific in its action, causing swelling only in the mitochondria of the parasite and not in those of the host cell.

The structure of the mitochondrion is closely linked to its metabolic functions (*3*), and any major disruption of this structure is undoubtedly accompanied by impairment of its associated metabolic processes. Although the mode of action of primaquine remains unknown, we feel that the morphological changes that we report are sufficiently severe to account for the strong action of this drug on the exoerythrocytic stages of malaria.

RICHARD L. BEAUDOIN
Department of Parasitology, Naval Medical Research Institute, Bethesda, Maryland 20014
MASAMICHI AIKAWA
Department of Experimental Pathology, Walter Reed Army Institute of Research, Washington, D.C. 20012

References and Notes

1. A. G. Davis, C. G. Huff, T. T. Palmer, *Exp. Parasitol.* **19**, 1 (1966).
2. P. K. Hepler, C. G. Huff, H. Sprinz, *J. Cell Biol.* **30**, 333 (1966).
3. A. L. Lehninger, *The Mitochondrion* (Benjamin, New York, 1964), pp. 180–204.
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19 March 1968

Plantlets from Aspen Tissue Cultures

Abstract. *After 2 weeks in the dark, leafy shoots were initiated on subcultured callus tissue of triploid quaking aspen on Wolter's medium without auxin and with 6-benzylaminopurine at 0.05, 0.10, 0.15, or 0.20 milligram per liter substituted for kinetin. Shoots transferred to Wolter's medium under light (323 milliphots) grew large roots and were isolated as plantlets.*

Plants have been reproduced from single isolated cells of carrot, tobacco, endive, parsley, and asparagus (*1*), but plantlets of trees have not been reported from either cell or tissue cultures. Roots and shoots, not directly attached to each other, grew from the same pieces of subcultured callus of European aspen and birch (*2*) and of triploid quaking aspen (*3*). I now report the growth of large roots from the base of leafy shoots initiated on aspen callus, and the isolation of plantlets.

Firm white callus was isolated (*4*) from root sprouts of triploid quaking aspen (*Populus tremuloides* Michx.) and subcultured monthly for 1 year on Wolter's defined medium (*5*). Twenty seed pieces (33 ± 5 mg) from stock tissue grown 5 weeks from subculture were distributed among ten 125-ml erlenmeyer flasks for each medium made by adding 6-benzylaminopurine (BA) at 0.05, 0.10, 0.15, or 0.20 mg/liter to Wolter's medium without auxin and kinetin.

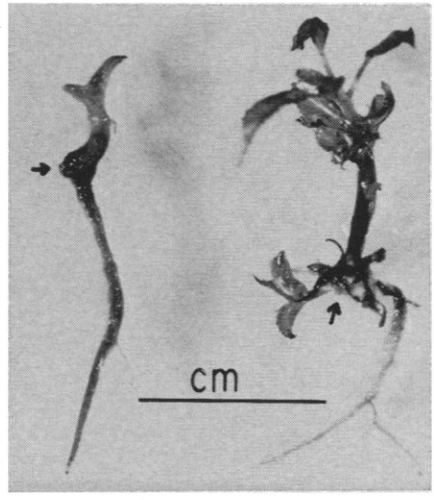

Fig. 1. Shoots, with attached roots, isolated from callus (arrows) as plantlets.

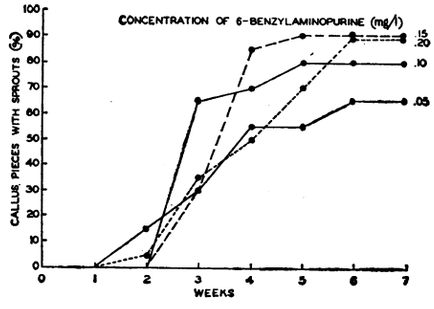

Fig. 2. Percentage of pieces of callus with shoots initiated on BA mediums without auxin.

Aerial shoots grew from cut surfaces of callus on 0.05-percent BA medium after 2 weeks in the dark at 27°C. Four shoots on separate pieces had large attached roots after 3 weeks when they were transferred to Wolter's medium (with 2,4-dichlorophenoxyacetic acid at 0.04 mg/liter and kinetin at 1.0 mg/liter) under 323-mphot light for 16 hours daily. Two plantlets (Fig. 1) dried during photography and two were placed in fixative for sectioning.

Vigorous shoots appeared on three pieces (15 percent) within 2 weeks and on 65 percent of the pieces within 6 weeks on medium containing BA at 0.05 mg/liter; smaller shoots grew from 30 to 65 percent within 3 weeks and from 80 to 95 percent of the callus pieces within 6 weeks on mediums containing more BA (Fig. 2). Shoots grew above and below the surface of mediums containing BA at 0.10, 0.15, or 0.20 mg/liter, but were not attached directly to roots. Shoots left on BA mediums in light or dark did not form roots.

Shoots grown from callus in the dark on medium containing BA at 0.05 mg/liter, in two subsequent experiments, are now on Wolter's medium in the light for initiation of roots. Roots but no shoots grew from callus in the dark on mediums made by substitution of 6-(γ,γ-dimethylallylamino) purine at 0.0001, 0.001, 0.01, 0.1, or 0 mg/liter for BA. The growth of aspen plantlets from callus tissue now opens the way for production of genetically identical trees from single cells.

LAWSON L. WINTON
Genetics and Physiology Group, Institute of Paper Chemistry, Appleton, Wisconsin 54911

References and Notes

1. F. C. Steward, M. O. Mapes, K. Mears, *Amer. J. Botany* **45**, 707 (1958); A. C. Braun, *Proc. Nat. Acad. Sci. U.S.* **45**, 932 (1959); I. K. Vasil, A. C. Hildebrandt, A. J. Riker, *Science* **146**, 76 (196→ I. K. Vasil and A. C. Hildebrandt, *Amer. J. Botany* **53**, 869 (1966); C. Wilmar and M. Hellendoorn, *Nature* **217**, 369 (1968).
2. C. Jacquiot, *Ann. Sci. Forestières* **21**, 317 (1964); *J. Inst. Wood Sci.* **16**, 22 (1966).

3. M. C. Mathes, *Forest Sci.* **10**, 35 (1964); L. L. Winton, *Proc. Executives' Conf. 30th* (1966), p. 17; K. E. Wolter, in preparation.
→ L. L. Winton, *Amer. J. Botany* **55**, 159 (1968); *Phyton* **25**, 15 (1968).
→ K. E. Wolter, *Amer. J. Botany* **53**, 263 (1966).
6. Supported by the Pioneering Research Committee, Institute of Paper Chemistry, Appleton, Wisconsin. I thank Susan Lebergen for her assistance; Karl Wolter, Forest Products Laboratory, USDA, Madison, Wisconsin, for suggestions and 6-benzylaminopurine; and Folke Skoog, Department of Botany, University of Wisconsin, Madison, for 6-(γ,γ-dimethylallylamino)purine.

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Reaction of Nitrosoguanidine (N-Methyl-N′-Nitro-N-Nitrosoguanidine) with Tobacco Mosaic Virus and Its RNA

Abstract. *"Nitrosoguanidine"* (N-*methyl*-N′-*nitro*-N-*nitrosoguanidine) *acts on polynucleotides in neutral aqueous solution, methylating guanine in the 7-position, and, to a much lesser extent, adenine. In 67 percent dimethylformamide the effect on the purines decreased, and what seems to be 3-methyl-cytosine appeared. In formamide solution no base changes were detected. Nitrosoguanidine had little mutagenic effect on tobacco mosaic virus RNA in water; it had greater effect in dimethylformamide, and even greater in formamide; it was a strong mutagen only when it acted on intact tobacco mosaic virus particles. Thus, neither the methylation of guanine nor the cytosine modification represents the main mutagenic event.*

N-Methyl-*N′*-nitro-*N*-nitrosoguanidine (nitrosoguanidine) reacts with the guanine residues of polynucleotides occurring in base-stacked array, but not with guanine-containing compounds of small molecular weight (*1*). It was only slightly mutagenic for tobacco mosaic virus RNA (TMV-RNA) under conditions that favored this guanine modification, but it was highly mutagenic under conditions that did not favor the guanine reaction. We now report (i) that the main reaction product is 7-methylguanine, and that adenine is to a lesser extent methylated; (ii) that this reaction proceeds in two steps: the binding of the reagent (possibly between guanine residues), favored by low temperatures; and the transfer of the methyl group, occurring only at elevated temperatures; (iii) that cytosine residues are methylated under conditions which favor mutagenesis and do not favor the G reaction; and (iv) that the most mutagenic action on TMV-RNA occurs under conditions causing minimum base changes even after prolonged reaction periods.

(i) When TMV-RNA was treated with nitrosoguanidine labeled with C^{14} in the methyl group, and the 5′-nucleotides obtained by treatment with snake venom diesterase (SV-DE) were chromatographed on paper, the typical spot showing white fluorescence (W) (*1*) was found to be radioactive (117,000 count/min). Several minor radioactive products were present in amounts too low for detection and characterization by ultraviolet spectrophotometry. Judged by the amounts of C^{14}, these comprised from 4 to 15 percent of the 7-methylguanylic acid fraction. As judged by its location, the strongest was a decomposition product of the reagent. The previously described fluorescent derivative of adenylic acid (*1*), possibly 7-methyladenylic acid, showed 4 percent of the radioactivity of 7-methylguanylic acid. Other products were probably 1,7-dimethylguanylic acid and 7-methylguanosine. A similar assortment of minor reaction products was detected spectrophotometrically when synthetic guanine-containing polynucleotides were treated with nitrosoguanidine and then were degraded with enzyme and chromatographed.

In contrast, when guanidine C^{14}-labeled nitrosoguanidine was used, the fluorescent guanine reaction product (W) was unlabeled, and no other radioactive spots were identified on the chromatogram. Thus, the only carbon that was added to the RNA in the course of the reaction was the methyl carbon.

The ultraviolet absorption maximum (277 nm), as well as the fluorescence excitation and emission maxima (292 to 298 nm, and 380 to 385 nm, respectively, the higher values being the result of slight contaminations with cytidylic acid) of the fluorescent product W corresponded to those of 7-methylguanylic acid. In turn, the spectrum of the product of acid hydrolysis of RNA treated with nitrosoguanidine or of W corresponded to those of 7-methylguanine. When 7-methylguanylic acid was used as marker in the chromatographic isolation of W, the two moved equally in both dimensions. When 7-methylguanine was compared chromatographically to the product of acid hydrolysis of W these also behaved alike (Table 1).

That 7-methylguanylic acid is a fluorescent component of an RNA has not been noted until quite recently (*2*), probably because analyses were usually preceded by acid hydrolysis, which yields the no-longer fluorescent 7-methylguanine.

7-Methylguanylic acid is surprisingly resistant to *Escherichia coli* phosphatase. Thus, treatment of SV-DE digests with amounts of the phosphatase that completely dephosphorylated the four typical nucleotides left much of the methylated nucleotide intact. However, with much more enzyme, most of the material was transformed to the still-fluorescent methylnucleoside ("Y"). Because 7-methylguanylic acid is more strongly basic than cytidylic acid it can be separated from all nucleotides by electrophoresis. Although the predominant action of nitrosoguanidine on RNA has now been identified as a methylation, this reagent differs from typical alkylating agents (such as dimethyl sulfate) in its conformational

Table 1. Chromatography of guanine nucleotides, nucleosides, and bases. Solvent I consisted of 75 ml of ethanol and 30 ml of 1*M* ammonium acetate *p*H 7.5. Solvent II was 80 ml of saturated ammonium sulfate, 18 ml water, and 2 ml of isopropanol. Solvent III was 68 ml of isopropanol, and 17.6 ml of concentrated HCl and 14.4 ml of water. Descending chromatography Whatman No. 3 MM paper was used for I and II, and Whatman No. 1 for III.

Compound	Ratio of movement to guanylic acid and guanine, respectively		
	Solvent I	Solvent II	Solvent III
"W"	1.2	1.3	
7-Methylguanylic acid	1.2	1.3	
1-Methylguanylic acid		0.55	
"Y" (nucleoside derived from "W")	2.6	1.3	
7-Methylguanosine	2.6	1.3	
1-Methylguanosine		0.5	
Guanosine	2.6	0.7	
Guanylic acid	1.0	1.0	
"W" hydrolyzed in 1*N* HCl 1 hour 100°C			1.33
7-Methylguanine			1.35
Adenine			1.16
Guanine			1.00