In comparing these actions, however, the effective dose of *N*-cyclohexyl linoleamide seems to be considerably smaller than that of the other substances.

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References and Notes

1. A suggested international nonproprietary name.
2. K. Toki and H. Nakatani, in *Proceedings of the Symposium on Drugs Affecting Lipid Metabolism*, Milan, 1965, in press; T. Imai, H. Nakatani, K. Toki, K. Matsuka, H. Fukushima, T. Seki, paper presented at 23rd International Congress of Physiological Sciences, Tokyo, 1965.
3. *The Pharmacopeia of the United States of America*, 17th revision, 1965 (distributed by Mack Pub. Co., Easton, Pa.), pp. 1075, 1076.
4. Silica gel G (Merck) and a mixture of petroleum ether, diethyl ether, and acetic acid (85 : 15 : 3, by volume) were used. Approximate R_F values of *N*-cyclohexyl linoleamide and several lipids components were as follows: *N*-cyclohexyl linoleamide, 0.26; free fatty acids, 0.35; triglycerides, 0.70; cholesterol esters, 1.0; and cholesterol, 0.27. For clear separation of cholesterol and *N*-cyclohexyl linoleamide, the plate, after drying, was developed once more with a mixture of petroleum ether, diethyl ether, and acetic acid (85 : 15 : 1, by volume).
5. H. Jeffay and J. Alvarez, *Anal. Chem.* **33**, 612 (1961).
6. J. L. Bollman, J. C. Cain, J. H. Grindlay, *J. Lab. Clin. Med.* **33**, 1349 (1948).
7. J. R. Senior, *J. Lipid Res.* **5**, 495 (1964).
8. H. H. Hernandez, D. W. Peterson, I. L. Chaikoff, W. G. Dauben, *Proc. Soc. Exp. Biol. Med.* **83**, 498 (1953); H. H. Hernandez and I. L. Chaikoff, *ibid.* **87**, 541 (1954).
9. The composition of cholesterol-free diet, in grams per 100 g, was as follows: casein, 20; hydrogenated coconut oil, 10; sucrose, 63.8; agar, 2; and adequate amount of vitamins and minerals. Cholesterol diet was made by substituting 1 percent of cholesterol and 0.5 percent of ox-bile extract for an equal amount of sucrose in the above diet.
10. R. G. Herrmann, *Proc. Soc. Exp. Biol. Med.* **94**, 503 (1957).
11. R. G. Gould and R. P. Cook, in *Cholesterol*, R. P. Cook, Ed. (Academic Press, New York, 1958), p. 241.
12. Numerous studies concerning plant sterols and the brain extracts were summarized by D. Steinberg, in *Advances in Pharmacology*, S. Garattini and P. A. Shore, Eds. (Academic Press, New York, 1962), vol. 1, p. 137; R. J. Jones, O. K. Reiss, M. F. Golden, in *Proceedings of the Symposium on Drugs Affecting Lipid Metabolism*, S. Garattini and R. Paoletti, Eds. (Elsevier, Amsterdam, 1961), p. 219.
13. We thank C. Naito of Tokyo University, and T. Imai, K. Toki, and our other colleagues of Sumitomo Chemical Co. Ltd. for their cooperation and advice.

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Chloroplast DNA from Tobacco Leaves

Abstract. *DNA from tobacco leaf chloroplasts was isolated as a single component with a buoyant density in CsCl of 1.702 compared to 1.697 for nuclear DNA. 5-Methylcytosine is present in nuclear DNA but absent in chloroplast DNA. Chloroplast DNA, with a guanine-cytosine content of 43 percent, has a melting temperature of 86°C and renatures completely on slow cooling, whereas nuclear DNA (melting temperature, 84°C; guanine-cytosine content, 40 percent) does not renature. About 9 percent of the total DNA in tobacco leaves is chloroplast DNA representing about 4.7 × 10^{-15} gram of DNA per chloroplast with a molecular weight of approximately 4 × 10^7.*

In a number of organisms, extranuclear (or satellite) DNA's differing in buoyant density from DNA obtained from nuclei have been found to be localized in two cytoplasmic particles, chloroplasts and mitochondria (*1*). Lyttleton and Peterson (*2*) concluded that in tobacco leaves, chloroplast DNA, if present, must be very similar to nuclear DNA in density, whereas Shipp *et al.* (*3*) reported chloroplast DNA of density 1.703 compared to nuclear DNA of density 1.690. However, Green and Gordon (*4*), using precautions to eliminate the possibility of a contribution of DNA from a bacterial contamination, have obtained a density of 1.696 for nuclear DNA and 1.706 for chloroplast DNA in tobacco seedlings. Satellite DNA's isolated from chloroplasts and mitochondria have been associated with variable amounts of nuclear DNA. Thus, the possibility of a second satellite component with a base composition virtually indistinguishable from that of nuclear DNA could not be ruled out. We now report the isolation of highly polymerized chloroplast DNA as a single component from purified chloroplast particles. Some of the properties which distinguish chloroplast DNA from nuclear DNA are also presented.

Lamina tissue of *Nicotiana tabacum* or *N. glutinosa* from leaves 5 to 7 cm in length was chopped by razor blades in Honda medium as described previously (*5*) in order to preserve the biphasic structure of chloroplasts in a recognizable condition. This homogenizing procedure also preserves nuclei in a condition closely resembling the in vivo state. After the homogenate was filtered through four layers of fine mesh cloth, the filtrate was centrifuged at 1000*g* for 15 minutes. The pellet of chloroplasts and nuclei corresponding to 15 g of fresh leaves was resuspended in 2 ml of homogenizing medium and then layered on a 25-ml discontinuous gradient of 60 (10 ml), 45 (10 ml), and 20 percent (5 ml) sucrose in Honda medium. The gradients were centrifuged for 2 hours at 25,000 rev/min in a Spinco SW 25-1 rotor, and the green-layer band at 45 percent sucrose was collected. Examination of this preparation with the phase microscope revealed well-preserved chloroplasts. When the preparation was stained with acridine orange and again examined by fluorescence microscopy, no intact or large fragments of nuclei could be detected. The pellet at the bottom of the tube consisted mainly of nuclei but was still contaminated with some unbroken cells and chloroplasts. This pellet served as the source of nuclear DNA without attempting further purification of the nuclei.

DNA was extracted from the original 1000*g* pellet of chloroplasts and nuclei; it was also extracted from the purified chloroplasts and from the enriched nuclear pellet. Each preparation was subjected to phenol extraction in the presence of 2 percent lauryl sulfate. After three phenol extractions, the aqueous phase was dialyzed against SSC (0.15*M* NaCl, 0.015*M* sodium citrate), treated with ribonuclease (50 μg/ml) for 2 to 3 hours, again extracted with phenol; the DNA was then precipitated with alcohol. At this stage DNA fibers were formed on a glass rod from all preparations except those of the purified chloroplasts where small amounts of DNA were collected by centrifugation. All three DNA preparations were then extensively dialyzed against SSC.

The buoyant density determined according to Schildkraut *et al.* (*6*) for nuclear DNA was 1.697 and 1.702 for chloroplast DNA, values resembling those of Green and Gordon (*4*). The density of nuclear DNA also agrees closely with the value of 1.698 reported by Lyttleton and Peterson (*2*). Density of spinach chloroplast DNA's differs from that of tobacco chloroplast DNA, the density of the former being 1.719 and 1.705 (*1*). The densitometer tracings of the ultraviolet photographs of tobacco leaf DNA's are presented in Fig. 1. The difference of

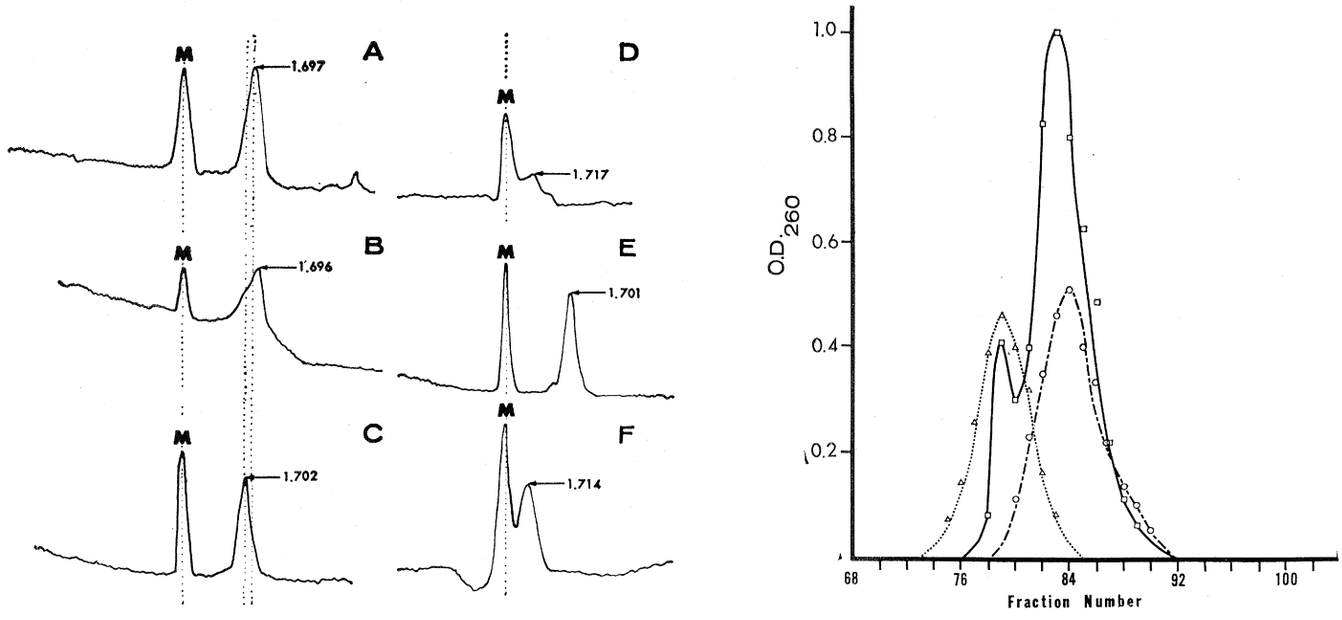

Fig. 1 (left). Densitometer tracings of ultraviolet photographs obtained from analytical density-gradient centrifugation of nucleic acid in CsCl. The gradients were centrifuged for 20 hours at 44,770 rev/min with *Myxococcus xanthus* DNA of density 1.727 as marker. *A*, nuclear DNA; *B*, DNA from mixture of nuclei and chloroplasts sedimented at 1000*g*; *C*, chloroplast DNA; *D*, heat-denatured chloroplast DNA; *E*, renatured chloroplast DNA; *F*, heat-denatured nuclear DNA. Fig. 2 (right). Preparative density-gradient centrifugation in CsCl of DNA obtained from nuclei (—○———○—), from the mixture of nuclei and chloroplasts sedimented at 1000*g* (—□—□—), and chloroplasts (---△---△---).

0.005 in the density of chloroplast and nuclear DNA, though small, was always reproducible. As shown by Fig. 1,*A* and *C*, there is only one component present in the DNA isolated from nuclei or chloroplasts which produces a very sharp band. However, the DNA extracted from the original 1000*g* pellet consisting of a mixture of nuclei and chloroplasts reveals a broad band (Fig. 1*B*) when centrifuged in CsCl, suggesting the presence of more than one DNA component. The major component corresponds in density with the DNA extracted from the enriched nuclear pellet, whereas the minor component would appear to have a density like that of the DNA extracted from the purified chloroplasts.

We were also able to resolve the chloroplast DNA from nuclear DNA by preparative density-gradient centrifugation in CsCl of final density of 1.710, using 150 μg samples of DNA extracted from purified chloroplasts and from the enriched nuclei. The solutions were centrifuged for 65 hours at 37,000 rev/min in the SW 39 Spinco rotor, and three-drop fractions from each tube were collected, yielding a total of 158 fractions from a total volume of 4.28 ml. After addition of 1.0 ml of SSC to each fraction, the optical density (O.D.) reading at 260 mμ was obtained. Chloroplast and nuclear DNA band at different positions in the gradient, the former having a peak at fraction 79, the latter at fraction 84 (Fig. 2). Both DNA's form bands as a single component. Figure 2 also shows the behavior of 250 μg of DNA extracted from the 1000*g* pellet consisting of a mixture of chloroplasts and nuclei and subjected to the same density-gradient treatment. Two components were resolved, the minor one banding at fraction 79 and the major one at fraction 83. In this latter case, integration of the areas beneath the curve indicates 8 percent of the total DNA as having been derived from the chloroplasts. Comparison of the amount of DNA, as determined by colorimetric method (*7*), extracted from purified chloroplasts with that extracted from the chloroplast-nuclear mixture in the pellet formed at 1000*g* also yields 9 percent chloroplast DNA, which represents about 4.7×10^{-15} g of DNA per chloroplast.

Chloroplast DNA on heating behaves as a single, sharply melting component with a melting temperature (T_m) of 86°C, dispersion of 6.6°C, and hyperchromicity ($O.D._{max}/O.D._{room\ temp.}$) of 1.32. The corresponding values for nuclear DNA are 84°, 6°, and 1.31. Chloroplast DNA was heated to 100°C in SSC, quickly cooled in ice, and analyzed for density. The densitometer tracings presented in Fig. 1*D* now show density of 1.717, an increase of 0.015. Another portion, after melting, was annealed in double-strength SSC for 2 hours at 60°C, slow-cooled to room temperature, and then analyzed for density. The chloroplast DNA renatured completely (Fig. 1*E*), with a final density of 1.701. Nuclear DNA similarly heated showed an increase of 0.017 in density on heating, but renaturation could not be achieved even after extended annealing at 60°C.

Chloroplast DNA pooled from a large number of individual isolations was analyzed for base composition after formic acid hydrolysis followed by paper chromatography (Table 1). The guanine-cytosine contents calculated from T_m and buoyant den-

Table 1. Base composition (molar percentage) of DNA from tobacco.

Component	Source of DNA	
	Nuclear (%)	Chloroplast (%)
Adenine	29.3	29.7
Guanine	23.5	23.2
Cytosine	12.2	18.7
Thymine	30.7	28.4
5-Methylcytosine	4.3	B.D.*
GC, via chromatography	40.0	41.9
GC, via density	37.8	42.9
GC, via T_m	35.9	40.8

* Below detection.

sity according to Marmur and Doty (*8*) and Schildkraut *et al.* (*6*) are also presented. There is close agreement for the base composition obtained by these three methods. A striking difference between the nuclear DNA and the chloroplast DNA is in the absence of 5-methylcytosine in the latter. To confirm this observation, 200 μg of chloroplast DNA was hydrolyzed and subjected to paper chromatography; the area on the paper corresponding to the 5-methylcytosine position, as ascertained from the corresponding nuclear DNA analysis, was cut out and eluted. Material absorbing at 260 mμ was not present.

Thus on the basis of density, T_m, renaturation, and base composition, chloroplast DNA represents a specific chloroplast entity. It resembles mitochondrial DNA at least in its renaturation behavior (*9*). Analyses indicate a molecular weight for tobacco chloroplast DNA that is close to 4×10^7 daltons. We have found that purified chloroplasts contain a DNA-dependent, RNA polymerase which has different properties from an RNA polymerase contained in nuclei. Whether chloroplast DNA functions as a template for RNA synthesis, in chloroplasts, as well as whether homologies exist between nuclear and chloroplast DNA's, is unknown (*10*).

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References and Notes

1. A. Gibor and S. Granick, *Science* **145**, 890 (1964); K. K. Tewari, J. Jayaraman, H. R. Mahler, in *Some Aspects of Yeast Metabolism*, R. K. Mills, Ed. (Oxford University Press, London, 1966); E. H. L. Chun, M. H. Vaughan, A. Rich, *J. Mol. Biol.* **7**, 130 (1963); R. Sager and M. Ishida, *Proc. Nat. Acad. Sci. U.S.* **50**, 725 (1963); M. Edelman, C. A. Cowan, H. T. Epstein, J. A. Schiff, *Proc. Nat. Acad. Sci. U.S.* **52**, 1214 (1964).
2. J. W. Lyttleton and G. P. Peterson, *Biochem. Biophys. Acta* **80**, 391 (1964).
3. W. S. Shipp, F. J. Kieras, R. Haselkorn, *Proc. Nat. Acad. Sci. U.S.* **54**, 207 (1965).
4. B. R. Green and M. P. Gordon, *Science* **152**, 1071 (1966).
5. D. Spencer and S. G. Wildman, *Biochemistry* **3**, 954 (1964).
6. C. L. Schildkraut, J. Marmur, P. Doty, *J. Mol. Biol.* **4**, 430 (1962).
7. K. Burton, *Biochem. J.* **62**, 315 (1956).
8. J. Marmur and P. Doty, *J. Mol. Biol.* **5**, 109 (1962).
9. K. K. Tewari, W. Votsch, H. R. Mahler, B. Mackler, *ibid.*, in press.
10. K. K. Tewari and S. G. Wildman, in preparation.
11. Research supported by contract AT (11-1)-34, project 8, Division of Biology and Medicine, AEC; and research grant AI 00536 from the NIH. We thank Dr. E. Rosenberg for the *Myxococcus xanthus* DNA.

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Contractile Cells in Human Seminiferous Tubules

Abstract. *Electron microscopic study of the peritubular connective tissue in human testis reveals the presence of "contractile-type" cells rather than of typical fibrocytes. Their cytoplasm has numerous fine filaments and other components characteristic of smooth muscle cells. The rough-surfaced endoplasmic reticulum, however, is relatively prominent. In some instances, the nuclear surface appears scalloped or folded, and the cell surface presents an irregular profile, similar to that of contracted cells.*

Since Stieve's (*1*) accounts of the structure of the human testis, the boundary tissue (tunica propria) of the seminiferous tubules has been regarded as a typical connective tissue, consisting primarily of fibrocytes, collagen, and elastic fibers. Recently, a contractile cell has been demonstrated by means of electron microscopy in the testis of the rat (*2*) and of the mouse (*3*). These cells form a distinct structure, similar to a sheath, in the connective tissue surrounding the tubular epithelium. When these cells are observed in routinely prepared specimens stained with hematoxylin and eosin, however, their nature is practically indistinguishable from that of the more peripheral layers of fibrocytes.

The presence of a contractile cell in the testis is consistent with observations by other investigators of rhythmic contractions in the testes of various laboratory animals (*4*). Only a few studies of the fine structure of the peritubular connective tissue in humans have been reported. These, however, have failed to identify a similar or comparable contractile component in the human testis (*5*). We now report the presence of a cell within the tunica propria of the seminiferous tubules which has most, if not all, of the morphological characteristics of a contracile cell.

We studied specimens of seminiferous tubules obtained by biopsy from six healthy individuals (22 to 39 years old). The tissues were obtained at operation and immediately fixed in a 3 percent glutaraldehyde solution buffered with phosphate. The specimens were further fixed in 1 percent osmium tetroxide (in phosphate buffer), dehydrated in ethanol, and embedded in Epon. Thin sections were stained with lead citrate (*6*) and were then examined and photographed with an RCA-EMU 2e electron microscope.

Examination of the cells within the peritubular connective tissue reveals that they are uniform in appearance and represent a single cell type. In most instances, there are three to four layers of cells surrounding the tubule epithelium, each layer being separated from the adjacent layer by varying amounts of collagen (Figs. 1 and 2). The shape of the peritubular cells is similar to fibrocytes seen in other tissues; the cytoplasm, however, is more abundant. The nucleus is elongate in shape, but in some specimens its surface is scalloped, as in Fig. 1, or marked by deep folds, an appearance which is characteristic of contracted smooth muscle cells. In those cells with folded nuclear surfaces, the surface contour of the cell is also irregular in profile (Fig. 1).

When viewed at higher magnifications much of the cytoplasm is seen to contain abundant, closely packed, fine filaments. The filaments, which are in the order of 50 to 60 Å in diameter, are arranged in a parallel array (Fig. 3).

At various sites within the filamentous areas of the cell, there are other, more dense structures (arrows, Figs. 2 and 4). These localized cytoplasmic densities are identical in appearance to the "dense bodies" (*7*) or "attachment devices" (*8*) seen in smooth muscle cells.

The nonfilamentous regions of the cytoplasm contain the usual cell organelles. The Golgi apparatus and centrioles are perinuclear in position, while mitochondria, free ribosomes, and elements of both the smooth- and rough-surfaced endoplasmic reticulum are more widely distributed throughout the cytoplasm.

The rough-surfaced endoplasmic reticulum consists of single profiles of variable length which are orientated along the breadth of the cell (see Fig. 2). The smooth endoplasmic reticulum consists largely of tubular membraneous elements. In addition, small intensely staining particles, slightly larger in size than ribosomes, are present. Their size and intensity of staining with lead indicate that these particles are glycogen granules.

The plasma membrane is studded with numerous vesicular invaginations of the type generally referred to as