Selective Depletion of Caudate Nucleus Dopamine and Serotonin During Chronic Manganese Dioxide Administration to Squirrel Monkeys[1]

Prolonged inhalation of dust containing manganese produces mental and extrapyramidal disturbances in man. The latter may resemble Parkinson's disease, Wilson's disease, or a mixture of both[2]. Attempts to produce an experimental animal model of this condition have been successful. Repeated administration of $MnCl_2$ to Rhesus monkeys produced disturbances of extrapyramidal motor system function associated with pathological changes in the basal ganglia resembling the pathological changes seen in man[3]. Chronic inhalation of MnO_2 dust by monkeys results in degeneration of Purkinje cells and granule cells of the cerebellum. These changes are associated with incoordination and fine intention tremor[4]. Repeated i.m. injection of MnO_2 in olive oil into Rhesus monkeys produced astroglial proliferation and neuronal degeneration primarily in the subthalamic nuclei and medial segment of the pallidum[5]. These animals had impaired coordination and flexion posture of the extremities.

Parkinson's disease is characterized by variable degrees of rigidity, tremor, and bradykinesia. The major biochemical deficit is depletion of brain monoamines, chiefly in the striatum[6,7]. Many of the features of Parkinson's disease are ameliorated by treatment with L-3,4-dihydroxyphenylalanine, DOPA[8,9]. This drug is also beneficial for chronic manganese intoxication[8].

The similarities between Parkinson's disease and chronic manganese poisoning prompted a study of the biogenic amine concentrations in monkey brain after chronic administration of MnO_2.

Methods. MnO_2 was finely ground in a mortar and olive oil added to form a suspension containing 200 mg MnO_2/ml. A total of 1 ml of suspension was injected s.c. at several sites in 15 squirrel monkeys (*Saimiri sciurea*). 5 control animals received 1 ml of olive oil. All animals received a s.c. injection of 50,000 units of potassium penicillin G following either MnO_2 or the oil vehicle. Animals were injected on 11 August 1967 and 19 September 1967. 2 weeks following the first injection 1 control and 4 MnO_2 treated monkeys died. Gross and microscopic examination of liver, kidney and brain tissue failed to reveal abnormalities. During November 1967, 5 of the remaining treated monkeys developed signs of toxicity. These monkeys (Group A) and 2 control animals were sacrificed by decapitation on 30 November 1967. The brains were divided midsaggitally; half studied for monoamine content and half for histological changes. The remaining 6 test (Group B) and 2 control monkeys were given a final injection on 30 November 1967 and were sacrificed on 30 January 1968.

The caudate nucleus, cerebrum and brain stem specimens exclusive of the cerebellum from Group A were assayed for norepinephrine and dopamine[10]. The caudate nuclei and cerebrum of the Group B monkeys were homogenized in 0.01 *N* HCl; norepinephrine and dopamine were assayed in half the homogenate and serotonin in the other half[11].

Brain tissue for histological examination was fixed in 10% neutral formalin and sections stained with hematoxylin-eosin (HE) and with phosphotungstic acid-hematoxylin. Liver and kidney sections were fixed and stained with HE.

Results and discussion. All monkeys in Group A exhibited signs of muscular rigidity, flexion posturing of the extremities, or fine rapid tremors of the distal extremities manifest on intention. 2 of the test monkeys were unable to climb about the cage when prodded; 1 exhibited an exaggerated startle reaction, hitting its head upon the cage top when approached. Several monkeys exhibited obstinate progression which occasionally reached near sommersault proportions.

Unlike the first group, Group B monkeys did not show uniform toxic features. 2 appeared normal, 3 showed slight hand tremor, and only 1 showed signs of gross toxicity. The latter animal had pronounced hand tremor and impaired equilibratory coordination function.

Tables I and II summarize the results of the chemical analysis of the 3 groups. The average norepinephrine and dopamine content of the control monkeys in both groups were computed together. The concentration of caudate dopamine was significantly reduced after chronic MnO_2 administration, whereas the cerebral and brain stem norepinephrine content was not affected. Moreover, dopamine depletion appeared related to the degree of toxicity, i.e. Group A monkeys exhibited the greatest disturbance of motor function and also showed the largest reduction in caudate nucleus dopamine. Caudate nucleus concentrations of serotonin were also reduced in association with MnO_2 treatment (Table II).

Cerebral cortex, caudate nucleus, pallidum, thalmus and hypothalamus, hippocampus, subthalamic nucleus, substantia nigra and cerebellum were examined as well as other areas of the lower brain stem. There were no changes in neurones or glial elements nor in the vascular supply in any of the regions studied. Variable, often mild,

Table I. Concentrations of norepinephrine and dopamine in brain after MnO_2 treatment

	Caudate nucleus Dopamine ($\mu g/g \pm$ S.E.)	Cerebrum Norepinephrine ($\mu g/g \pm$ S.E.)	Brain stem Norepinephrine ($\mu g/g \pm$ S.E.)
Control (4)	2.90 ± 0.28	0.17 ± 0.01	0.52 ± 0.06
Group A (5)	1.29 ± 0.33[a]	0.18 ± 0.01	0.49 ± 0.03
Group B (6)	2.17 ± 0.13[b]	0.22 ± 0.03	0.55 ± 0.01

Number of observations in brackets. [a] $P < 0.01$, or [b] $P < 0.05$, when compared with control animals.

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vacuolar changes in liver cells were observed, however, these changes were noted in both test groups and did not correlate with the severity of clinical symptoms.

The finding that 2 conditions (one spontaneous and idiopathic, the other experimental) with manifestations of extrapyramidal motor dysfunction are associated with a deficiency of dopamine and serotonin in the caudate suggests that this deficiency may play a significant role in producing this dysfunction. In the present studies, the concentration of norepinephrine and serotonin in cerebrum and brain stem appeared essentially normal after MnO_2 treatment. Therefore, failure of the enzyme systems responsible for their formation and destruction in caudate was probably not the primary reason for depletion. Furthermore, it is important to recognize that clinical and biochemical abnormalities may appear before histopathological changes in brain can be demonstrated, and that the presence of histological changes in specific locations may not explain the clinical manifestations[12].

Table II. Concentrations of serotonin in caudate nucleus and cerebrum after MnO_2 treatment

	Caudate nucleus (μg/g)	Cerebrum (μg/g)
Control (2)	0.77 (0.66–0.88)[a]	0.19 (0.18–0.20)
Group B (6)	0.12 ± 0.05[b]	0.19 ± 0.03

Number of observations in brackets. [a] Mean (range). [b] Mean ± S.E.

Riassunto. Scimmie scoiattolo trattate cronicamente con MnO_2 presentano disturbi del sistema extrapiramidale ed una riduzione delle concentrazioni di serotonina e dopamina nel nucleo caudato. L'intensità dei disturbi extrapiramidali e la riduzione della concentrazione delle amine nel caudato appaiono correlate.

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Biosynthesis of Aflatoxins by Cell-Free Preparations from *Aspergillus flavus*

The aflatoxins are a group of hepatotoxic metabolites produced by *Aspergillus flavus* which infects foodstuffs, especially groundnuts[1]. Though a large number of reports have appeared on their occurrence in contaminated foodstuffs, their structure and toxicity, no major effort seems to have been made to investigate their biosynthesis. Mateles et al.[2,3] have studied the incorporation of some radioactive substrates into aflatoxins by the resting mycelium of *A. flavus* and Biollaz et al.[4] have determined the labelling pattern of aflatoxin B_1 so formed.

In the present investigation, the incorporation of some ^{14}C-labelled compounds into aflatoxins has been studied using a cell-free system prepared from *A. flavus* with a view to obtaining information on the mechanism of aflatoxin biosynthesis.

Materials and methods. Aspergillus flavus ATCC 15517 used in this study was maintained on a glucose peptone agar medium. Spores of 7-day growth on this medium were transferred to a 20% sucrose–2% yeast extract medium[5] in 500 ml Erlenmeyer flasks and incubated either on a rotary shaker for 5 days at 30 °C or without shaking for 15 days at 25 °C. The mycelial growth was filtered, washed with distilled water and ground up in a glass mortar with acid-washed sand and cold 0.05*M* phosphate buffer, pH 6.5. Subcellular fractionation was carried out in an International Refrigerated Centrifuge Model PR 2, by standard procedures[6]. The reconstituted homogenate was prepared by mixing equivalent amounts of nuclear and mitochondrial fractions with $^1/_{10}$ of the corresponding original volume of the supernatant.

As preliminary experiments indicated that the ability to incorporate acetate-1-^{14}C was found mostly in the mitochondrial fraction, either the reconstituted homogenate or the mitochondrial fraction was incubated in Erlenmeyer flasks for 6 h at 30 °C on a rotary shaker with the addition of suitable substrates and co-factors as given below: mitochondrial fraction or reconstituted homogenate in 0.05*M* phosphate buffer pH 6.5, 10 ml, glucose 60 mg, Difco yeast extract 40 mg, isocitrate 1 mg, ATP 1 mg, NADPH 0.5 mg and acetate-1-^{14}C (0.22 mc/m*M*) 10 μc, or DL-leucine-U-^{14}C (4.55 mc/m*M*) 8 μc or DL-mevalonic acid-2-^{14}C-lactone (3.35 mc/m*M*) 8 μc. At the end of 6 h, a carrier of a mixture of aflatoxins in 0.2 ml propylene glycol was added to the incubation mixture which was then extracted with chloroform and the extract was dried over anhydrous sodium sulphate, evaporated under reduced pressure and made up to 2 ml. An aliquot of this was subjected to thin-layer chromatography on Silica gel G (Merck) with 2% methanol in

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