Table 2. Adrenal catecholamine levels after administration of L-dopa. These were the same animals as used in Table 1.

Group	Catecholamine (micrograms per adrenal pair)			
	Epinephrine	Norepinephrine	Dopa + dopamine	Total
Control	21.40 ± 1.31	7.3 ± 0.83	1.91 ± 0.26	30.61 ± 0.93
L-Dopa (4 days)	23.43 ± 1.34	5.5 ± 1.4	4.3 ± 0.52*	33.20 ± 0.97
L-Dopa (7 days)	23.43 ± 0.19	6.4 ± 0.88	4.5 ± 0.35†	34.33 ± 1.36

* Significantly different from controls ($P < .005$). † Significantly different from control ($P < .001$).

ganglionic sympathetic nerve activity after L-dopa administration has been reported (*17*). This effect may be more directly responsible for lowering tyrosine hydroxylase. Thus prolonged changes in the intensity of nerve activity may directly influence the level of tyrosine hydroxylase in the tissues. Some support for this mechanism has been provided by work with genetically hypertensive rats. Louis *et al.* (*18*) have suggested that these animals have diminished sympathetic nerve activity which may represent an attempt to compensate for their hypertension. Tarver and Spector (*19*) have demonstrated that in these animals the level of tyrosine hydroxylase in blood vessels is decreased. On the other hand splanchnic nerve transection in the rat does not lower tyrosine hydroxylase in the denervated adrenal gland (*13*), a finding that is admittedly difficult to reconcile with the above hypothesis. Thus the mechanism of L-dopa's action on this key enzyme in norepinephrine synthesis remains in doubt.

In addition to that of tyrosine hydroxylase, activities of other enzymes taking part in catecholamine biosynthesis and degradation may be altered by L-dopa administration. Tarver and Spector (*19*) have reported that monoamine oxidase in the tissues is increased in animals given L-dopa. We have also examined aromatic L-amino acid decarboxylase in several tissues of rats given L-dopa for 7 days. The decarboxylase levels were unaltered in adrenals and brain, but were reduced by 30 percent in the kidney. Changes in dopamine-β-hydroxylase and catechol-*O*-methyltransferase have not yet been measured in animals on prolonged L-dopa treatment. The effects of tyrosine hydroxylase may be of greatest significance because of the key regulatory role of this enzyme in catecholamine biosynthesis.

The possibility that L-dopa produces a general inhibition of protein synthesis seems unlikely. The activities of brain tyrosine hydroxylase and of aromatic L-amino acid decarboxylase in several tissues, including the adrenal gland, were unaltered at a time when adrenal tyrosine hydroxylase was diminished. In addition, the activity of a third enzyme, monoamine oxidase, increases after similar treatment with L-dopa (*19*).

Our results may have significance in dealing with Parkinsonian patients on L-dopa therapy. If L-dopa lowers tyrosine hydroxylase in the peripheral tissues of these patients, sudden withdrawal of the drug may leave the patients with a diminished capacity for synthesizing norepinephrine until enzyme levels return to normal.

WALLACE DAIRMAN
SIDNEY UDENFRIEND
Roche Institute of Molecular Biology, Nutley, New Jersey 07110

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Scanning Electron Microscopic Observations of Surface Structure of Isolated Human Chromosomes

Abstract. *Isolated human chromosomes dried by the critical-point method have been assumed to retain their original three-dimensional shape when viewed under a transmission electron microscope. Our scanning electron microscopic study confirms this interpretation and reveals an appearance like that of a skein of yarn. The existence of fiber bridges between chromatid pairs and among chromosomes is demonstrated.*

The critical-point drying method (*1*) permits the removal of water from sensitive (fragile) objects without the passage of an air-liquid interphase through it. In drying, the object is not affected by surface tension. Also eukaryotic chromosomes dried by such a technique and examined by transmission electron microscopy appear to be preserved in their original three-dimensional extension (*2, 3*). This belief was supported by quantitative evaluation of contrast (*3*).

The great depth of focus of scanning electron microscopy has allowed us to examine in a direct manner the three-dimensional aspect of chromosomes dried by the critical-point method. Human chromosomes appeared as a skein of chromatin fibers, which confirmed previous concepts of chromosome conformation.

Peripheral lymphocytes from human male donors were cultured according to standard techniques. Cells were harvested by centrifugation, treated with hypotonic Hanks solution for 10 minutes, and applied to the surface of distilled water in a Langmuir trough. Surface tension disrupted the cells causing the release of their chromosomes, which we picked up by touching Formvar-coated aluminum disks to the surface of the water. Chromosomes adhere better to Formvar-coated disks than to uncoated disks. The wet chromosomes were quickly submersed in 30 percent ethanol, dehydrated in

an ascending series of ethanol solutions, transferred to amyl acetate, and finally dried by the critical-point method.

The specimens were subjected to rotatory gold paladium shadowing that resulted in the deposition of a coating approximately 150 Å thick. A Cambridge Mark IIA scanning electron microscope was used to examine the shadowed specimens. The scanned image was preserved on Polaroid PN55 film.

Over 30 partial metaphase spreads were examined. The chromosomes in each had a mottled appearance at low magnifications (Fig. 1). Many fibers of apparently varying diameters were seen to radiate from the body of the chromosome. At higher magnification (Fig. 2), the typical fibrous composition of human chromosomes is resolved. Tortuous bumpy fibers of rather even overall diameters loop from the interior to the surface of the chromosome and form its highly uneven surface.

These fibers are organized in the three commonly identifiable configurations—acrocentric, metacentric, and submetacentric (Fig. 1). Tortuous, looping fibers and straight radiating fibers were the only structural elements detectable. Occasionally remnants of small mitochondria were found attached to the irregular surface of the chromosome as at the telomere of the metacentric chromosome (Fig. 1).

At the highest magnification used in this investigation (×28,000), an average fiber diameter of roughly 700 Å was found after the rotatory shadowing procedure. Unshadowed fibers of this preparation measured approximately 400 Å in diameter. From these data a thickness of about 150 Å is calculated for the conductive gold palladium metal layer.

A submetacentric chromosome oriented horizontally can be seen in Fig. 2. The long arms of the chromosome are seen along the upper and lower edge of the micrograph, respectively. One short arm extends to the right, and the other extends toward the observer. A few fibers radiate from the chromatids, and one can be seen clearly to connect the long arms. In many instances, an extensive enmeshment of the fibers from two chromatids is present. Often this enmeshment makes it difficult to distinguish individual chromatids (Fig. 1). In none of the preparations studied could any difference in the structure of the fiber or its arrangement be discovered at the telomeres, along the chromatid, or at the centromere.

Scanning electron microscopy confirms the concept that a chromatid is essentially a rod that has a varying but roughly circular cross section. Light microscopy indicates that the body of a chromosome possesses a distinct border, which appears to be the result of fixation, staining, and limited resolution.

When the decreased packing density of fibers at the periphery of the chromosome is disregarded, a calculation of the overall packing density of fibers in the volume of a chromatid may be made. From the total dry mass of 10.61×10^{-13} g of a submetacentric Group E chromosome, determined by

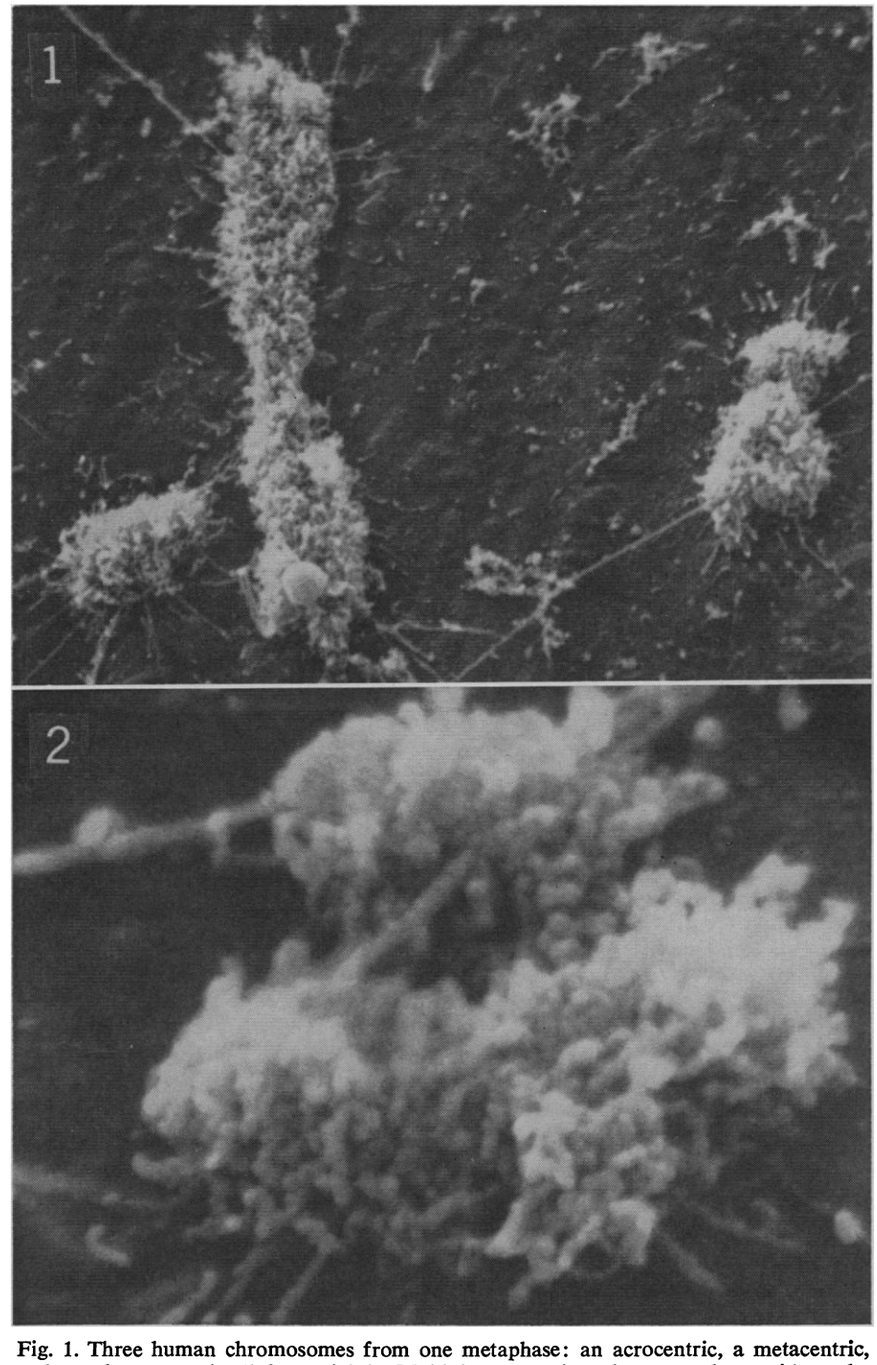

Fig. 1. Three human chromosomes from one metaphase: an acrocentric, a metacentric, and a submetacentric (left to right). Multiple connections between chromatids make the distinction of individual chromatids difficult. Note long and short connecting fibers between chromosomes (× 6,700, AFIP negative 70-9348). Fig. 2. Single submetacentric chromosome illustrates the skein of chromatin fibers (× 28,000, AFIP negative 70-9347).

quantitative electron microscopy (*4*), and the average dry mass of the fiber of 11.61×10^{-16} g/μm, a total fiber length of 457 μm per chromatid is calculated. A measured radius of 0.3025 μm and a measured length of 3.025 μm yield a volume of 0.87 μm^3 for a chromatid. Knowledge of fiber diameter (365 Å) and fiber length (457 μm) permits calculation of total fiber volume per chromatid which is 0.48 μm^3. Fiber volume divided by chromatid volume yields 0.55; that is, 55 percent of the volume of a chromosome is occupied by chromatin fibers. Preliminary calculations for some other chromosomes for which detailed quantitative evaluations of total and fiber masses and dimensions are available (*3*, *5*) indicate that the percentage of fiber packing in human metaphase chromosomes varies from 30 to 70 percent.

For scanning electron microscopy, electrically nonconducting objects should be coated with a suitable conductor that permits the greatest exposure of the fine structure of the object. Deposition of 150 Å of metal undoubtedly obscured fine structural detail, but we were able to inspect the three-dimensional aspect of chromosomes dried by the critical-point method in a more useful and demonstrable manner than is possible by stereoscopic electron micrography. Our study supports the folded fiber model of chromosome structure proposed by DuPraw and Rae (*6*).

H. M. Golomb
G. F. Bahr

Biophysics Branch,
Armed Forces Institute of Pathology,
Washington, D.C. 20305

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Primitive Earth Synthesis of Nicotinic Acid Derivatives

Abstract. *Nicotinonitrile, 2-cyanopyridine, and 4-cyanopyridine can be synthesized under primitive earth conditions by the action of electric discharges on ethylene and ammonia. The electric discharge first synthesizes pyridine and hydrogen cyanide, which react in the discharge to form the cyanopyridines. Nicotinonitrile would have hydrolyzed in the primitive ocean to nicotinamide and nicotinic acid.*

The synthesis under primitive earth conditions of organic compounds such as amino acids, purines, and pyrimidines has been demonstrated by a number of investigators, but the synthesis of a vitamin has not been reported until recently (*1*). We find that nicotinonitrile (3-cyanopyridine) can be synthesized in small yield by the action of electric discharges on mixtures of various hydrocarbons and ammonia or nitrogen. Nicotinamide and nicotinic acid would be obtained by hydrolysis of the nitrile.

A mixture of ethylene (at a pressure of 330 mm) and ammonia (at a pressure of 120 mm) in a 3-liter flask containing tungsten electrodes (*2*) was sparked with a corona discharge for 4 hours. The compounds volatile at −78°C were discarded, and the remaining yellow liquid was distilled into a trap cooled to +78°C. The yellow distillates from three similar experiments were combined and chromatographed on a 5-foot (152-cm) Porapak Q gas chromatograph column at 170°C. The peaks corresponding to 3-, 2-, and 4-cyanopyridine were collected and rechromatographed on a 5-foot, ⅛-inch (0.29-cm) SE-30 column at 50°C. The cyanopyridine peaks were again combined and rechromatographed on the Porapak Q column. The cyanopyridine peaks were collected separately and their ultraviolet spectra were measured with a spectrophotometer (Cary model 15). The spectra corresponded to known samples of 3-, 2-, and 4-cyanopyridine. The percentage yields of nicotinonitrile, 2-cyanopyridine, and 4-cyanopyridine were 7×10^{-4}, 4×10^{-4}, and 1.3×10^{-4}, respectively, based on the ethylene carbon.

It seemed likely that the cyanopyridines were formed from pyridine and cyanogen radicals. Therefore, the yield of pyridine from the reaction of various hydrocarbons and nitrogen or ammonia was investigated (Table 1). The yields of pyridine vary from 10^{-4} to 0.07 percent. Electric discharges are more efficient by a factor of 2 to 20 than pyrolysis (*3*) for the hydrocarbon-ammonia mixtures. The yield of pyridine is about the same with nitrogen as with ammonia if an electric discharge is used, but the hydrocarbon-nitrogen mixtures require a longer sparking time.

The yield of pyridine from the sparking of a mixture of methane and nitrogen over a water surface was 4×10^{-2} percent, greater by a factor of 7 than the yield in the absence of a water surface. It is not clear why the yield is greater in the presence of a water surface.

Pyridine synthesized in the atmosphere would dissolve in the primitive ocean as pyridine rather than as the pyridinium ion ($pK_a = 5.4$). However, pyridine would not be reactive in the ocean, and, since it is more volatile than water in dilute aqueous solution (*4*), a substantial fraction would remain in the atmosphere where it would be subject to the effects of electric discharges and other sources of energy. Hydrogen cyanide, which is obtained in high yields from electric discharges, is also more volatile than water and would be present in the atmosphere (*5*). The reaction of these two compounds was simulated by sparking a mixture of pyridine (6.2×10^{-3} mole) and hydrogen cyanide (1.6×10^{-2} mole) for 3 hours; the products were distilled as before. The distillate was injected into a combination gas chromatograph–mass spectrometer, and the products were identified by their retention times and mass spectra. The percentage yields of nicotinonitrile, 2-cyanopyridine, and 4-cyanopyridine were 1.4, 0.85, and 0.23, respectively, based on the added pyridine. The percentage yields based on the pyridine used were 2.8, 1.7, and 0.48, respectively. These results can account for the cyanopyridine yields from the sparking of ethylene and ammonia. The yield of pyridine was 5×10^{-2} percent in this experiment, and the cyanopyridine yields are about 1 percent of the pyridine yield.

Pyrolysis and ultraviolet light are less effective than electric discharges in bringing about the synthesis of cyanopyridines. A mixture of pyridine and hydrogen cyanide was pyrolyzed with a hot wire at 1200°C for 45 minutes. The