would suggest that cytosine modification represents the mutagenic event. However, when TMV-RNA was treated with nitrosoguanidine in the presence of dispersing solvents, the cytosine reaction was observed only in dimethylformamide, while the greatest numbers of mutants was obtained in formamide where, even after prolonged reaction periods, no base changes were detected (Tables 3 and 4). Thus, the high mutagenic action of nitrosoguanidine appears not to be attributable to either the observed guanine methylation or to the recently identified cytosine 3-methylation. The possibility that the mutagenesis of nitroguanidine acting on intact TMV is due to deamination has not yet been investigated. The nature of the amino acid replacements observed in the coat proteins of nitrosoguanidine mutants of TMV is not in conflict with such a mechanism. The chemical nature and biological significance of the observed adenine modification also require further study.

After this paper had been prepared for publication, we learned that Chandra *et al.* (*4*) had studied the template activity of nitrosoguanidine-treated polynucleotides and the transfer activity of treated sRNA, and had offered tentative conclusions that agree with our data. Other data on the action of nitrosoguanidine on DNA (*5*) gives evidence of the formation of small amounts (no quantitative data given) of 7-methylguanine.

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References and Notes

1. B. Singer and H. Fraenkel-Conrat, *Proc. Nat. Acad. Sci. U.S.* **58**, 234 (1967).
2. D. B. Dunn, *Biochem. J.* **86**, 14P (1963); M. Leng, F. Pochon, A. M. Michelson, in preparation.
3. H. Fraenkel-Conrat, *Biochim. Biophys. Acta* **49**, 169 (1961); B. Singer and H. Fraenkel-Conrat, *Progr. Nucleic Acid Res. Mol. Biol.*, in press.
4. P. Chandra, A. Wacker, R. Süssmuth, F. Lingens, *Z. Naturforsh.* **22b**, 512 (1967).
5. R. R. McCalla, *Biochim. Biophys. Acta* **155**, 114 (1968).
6. H. Fraenkel-Conrat *et al.*, unpublished results.
7. We thank Mrs. Ann Bradley for technical assistance. Supported by NSF research grants GB 3107 and GB 6209X and by NASA grant NsG 497.

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Motor Effects of Copper in the Caudate Nucleus: Reversible Lesions with Ion-Exchange Resin Beads

Abstract. *A method employing the use of ion-exchange resin beads is described for the punctate introduction of discrete amounts of various anions, cations, or zwitterions into given brain regions. A series of experiments utilizing the method to introduce ionic copper into the caudate nucleus with the resulting motor manifestations are discussed.*

The concentrations of ionic copper in the basal ganglia, substantia nigra, and nucleus ruber are, in general, higher than those in other areas of the brain (*1*). For this reason, there is considerable interest in the function of this metal in the extrapyramidal system (*2*). Ionic copper has particular relevance to the neuropathology of Wilson's disease, in which abnormally high concentrations of Cu^{2+} are found in the lenticular nucleus, and experimental methods have been developed to systematically alter the copper metabolism of various regions of the nervous system. For example, intraperitoneal injection of various concentrations of a copper sulfate solution resulted in an increase in the Cu^{2+} concentrations in the caudate and lenticular nuclei of guinea pigs. Highest uptake was observed in the caudate nucleus (*3*). No motor involvement has been reported, however. In cats, intraventricular injection of a complex of copper and albumin produces marked motor manifestations ranging from clonic and tonic seizures to rigidity and quadriplegia (*4*). Changes in copper metabolism in the nervous system have also been attempted through modification of the diet (*5*). Pregnant ewes maintained on a diet supplemented with ammonium molybdate bore lambs with ataxia or incoordination of the hind limbs, although no deficit was observed in the ewes. Direct and discrete application of copper complexes to substantia nigra, nucleus ruber, or targets in the basal ganglia has not been attempted.

The disadvantages of the techniques used in the earlier studies are several. Intraperitoneal introduction of chemical agents does not permit study of their metabolism in discrete areas, since the compounds can diffusely affect a number of neural and nonneural structures not under experimental control. The special problem of passage across the blood-brain barrier is also encountered. Intraventricular injection is slightly more specific, the agents introduced affecting only those structures which border the ventricles. Heavy-metal salts directly applied to particular targets can be localized, but the presence of both the anions and the cations makes difficult the assessment of their relative or interactive effects if both are biochemically active. Introduction of compounds in solution for the purpose of chemical localization in brain has a special disadvantage. Even if punctate application of the solution is successful, diffusion as a consequence of capillary action around the guide cannula is not uncommon, not only making the localization of effect difficult, but making more uncertain the amount of chemical applied at a given site.

Punctate application of a given ion-exchange resin bead loaded with the particular anion, cation, or zwitterion for which it is selective offers some advantages for the study of certain aspects of nervous system metabolism. Accurate amounts of the charged particle can be stereotaxically deposited through cannulas surgically placed at the desired location in brain. The resin portion of the complex is inert and will not enter into biochemical reaction. The ion-resin complex is solid, and it can be accurately placed without loss by capillary action of effective material from the site of application. As a solid, it is also relatively easy to handle. Of course, the disadvantage of tissue displacement is common to all methods.

Our interest in an effective method for the introduction of ionic copper into the brain originates from studies on the effects of caudate lesions on a variety of behaviors (*6*). In these earlier studies, bilateral caudate ablations in cats effected a loss of the ability to inhibit a previously learned instrumental feeding response. Others have also found that ablations and electrical and chemical stimulation of the caudate result in distinct behaviors similar to those we observed. Ablation of the caudate brings about ipsilateral turning if the lesion is unilateral and obstinate progression if the lesion is bilateral (*6–8*). However, electrical stimulation of the caudate evokes contralateral turning (*8*), as does application of acetylcholine or diisopropyl fluorophosphate (*9, 10*) whereas injection of alumina cream into the caudate produces a catatonic state in cats (*11*).

We used beads (0.074 to 0.037 mm) of Dowex 50W cross-linked with 8 percent devinylbenzene (50W × 8) to introduce Cu^{2+} into the caudate nuclei of cats. A 1*M* solution of $CuSO_4$ was continuously passed through a column (5 by 0.5 inch) of Dowex 50W × 8; the Cu^{2+} became attached to the resin beads, forming a copper-resin complex. The column was then washed with distilled and deionized water to remove excess $CuSO_4$, and the beads were dried and stored until use.

The ion-resin complex was deposited by means of a system of two concentric cannulas. A guide cannula was stereotaxically implanted in the caudate nucleus. Then a given amount of beads, determined by weight and volume, was tamped into the end of the injection cannula. When in place, the injection cannula protruded 0.5 mm beyond the guide canula. The beads were introduced into the tissue when the stylus of the injection cannula was depressed. Beads were injected into both caudate nuclei of awake, unanesthetized animals in which guide cannulas had been implanted approximately 2 to 3 weeks earlier. The amount of Cu^{2+} introduced at any one time was spectrophotometrically estimated to be 50 to 60 μg (*12*).

Deposition of 50 to 60 μg of Cu^{2+} in both caudate nuclei produced obstinate progression which reached its peak approximately 8 hours after the injection (Fig. 1). However, spontaneous treading was evident from 4 to 6 hours after introduction of the beads. Listlessness also developed early. This progression of symptoms perhaps reflects the increasing amount of Cu^{2+} exchanged. In addition to showing obstinate progression, the animals often stood motionless for prolonged periods (4 to 5 minutes) before spontaneously and lethargically walking again. At any time, any restraining force elicited resistance, treading, and subsequent obstinate progression. When an animal reached a wall, it made no attempt to turn or to avoid it; treading continued even though no forward movement was possible. These cats also walked off a 4-foot-high table without apparent concern for the obvious consequences. However, the righting reflex appeared normal. These effects subsided over a 3-day period and were completely absent after the 4th day.

Control injections, in the same animals, of beads containing sodium ions, mercuric, or hydrogen ions approximately 15 days apart did not produce obstinate progression. In any given animal, a second injection of the copper-resin complex 75 days after the first again produced the above described motor effects. It is, therefore, unlikely that the volume of tissue displaced by the beads or cannulas had any effects on the animals' behavior. The observed effects were quite specific to copper, at least in the concentration applied.

In other studies (*13*) these clinical findings have been quantified. Four groups of rats were compared for turning tendencies in terms of percentage of avoidance in a T-maze. Three groups of rats with cannulas implanted bilaterally in the caudate nucleus were injected on one side with (i) 50 to 60 μg of Cu^{2+}, (ii) 50 to 60 μg of Zn^{2+}, or (iii) 50 to 60 μg of H^+; rats bilaterally implanted with cannulas in the cortex overlying the hippocampus and injected on one side with Cu^{2+} made up the fourth group. Rats with Cu^{2+} injected in the caudate (group 1) significantly failed to inhibit ipsilateral turning to avoid an aversive stimulus as compared with the three groups of animals under control conditions (groups 2, 3, and 4). This finding indicates that, as in the cat studies, the effect is specific to cupric ion. Histological examination revealed some cavitation and demyelination in the area of implantation. A slight inflammatory reaction was noted in all sections stained with hematoxylin and eosin.

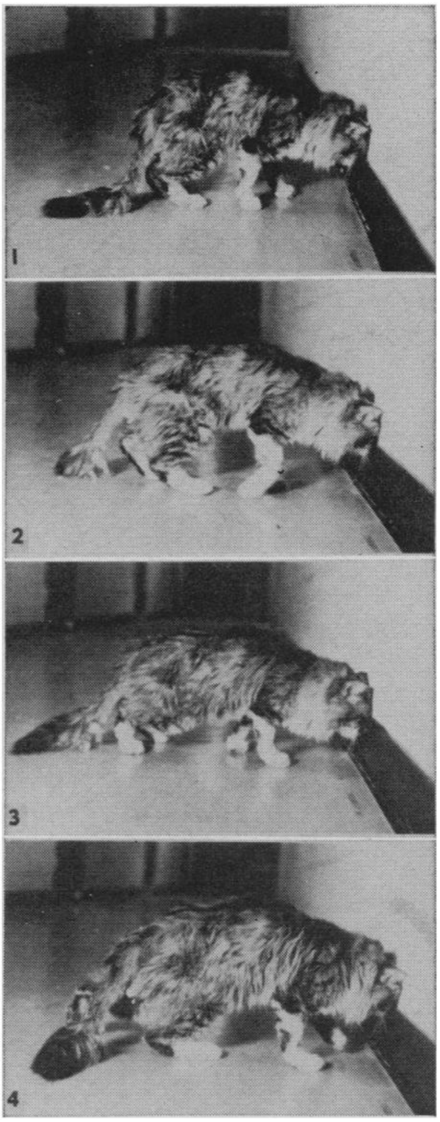

Fig. 1. Cat showing classical obstinate progression after deposit of copper-loaded (Cu^{2+}) ion-exchange resin beads into the head of the caudate nucleus on both sides. Note the characteristic hunching and treading as well as the butting and pressing of the head against the wall. This behavior is maintained until the head slips off the wall to either side. Normal slow walking then ensues until another immobile surface is encountered. Pictures were taken 8 hours after injection.

The use of ion-exchange resin beads to introduce metals into various regions of the nervous system appears to be a useful technique in studying the biochemistry of brain and behavior. By properly choosing the ion-exchange resin and employing the techniques described, many other charged particles (amino acids, inorganic anions and cations) could be introduced into different neural regions. Further, from our experiments we conclude that copper may have a significant and specific function in the metabolism of the caudate nucleus.

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References and Notes

1. R. H. S. Thompson, in *Regional Neurochemistry*, S. S. Kety and J. Elkes, Eds. (Pergamon Press, New York, 1961), p. 102.
2. J. P. Green, *Advances in Pharmacology* (Academic Press, New York, 1962), p. 349.
3. J. Holbrook, *J. Neurochem.* **7**, 60 (1961).
4. F. S. Vogel and J. W. Evans, *J. Exp. Med.* **113**, 997 (1961).
5. C. F. Mills and B. F. Fell, *Nature* **185**, 20 (1960).
6. S. S. Fox, D. P. Kimble, M. E. Lickey, *J. Comp. Physiol. Psychol.* **58**, 380 (1964).
7. F. A. Mettler, in *Diseases of the Basal Ganglia*, Association for Research in Nervous and Mental Diseases (Williams and Wilkins, Baltimore, 1942), vol. 11, p. 150.
8. A. M. Laursen, *Acta Physiol. Scand. Suppl.* **211**, 59 (1963).
9. R. P. White and H. E. Himwich, *J. Neurophysiol.* **20**, 81 (1957).
10. J. R. Stevens, C. Kim, P. D. McLean, *Arch. Neurol.* **4**, 47 (1961).
11. E. A. Spiegel and E. G. Szekely, *ibid.*, p. 55.
12. The unknown amount of Cu^{2+} introduced was reacted with 0.9 percent aqueous solution of sodium diethyldithiocarbamate and optically

compared with known concentrations on a Beckman DU spectrophotometer at a wave length of 437 nm.
13. L. Butcher and S. S. Fox, *Proc. Amer. Psychol. Ass.* **73**, 97 (1965).

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Stable Substoichiometric Cerium Oxide Formed in an Air Plasma

Abstract. *Cerium dioxide was reduced to a stable, substoichiometric monophase in an air plasma. This phase has been identified as* $CeO_{1.94}$ *by thermal gravimetric analysis. The existence of a stable, reduced phase of cerium oxide can be explained by interpretation of both theoretical and experimentally determined crystal data.*

Cerium dioxide, CeO_2, with a melting point of 2750°C, has attractive properties as a high-temperature refractory. It is stable in air but reduces to Ce_2O_3 when heated in reducing atmospheres for prolonged periods (*1*). The reduction of CeO_2 with hydrogen occurs appreciably at temperatures above 500°C (*2*).

Ceria and oxides of many other heavy metals can be reduced to oxygen-deficient compounds by heating them in inert or reducing atmospheres. For example, CeO_2 is reduced to numerous substoichiometric forms by heating in a vacuum at elevated temperatures (*3*); ZrO_2 is reduced to $ZrO_{1.96}$ by heating at 900°C at 10^{-3} torr for 1 hour (*4*); and ZrO_2 microspheres which are deficient in oxygen may be prepared in an argon plasma (*5*).

Stoichiometric CeO_2 particles were spheroidized by an inductively coupled plasma torch sustained by a 50-, 25-, and 10-kw induction heater modified to operate between 5 and 7 Mc/sec. The plasma gas employed with the 50-kw and 25-kw torches was air or O_2; the mixtures in the 10-kw system consisted of 50 percent Ar and 50 percent air or 50 percent Ar and 50 percent O_2. The operation of these plasma torches and the mechanism used to feed particles into the plasma has been described (*6*). Blue-black microspheres were obtained in the high-powered plasma systems but only ivory-colored microspheres were obtained in the 10-kw system. The spheroidized product was collected in air at room temperature.

Thermal gravimetric analyses from ambient to 1000°C were performed on representative samples of each of the cerium dioxide products. No weight increase was obtained on the ivory-colored microspheres. In duplicate samples a weight increase corresponding to an initial formula of $CeO_{1.940 \pm 0.005}$ was obtained on the blue-black microspheres. The $CeO_{1.940}$ can be oxidized by heating in air at 500°C for several hours; ivory-colored CeO_2 microspheres are produced.

Ceramographic cross sections of $CeO_{1.940}$ microspheres were optically examined at 200-power magnification. This examination showed that a homogeneous monophase was present.

X-ray diffraction powder patterns of the blue-black microspheres indicate a single face-centered cubic phase with the fluorite structure having a lattice constant of 5.4098 ± 0.0007 Å. The ivory-colored microspheres, whether from the original material or from oxidation of the blue-black microspheres, show this same face-centered cubic monophase with a lattice parameter of 5.404 ± 0.005 Å. These parameters were refined by the least-squares method (*7*) and the errors are given as standard deviations.

From x-ray diffraction studies on samples which varied between $CeO_{2.00}$ and $CeO_{1.82}$ in overall stoichiometry and which were variously quenched from 800° to 1200°C, Bevan (*3, 8*) found evidence of a face-centered cubic phase that had an unchanging lattice parameter (from a statistical viewpoint) down to an overall composition of $CeO_{1.83}$. The exact composition of this cubic phase is uncertain according to Bevan's data.

Our studies indicate that stable, monophase $CeO_{1.94}$ microspheres can be produced in an induction-coupled plasma torch under oxidizing conditions and that these microspheres will retain their nonstoichiometric nature even at 250°C. [Oxygen-deficient CeO_2 prepared in an inert or reducing atmosphere or in a vacuum contained, in general, two phases (*3*).] The absence of the $CeO_{1.94}$ prepared in the 10-kw plasma system indicates that the reduction is energy-dependent.

This detectable change of stoichiometry (CeO_2 to $CeO_{1.94}$) in the 25- and 50-kw system without notable modification of the fluorite structure or even significant alteration in lattice parameter is crystallographically favorable because the radius ratio of Ce^{4+} to O^{2-} [1.01 and 1.40 Å, respectively (*9*)] is below the ideal minimum for spherical ion contact of 0.73. Apparently, the gain from relief of anion-anion overlap by in-site reduction of a portion of Ce^{4+} ions to form larger Ce^{3+} ions (radius, 1.11 Å) and the concurrent oxidation of equivalent O^{2-} ions so nearly offsets the unfavorable energetics of Ce^{3+} relative to Ce^{4+} that in the higher-powered plasma environments, at least, a relatively stable, metastable, substoichiometric product can be obtained.

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References and Notes

1. I. E. Campbell, *High Temperature Technology* (Wiley, New York, 1956), p. 66.
2. E. Ryshkewitch, *Oxide Ceramics* (Academic Press, New York, 1960), p. 430.
3. D. J. M. Bevan, *J. Inorg. Nucl. Chem.* **1**, 49 (1955).
4. J. Livage and C. Mazieres, "Amorphous zirconias and nonstoichiometric zirconias," *AEC-TR-6692* (U.S. Government Printing Office, Washington, D.C., 1965).
5. D. B. Sullenger and K. D. Phipps, "Plasma spheroidized zirconia," *USAEC Rept. MLM-1205* (U.S. Government Printing Office, Washington, D.C., 1964).
6. T. B. Reed, *J. Appl. Phys.* **32**, 821 (1961).
7. Mound Lab. Program No. 114.
8. D. J. M. Bevan and J. Kordis, *J. Inorg. Nucl. Chem.* **26**, 1509 (1964).
9. L. Pauling, *Nature of the Chemical Bond* (Cornell Univ. Press, Ithaca, N.Y., ed. 3, 1960), p. 541.

10. We thank S. G. Abrahamson and B. R. Kokenge for comments and criticisms. Sponsored by AEC under contract AT-33-1-GEN-53 with Monsanto Research Corporation.

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Cholesterol: Treadmill Activity Accelerates Oxidation in Rats

Abstract. *Cholesterol-26-*^{14}C *was injected intravenously into male and female rats of two different strains. Recovery of radioactivity from the expired air was increased by treadmill activity.*

When cholesterol-26-^{14}C is injected intravenously into rats, 30 percent of the radioactivity can be recovered as $^{14}CO_2$ in the expired air within the first 24 hours (*1*). Oxidation of the side chain of cholesterol, similarly demonstrated in man (*2*), is implicated in the synthesis of bile acids (*3*), which play an important role in the elimination of cholesterol from the body (*4*). We have demonstrated that treadmill activity increases the rate of oxidation of cholesterol in rats.

Male and female rats of the Sprague-Dawley and Wistar strains were used. One milliliter of 0.9-percent solution of NaCl was added to 100 μl of methanol containing 1 mg of Tween-80 (Atlas