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MANGANESE POISONING : A METABOLIC DISORDER

Participating Institutions:

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Study of the clinical and metabolic aspects of manganese poisoning. Findings include: levodopa is capable of correcting symptoms of extrapyramidal damage induced by manganese; susceptibility to manganese poisoning is linked to increased intestinal absorption of manganese in anemic individuals, with a positive correlation demonstrated for iron and manganese absorption; parallel pathophysiology is found between manganese poisoning and Parkinson's disease. Other areas of investigation include: long term treatment of manganic patients, mechanism for intermittent loss of control and involuntary movements induced by levodopa, and study of interaction of manganese and dopa and permeability of blood brain barrier to manganese.

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Final Report

on

Research Project

MANGANESE POISONING : A METABOLIC DISORDER

Principal Investigator:

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Conducted over the period October 1, 1970-July 31, 1974, years 08-10
This follows a report for years 01-07 of October 1, 1970

Our report for year 01-07 of this grant defines the clinical and metabolic aspects of manganese poisoning. This work culminated with the discovery that levodopa was able to correct the symptoms of extrapyramidal damage induced by manganese.

Susceptibility to manganese poisoning was linked to increased intestinal absorption of manganese in anemic individuals. While manganese ore dust is inhaled it is eventually degluted, thus the route of entry becomes the gastrointestinal tract. A positive correlation was demonstrated for iron and manganese absorption.

Parallel studies of parkinsonism and manganese poisoning proved to be highly rewarding. In Parkinson's disease depigmentation of the Substantia Nigra is accompanied by loss of dopamine in the caudate nucleus. Similarity of symptoms in manganese poisoning led to the hypothesis that a similar pathophysiologic basis was present. Levodopa had corrected the symptoms of extrapyramidal damage in parkinsonism by replenishing dopamine and it did so also in manganism. Thus important informations have been gathered at the bedside while improving a crippling disease.

The beginning of these three last years were marked by the emergence of differences to the response of levodopa between parkinsonian and manganic patients. These differences related to the side effects of levodopa in parkinsonians, that were notably absent in manganism. The most important side effects were involuntary choreoathetoid movement, psychotic phenomena and intermittent loss of effect of levodopa.

Thus, the major goals for this period became a) observations on long term treatment of manganic patients and induction of side effects. These continued during the three years to be absent. b) elucidation of mechanism for intermittent loss of control and involuntary movements induced by levodopa. Possible therapeutic approaches to these complications. c) experimental studies pertaining interaction of manganese and dopa, permeability of blood brain barrier to manganese and susceptibility to manganese poisoning and finally, teratologic effects of levodopa.

A. Observations on Long Term Treatment with Levodopa.

In manganic patients the response to levodopa has been a function of the neurological pattern of symptoms. Rigid, hypokinetic patients with loss of postural reflexes and impairment of gait have responded to doses greater than 3 g/day with marked to total reduction of rigidity, improvement of postural reflexes and gait and correction of hypokinesia but with no improvement of speech. The therapeutic effects lasted while L-Dopa was given but the symptoms reemerged after 7-10 days on placebo therapy. Notably absent in these patients have been side effects such as involuntary movements, mental aberrations or intermittent loss of therapeutic effect. Several of these patients returned to minor menial jobs.

Dystonic manganic patients were given doses of 4-5 g of L-dopa/day and showed improvement of dystonia and diminution of passive muscular tonus. However, physical strain and emotional stress can trigger the appearance of dystonic crisis. After a period of 3-4 months, L-dopa loses its effectiveness, and dystonia reemerges with greater intensity than that of the pretreatment level. Placebo administration for 10-30 days caused this abnormality to regress, and L-dopa therapy was reinstituted with the same therapeutic effects as before.

Another reaction to levodopa appeared in a patient who did not have rigidity but did have muscular hypotonus, tremor, slowness and impaired postural reflexes. Treatment with 1.2 g of L-dopa per day caused a marked aggravation of hypotonia, impairment of postural reflexes and further impairment of gait; 3 g/day also caused worsening of tremor. Placebo administration restored pretreatment levels after 48 hours.

A.1. Materials and Methods.

A.1.1. Patients.

Eight patients with chronic manganese poisoning were studied, their age ranging from 26-70 years. The time of exposure ranged between 7 months and 20 years and the period of illness ranged between 3 years to 26 years at the moment of onset of treatment. The patients were studied as in-patients in the Neurologic Center of the Catholic University, Santiago, Chile during the initial period of observation that lasted between 3 and 11 months. Afterwards they were granted extended periods of leave once the clinical picture and the clinical effects were fully assessed. The patients were made aware of the nature and ill side effects of this treatment but not of the timing period of levodopa or placebo administration. They were treated for periods that ranged between 39 days and 5 years.

TABLE I

	Period of Treatment Total	L-Dopa	Period Placebo	Maximal Daily Dose gm	Optimal Daily Dose gm
1	4.5 y	1.3 y	2	7.2	4.2
2	198 d	141 d	3	6	3.0
3	4.5 y	1.3 y	2	7.8	3.6
4	4.3 y	3 y	2	8.0	3.0
5	4.5 y	157 d	1	7.8	4.8
6	x	39 d	2	4.8	0
7	5 y	3 y	8	7.2	4
8	5 y	4.3 y	10	8.4	4.5

A.1.2. Drugs and Dosages.

L-dihydroxiphenylalanine (L-Dopa) was obtained from the Nutritional Biochemical Corporation and was made up in pink capsules containing 100 and 500 mg. The same capsules filled with lactose, served as placebo.

Within the period that covers this report all these patients were treated on the optimal daily dose that ranged between 3 and 4.8 g per day. Levodopa was administered in equal doses 6 i.d. starting at 7:00 AM. The maximal daily doses administered ranged between 4.8 and 8 g (Table 1). Frequent periods of placebo were interposed and these ranged between 2 and 10 periods of placebo (Table I).

A.1.3. Laboratory Tests.

Urine analysis, hematocrit, red and white blood cells total and differential counting were performed at least 3 times a year. Blood analysis were completed by means of hepatic tests; namely, billirubin concentration, flocullation test, choloidal red and zinc sulphate test; alkaline phosphatase concentration. Renal studies included creatinine.

A.1.4. Clinical Evaluation.

Motor function was evaluated once a day or several times a day during special periods of study by means of a modified scoring system for parkinsonism that yielded zero for maximal abnormality and 100 maximal disability.

A.1.5. Results.

T A B L E II

SCORING MANGANESE POISONING

CASE Nº	RIGIDITY	POSTURAL REFLEXES	HYPOKINESIA	FACIAL EXPRESSION	DISARTHRIA	DYSTONIA
1	75 (N)	50 (N)	50 (N)	25 (N)	0	-
2	50	-	25 (N)	25 (N)	0	-
3	50 (N)	50 (N)	50 (N)	50 (N)	0	-
4	50	50	50 (N)	25	0	-
5	25 (N)	25	25	25	25	-
6	-	25	25	25	0	75
7	-	50	-	-	0	50
8	-	-	-	-	0	50

(N) = Normality.

% Improvement: 25% = + dif.
50% = ++ dif.
75% = +++ dif.
100% = ++++ dif.

Table II summarizes our findings on rigidity, postural reflexes, hypokinesia, facial expression, disarthria and dystonia. The results are expressed as % of change of improvement from basal level. If the basal level was 50% impaired, a 50% improvement led to normality (N, case 3). The results of levodopa were marked in the patients exhibiting rigidity, three of them reached normality. Also significant results were gathered with regard to the correction of postural reflexes and hypokinesia. Facial expression in these patients did improve, but disarthria present in one patient (5) did not change. In dystonia, there was a 75-50% improvement of this symtpoms without reaching normality.

A.1.6. Side Effects.

Levodopa showed during the period of maximum doses side effects consisting mostly in nausea and vomiting but these were notably absent during the period of treatment on optimal doses. There was a 75 and 62% of frequency of these symptoms: Orthostatism, dyskinesia, and psychosis were absent (Table III).

TABLE III

SIDE EFFECTS LEVODOPA. MANGANESE POISONING.

	Nausea	Vomiting	Orthostatism	Dyskinesia	Psychosis
1	++	+	-	-	-
2	++	+	-	-	-
3	+	+	-	-	-
4	-	-	-	-	-
5	-	-	-	-	-
6	+	-	-	-	-
7	+	+	-	-	-
8	+	+	-	-	-
Total	75	62	0	0	0
Parkinsonism (52)	53%	21%	13%	36%	4%

In order to compare these effects a population of 52 Chilean parkinson patients were treated for periods of up to 16 months with levodopa, the maintenance dose being lower than the one for parkinsonian patients. There was an incident of 53% and 21% of nausea and vomiting respectively, however, orthostatism was present in 13% of the cases, dyskinesia or involuntary movements 36% and psychosis in 4% of the cases.

In summary, 8 manganic patients were treated for a period of 39 days to 5 years with levodopa, the symptoms of rigidity, postural reflexes, hyperkinesia and loss of facial expression responded markedly and in a sustained way. There was no effect on dysarthria. The effects on dystonia were marked with loss of effect after periods of 2 to 3 months of treatment or whenever stressful situations occurred.

There was a notable absence of involuntary movements and psychotic episodes in these patients.

B. Elucidation of mechanism for intermittent loss of control of parkinsonism by levodopa. Improvements of treatment.

Parallel studies of manganic patients and parkinsonian patients were conducted in order to elucidate the mechanisms by which there is episodic loss of symptomatic control (off-on phenomenon). We thought that this may be due to diminished entrance of levodopa into the brain. We linked this phenomenon with interception of the amino acid levodopa in peripheral tissues perhaps due to competition with alimentary amino acids. The possibility of this explanation was strengthened when the off-on phenomenon was diminished by restriction of protein intake in some patients receiving levodopa.

We studied therefore the influence of protein ingestion on the therapeutic efficacy, and metabolic effects of levodopa in Parkinson's disease and manganese poisoning.

B.1. Materials and Methods.

B.1.1. Patients.

1. Parkinsonian patients. Eight patients with idiopathic Parkinson's disease were studied at the Medical Research Center of Brookhaven National Laboratory. Their neurologic status varied between stability and almost total instability of symptomatic control. All were studied as in-patients in a metabolic ward. Once the acute study was finished 7 patients were kept for periods of 2 months to 1 year on a restricted protein diet.

B.1.2. Results.

The neurological scores (normal 0, maximal disability 100) on 2 g protein/kg body weight were at A.M. 27.8 ± 2.1 (SEM) and at 3 P.M. 46 ± 2 ($p < 0.001$). On 10 g protein/d scores were 24 ± 2 at 8 A.M. and 24 ± 2 at 3 P.M.

T A B L E IV

GROUP I

PROTEIN INTAKE	8 am SCORE		3 pm SCORE		
	Mean ± SEM	p_1	Mean ± SEM	p	p_1
1 g/kg/d	23.4 ± 1.7		33.5 ± 2.7	+	
1 g/kg/d (8 meals)	25.1 ± 1.8	NS	32.9 ± 2.0	Δ	NS
10 g/d	24.6 ± 2.1	NS	24.1 ± 2.7	NS	Δ
2 g/kg/d	27.8 ± 2.1	NS	46.7 ± 2.6	+	+
0.5 g/kg/d (8 meals)	21.8 ± 1.5	NS	30.1 ± 3.4	Δ	NS

p = 8 am vs 3 pm

p_1 = vs 1 g/kg/d protein intake

NS = not significant

Δ = $p < 0.05$

\+ = $p < 0.001$

In 7 patients maintained on .5 g protein/kg body weight/d for 2 months during one year levodopa requirement diminished progressively (Table I). On MK-486 the hydrazine of α-methyldopa, a peripheral blocker of decarboxylases the significant impairment of function observed during the high protein diet was markedly diminished.

This study confirms that a high protein meal can block the effects of levodopa in some patients with Parkinson's disease. Since doubling the protein intake usually offered in hospital diets, diminished both the therapeutic actions and cerebral side effects of levodopa in symptomatically unstable patients. This effect was evident in the long term as well as short term investigations. Restricted protein intake diminished also the percent of levodopa excreted as homovanillic acid while the high protein diet increased it. Furthermore, peripheral inhibition of decarboxylation of levodopa by MK-486 blocked the off-on phenomenon induced by a high protein diet. These latter findings argue against the assumption that competition between the amino acid levodopa and large amounts of neutral amino acids provided by the diet was the only explanation for the off-on phenomenon. They favor the assumption that fluctuating peripheral decarboxylation of levodopa causes fluctuations of the fraction of levodopa entering the brain thus contributing to the off-on phenomenon. We concluded that this regimen plus MK-486 was useful in treating unstable parkinsonian patients.

B.2. Manganic patients.

Six manganic in-patients were subjected to identical dietary regimens; namely, high protein diet consisting of 2 g/kg/d and a low protein diet consisting of 10 g protein/d. Patients were scored and ergometric measurements were performed repeatedly through the day. No significant changes were observed during treatment with both diets. Homovanillic acid excretion also failed to show differences; namely, 9 ± 6% of levodopa excreted in 24 hrs on the hyperproteinic regimen and 8 ± 3.5% on hypoproteinic regimen.

B.3. Growth hormones released by levodopa in manganic and parkinsonian patients.

Late metabolic effects of levodopa treatment but not as yet linked to the control of Parkinson's disease are dose attributed to the release of growth hormone. In fasting normal subjects or patients with Parkinson's disease Boyd, et al found that levodopa raised the serum growth hormone level through hypothalamic mechanisms. It seemed pertinent to us to study this cerebral effect in non-fasting patients

for we had found that pharmacological doses of this hormone injected into mice promoted, potentiated and prolonged all cerebral effects of levodopa suggesting that the hormone released during treatment might have similar effects. Serum growth hormone levels were therefore investigated during dietary modifications of symptomatic control.

B.3.1. Parkinsonian patients.

These consisted of 17 patients in whom serum levels of growth hormone were followed for 8-24 hrs. Growth hormone levels were determined every 30 minutes while the patients were on optimal amounts of levodopa. Six of these patients were studied during 8 hrs. on the third day of having received protein intake of either 1 g /kg/d or 2 g/kg/d.

B.3.2. Results.

Patients off levodopa for one week showed almost constant levels of growth hormone with a few minor rises mostly at night. In treated patients several of the doses of levodopa were followed 30-40 minutes later by a ten-fold or higher rise of serum growth hormone lasting between 1 and 1 1/2 hrs.

TABLE V

GROWTH HORMONE

Mean ± SEM of Serum Concentration (ng/ml) over 8 hours

	TOTAL		NET RISE	
	Radio-immunoassay	Double Antibody	Radio-immunoassay	Double Antibody
1) Untreated (8)	3.8 ± 0.5	2.2 ± 0.4	1.1 ± 0.3	1.1 ± 0.2
2) Levodopa (6)	5.6 ± 0.6	8.5 ± 0.1	3.4 ± 0.6	6.4 ± 0.1
$p <$	0.06	0.001	0.007	0.001

The six patients who received levodopa in optimal amounts had mean concentrations of growth hormone significantly higher on the former as opposed to the 2 g/kg d of protein diet (Table VI).

TABLE VI

GROWTH HORMONE

Mean Serum Concentration (ng/ml) over 8 hours

1 g/kg protein levodopa (6)	6 ± 0.9	4.5 ± 1.0
2 g/kg protein levodopa (6)	3.2 ± 0.7	2 ± 0.7
$p <$	0.05	0.1

Since growth hormone can increase the incorporation of amino acids into proteins and its release is promoted by levodopa, the question arises whether this hormone plays a part in the control of parkinsonism, especially as we found that pharmacological doses of growth hormone potentiated the cerebral effects of levodopa in mice. The present work does not answer the question fully because it did not release direct correlations between individual rises in serum growth hormone and episodes of either excessive or diminished therapeutic effects. It did show however that the abnormally flat patterns and lower levels of serum growth hormone in untreated patients became less abnormal while the patients were being treated provided that the protein intake was less than 2 g/kg/d. This trend toward the normal might in the long run benefit the patients particularly with regards to osteoporosis.

B.3.3. Manganic patients.

Six normal controls and six manganic patients were studied during 8 hrs of sleep. Blood samples were drawn from an indwelling catheter and the normal shown to have a mean concentration of growth hormone of 5.9 nanograms/ml ± 1.6 (SEM). Six untreated manganic patients showed concentrations of growth hormone during sleep of 1.8 ± 0.3 nanograms/ml $p < 0.02$. The same patients on optimal doses of levodopa had a nocturnal growth hormone concentration of 2.7 ± 0.7 $p < 0.002$, but not statistically different from the basal levels of these same individuals. However, when levodopa was administered prior to sleep the concentration of growth hormone increased to 4.1 ± 0.8 not significantly different from the controls but statistically significant from the basal levels of these same patients $p < 0.05$. These findings show a similarity in these results with the ones observed with parkinsonian patients during sleep.

B.4. Electroencephalographic findings during sleep in manganic patients.

In 11 normal individuals and in 11 occasions in 6 manganic patients rapid eye movement periods were evaluated by electroencephalography. The percentage of REM period in normals was 13.9 ± 5.8% (standard deviation) of the total sleep. In untreated manganic patients, this type of sleep diminished to 5.4% ± 2 while the same individuals on levodopa had a percentage of total sleep spent in REM phase of 8.2 ± 2.6%.

B.5. Decarboxylation of D-L-dopa ^{14}C and tyrosine ^{14}C.

The studies of protein intake and central effects of levodopa showed in parkinsonian patients an increase of peripheral decarboxylation on high protein diet. In order to evaluate peripheral decarboxylation of these amino acids, measurements of $^{14}CO_2$ were performed in exhaled air. In 14 parkinsonian patients at the Catholic University in Chile and also in 7 patients with chronic manganese poisoning. Six healthy manganese miners and six normal males were also studied. One minute after intravenous injection of ^{14}C labeled D-L-dopa and L-tyrosine a surge of radioactivity was detected reaching a maximum after 10 minutes, thereafter radioactivity diminished until it reached a level of 10% of maximum at 1 hour and 0% at 24 hrs. No significant differences in rate of decarboxylation was observed between the four different groups studied (Table VII).

T A B L E VII

DECARBOXYLATION D-L-DOPA ^{14}C. % Excreted 1st hour

		% ± SEM	MK-486 %	p <
Control	(7)	11.8 ± 1.2	4.7 ± 0.9	0.001
Parkinsonians	(14)	10.3 ± 0.69	4.5 ± 0.4	0.001
Manganics	(9)	9.9 ± 0.9	5.1 ± 1.08	0.01
Miners	(6)	16.2 ± 1.0	9.5 ± 1.4	0.01

A 50% diminution of this decarboxylation was observed when MK-486 the peripheral inhibitor of decarboxylase was given prior to the test. The decarboxylation of L-tyrosine the precursor of dopa was studied in these same four groups of patients. The rate of decarboxylation was less than the one observed for levodopa (Table VIII).

T A B L E VIII

DECARBOXYLATION D-L-DOPA ^{14}C. % Excreted 1st hour

		BASAL ± SEM	MK-486	p
Control	(7)	5.0 ± 0.3	5.2 ± 0.3	N.S.
Parkinsonians	(6)	7.0 ± 0.6	7.2 ± 0.6	N.S.
Manganics	(9)	6.5 ± 0.5	6.0 ± 0.6	N.S.
Miners	(7)	6.0 ± 0.8	8.4 ± 0.7	N.S.

While MK-486 failed to inhibit this rate of decarboxylation, the measurements performed in these experiments are in agreement with the indirect measurements of decarboxylation by analysis of the percentage of levodopa excreted as the methylated metabolic homovanillic acid. In 24 hours approximately 20% of levodopa is excreted through this metabolic route. Furthermore, partial blockage of decarboxylation by MK-486 resulted in approximately 50% reduction of dopa decarboxylation but not of tyrosine.

C.1. Involuntary movements in parkinsonian patients with levodopa.

At the Catholic University in Santiago, 6 parkinsonian patients that showed intense dyskinesia induced by levodopa were studied by means of an inhibitor of the enzyme dopamine β-hydroxylase, this enzyme hydroxylases dopamine to noradrenaline. Fusaric acid is an inhibitor of this enzyme with effects on the metabolism of serotonin also. In these 6 patients there was a temporary significant reduction of the involuntary movement without exacerbation of the parkinsonism. In one manganic dystonic patient during a period of refractoriness to levodopa, fusaric acid was used. There was a considerable temporary reduction of dystonia with loss of effect however after 30 days of administration.

No further efforts were pursued in this line by the group in Santiago due to the transient nature of the central nervous system effects observed with this agent.

D.1. Susceptibility to manganese poisoning.

We have previously stated that the estimated incidence of manganism varies between 25 and 4% of the exposed population occurring after variable periods of exposure to inhalation of ore dust (6 mo - 24 yrs). Possible individual susceptibility has been related to variations of intestinal absorption of manganese. Individuals with increased iron absorption have increased manganese absorption as well. Absorption of ^{54}Mn in normals is 3% ± .5, in anemic patients the rate is 7.5% ± 2. ^{59}Fe absorption was 11% ± 10 and 64% ± 22 respectively. Information on intestinal absorption of Mn in infants is not available.

In iron deficient rats, the binding capacity of manganese (transferrin) is increased approximately 100% as is the entrance of Mn into the brain. This appears coupled with transport of Mn to the blood brain barrier by transferrin and would link therefore increased Mn binding capacity to increased entrance of ^{54}Mn to the CNS.

In iron deficient rats, Mn binding capacity of transferrin is increased after 24 hrs equilibrium dialysis against buffer barbital, iron deficient plasma (6) retained 48% ± 9 of $^{54}Mn\ Cl_2$ carrier free, while normal plasma (6) retained only 21% ± 6 $p < 0.001$. This might be pertinent to the entrance of Mn into the brain. This is a slow process that reaches the maximum of 1% of injected dose at 30 days after injection.

In order to test the iron deficiency hypothesis further, $^{54}Mn\ Cl_2$ was injected intraperitoneally to iron deficient rats and the animals were decapitated 5 days later. Specific activity of ^{54}Mn was determined by measuring ^{54}Mn in a well counter and ^{55}Mn by neutron activation analysis at Brookhaven National Laboratory. Specific activity in the brain stem was 191 ± 74 cpm/ug ^{55}Mn/g in normals (6) and 360 ± 58 in iron deficient rats (22) $p < 0.001$. Basal ganglia specific activity was 135 ± 61 in normals and 305 ± 18 in deficient rats $p < 0.001$, the medulla had 126 ± 45 and 300 ± 56 respectively $p < 0.001$.

In iron deficient rats, entrance of ^{54}Mn into the brain is enhanced and appears coupled to its transport to the blood brain barrier by transferrin. It links therefore increased plasma Mn binding capacity to increased entrance of ^{54}Mn into the CNS. Another factor enhancing entrance of manganese into the brain is age. Newborn rats and infants, and fetuses on 18 days of gestation shows 4 times greater entrance of $^{54}Mn\ Cl_2$ into the brain than other rats. This later observation is possibly linked to a maturity of the blood brain barrier.

In order to assess the effects of increased manganese into the brain, correlations between manganese intake and concentrations of catecholamines, noradrenaline and dopamine in brain of rats were sought.

D.2. Correlation of brain catecholamine levels and age of the animals and dietary intake of manganese.

Sprague-Dowley rats of the vivarium of the Catholic University in Santiago were studied. The animals were fed Hurley diet, that is a manganese only deficient diet plus control amounts of manganese chloride. Animals were sacrificed at preset intervals and the concentrations of dopamine.

D.2.1. Results.

TABLE IX

Variations of catecholamine concentration in brain at different ages of the rat

HURLEY DIET Groups		N° det.	N° brains	NORADRENALINE mug/brain x ± SE	DOPAMINE mug/brain x ± SE
1. Rats 22 days of gestation		3	42	3.18 ± 0.34	16.2 ± 2.53
2. Control 12 hrs		3	28	24.7 ± 3.68	26.6 ± 6.1
3. Control 24 hrs	(A)	3	33	27.5 ± 5.2	47.4 ± 7.6
	(B)	6	54	20.5 ± 1.00	40.5 ± 3.27
4. Control 20 days	(A)	3	3	216.1 ± 34.3	298.9 ± 46.9
	(B)	6	54	161.1 ± 18.9	237.0 ± 18.3

Table IX shows the linear increase of dopamine and noradrenaline in newborn rats at 22 days of gestation, 12 hrs, 24 hrs and 20 days.

TABLE X

Catecholamine in brain of pregnant rats

Groups	Diet	N° det.	NORADRENALINE mug/brain x ± SE	DOPAMINE mug/brain x ± SE
1	Control Hurley	8	198.2 ± 31.16	528.9 ± 69.54
2	Hurley + 1 mg Mn	6	448.6 ± 42.2	848.1 ± 87.87
3	Hurley + 10 mg Mn	6	561.9 ± 42.9	953.3 ± 103.4

Table X shows a significant diminution of both noradrenaline and dopamine in the manganese deficient brains of pregnant rats.

TABLE XI

Catecholamine in brain of newborn rats

Group	Diet	N° det.	N° brains	NORADRENLINE mug/brain $\bar{x} \pm SE$	DOPAMINE mug/brain $\bar{x} \pm SE$
1	Control Hurley	6	65	11.6 ± 0.83	38.9 ± 2.7
2	Hurley + 1 mg Mn	6	51	19.9 ± 2.9	51.0 ± 5.9
3	Hurley + 10 mg Mn	5	51	20.3 ± 1.7	30.6 ± 6.1

Table XI shows a diminution of noradrenaline and dopamine concentrations in brains of newborn rats from mother fed manganese deficient diet during gestation and also a significant decrease of dopamine concentration in brains of newborns from mothers that received 10 mg of manganese per day during gestation.

D.3. Teratologic effects of levodopa.

In comparison with controls from saline treated mothers, newborn rats from mothers receiving levodopa during gestation showed diminished tolerance to cold and had a higher incidence of hemorrhages in the thoracic brown fat and they were cannibalized by their mothers more often.

The resulting impairment of thermogenesis was changed by manganese administration which was tried because a metabolic interrelationship between the amino acid and the metal had been previously found. Sprague-Dowley virgin female rats were used. Their mating was carefully timed and one day prior to estimated delivery they were isolated for observation. Mortality rates of the offsprings during the first 12 hours after birth were recorded. Inspection of offsprings revealed a blue spot which appeared a few hours after birth between the scapula of some rats born of mothers treated with levodopa during gestation. Upon dissection the spot was found to indicate extensive subcapsular hemorrhages restricted specifically to the brown fat. Detailed macroscopic and microscopic examination of the other tissues showed no abnormalities. Hemorrhages were essentially absent during intrauterine life. Table XII shows increase incidence of the blue spot in new borns after levodopa administration to their mothers

which also increased a spontaneous postnatal mortality.

The assumption that postnatal survival had been jeopardized by impairing the thermogenic contributon of brown fat was tested by placing representative newborns in the cold (10°C) until death. Survival was markedly reduced in blue spot animals and less so in the rest of the offspring of levodopa treated mothers in comparison with those of saline treated ones. In 18 animals, the 50% survival was 25 hours while 12 animals with brown fat hemorrhage had a 50% survival of 7 $\pm$ 3 hrs.

The incidence of hemorrhage in the brown fat was markedly diminished by the addition of $MnCl_2$ into the regimen (Table XII).

The results of these experiments indicate that levodopa administration to rats during pregnancy can damage selectively the brown fat of the newborns and thus decrease their survival in the cold. The selective cannibalization of such offsprings by their mothers seems to be an indirect consequence of levodopa administration. Co-administration of manganese appears to have a preventive effect on the damage to brown fat induced by levodopa. These procedures offer a laboratory model for inducing defective thermoregulation in early life and studying its consequences.

TABLE XII

BROWN FAT HEMORRHAGE IN NEWBORN RATS FROM LEVODOPA-TREATED MOTHERS

		Percent of newborns with BF Hemorrhage	Percent Offspring born dead	A + B	N° of Animals	N° of Litters
1)	Dopa 1 mg/g 1-5 day	14.7	1.6	16.3	61	5
2)	Dopa 1 mg/g 1-22 day	16.5	7.7	24.2	258	24
3)	Dopa 1 mg/g 1-10 day	14.8	6.5	21.3	129	14
4)	Dopa 0.1 mg/g 1-10 day	12.0	-	12.0	50	6
5)	Dopa 1 mg/g 15 days before pregnancy	3.2	0.7	3.9	140	14
6)	Dopa 1 mg/g 1-22 day $MnCl_2$ 0.2 mg/g	7.5	20.9	28.4	172	18
7)	Dopa 1 mg/g 1-22 days $MnCl_2$ 0.02 mg/g	3.7	7.5	11.2	53	5
8)	Dopa 1 mg/g 1-22 day Mn deficient diet (milk)	28	53	81	51	5
9)	Control	2.94	-	2.94	339	34
	TOTAL				1253	125

Brown fat hemorrhage for 1 vs. 9, 2 vs. 9, 3 vs 9, 4 vs. 9 and 8 vs. 9, $p < 0.001$; 6 vs. 9 $p < 0.05$; and 5 vs. 9 and 7 vs. 9 not significant

E. Technological procedures developed during this project.

E.1. Ergometry in the clinical evaluation of parkinsonism and manganese poisoning.

Ergography had been shown by Schwab, England and Peterson to serve in the clinical differentiation of parkinsonism from other disorders which also cause slowness and fatigability. In parkinsonism itself, fatigue was evidenced by declining amplitude of the ergographic excursions and was convincingly related to rigidity. Akinesia was reflected in the sudden rise of these excursions following a command at the end of the test. The disadvantages of ergography are that the patterns recorded do not measure directly the output of work, they are not directly amenable to this study's particular analysis and they become exceedingly complex when dyskinetic patients cannot fully relax their fingers or when festinating, one cannot follow the signal to contract their muscles.

In contrast automated ergometry can measure sequentially the several parameters pertaining to output of work during willfully selected units of time. It can therefore have all the advantages of ergography without some of the disadvantages and could become useful in studying the rapidly changing neurological status particularly among levodopa treated patients. Using an automated ergometer of our construction we have conducted ergometric measurements during semi-daily neurological examinations of 12 patients for a maximum of one year in the metabolic ward. We have shown whenever the neurological status was steady, so was the output of work; whereas, when the neurological status was changing, so were the ergometric measurements.

The ergometer constructed at BNL was able to measure and record automatically the following parameters: 1) the sum of positive excursion, 2) the slope described by the peaks of these excursions, 3) the degree by which negative downward excursions failed to reach the base line, and 4) the number of excursions per unit time period. Correlations of these measurements with neurological scoring showed a linear relationship with a coefficient of correlation of .85, thus providing the applicability of ergometry on recording changes in the clinical status of patients with parkinsonism.

F. Negative results.

In order to evaluate the degree of exposure of healthy manganese miners in the mine district of Andacollo, Chile, 20 miners were requested on site to collect 24 hour feces in order to measure manganese content by neutron activation analysis. The low cultural background of these individuals and the impossibility of control of these measurements in that environment precluded us from getting this important and meaningful information. The range of 24 hour excretion of manganese observed varied between 3 mg and 56 mg.

G. Personal publications.

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Nature 239:286-287, 1972.

2. MENA I., COURT J., COTZIAS G.C.
"Levodopa, involuntary movements and fusaric acid."
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3. MENA I., LOPEZ G., HORIUCHI K., ARANDA L.
"La barrera hematocefalica y oligoelementos: Manganeso."
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4. COTZIAS G.C., TANG L.C. and MENA I.
"Effects of inhibitors and stimulators of protein synthesis on the cerebral actions of levodopa."
Neuroscience Research, Vol. 6, I.J. Kopin and S. ehrenpreis (Eds.) Academic Press, N.Y. 97-107, 1973.

5. COTZIAS G.C., PAPAVASILIOU P.S. and MENA I.
"L-meta-tyrosine and parkinsonism"
JAMA 223 (1) 83, 1973.

6. GILLESPIE N.G., MENA I., COTZIAS G.C. and BELL M.A.
"Diets for modifying responses to levodopa in parkinsonism."
J. Amer. Dietetic Assoc. 62 (5) 525-528, 1973.

7. COTZIAS G.C., MENA I. and PAPVASILIOU P.S.
"Overview of present treatment of parkinsonism with levodopa in the treatment of Parkinson's disease - the role of dopa decarboxylase inhibitors."
M.D. Yahr, Ed., Raven Press, N.Y., Adv. Neurol. 2:265-277, 1973.

8. PAPVASILIOU P.S., COTZIAS G.C., MENA I., and BELL M.A.
"Oxybate sodium for parkinsonism."
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9. MENA I.
"Manganese poisoning: Its therapy and relationship between metabolism of manganese and that of biogenic amines."
Yearbook Science and Technology, 1973.

10. COTZIAS G.C., PAPAVASILIOU P.S., MENA I., TANG L.C. and MILLER S.T.
"Manganese and catecholamines."
Trans 2nd U.S.-Canadian Conf. on Parkinson's Disease, Raven Press 235-244, 1973.

11. MENA I., COTZIAS G.C., PAPAVASILIOU P.S., MENDEZ J.S. and BROWN F.C.
"Chronic treatment with levodopa and growth hormone release."
Trans 2nd U.S.-Canadian Conf. on Parkinson's Disease, Raven Press 471-477, 1973.

12. MENA I., COTZIAS G.C., BELL M.A. and POTTER D.W.
"ERgometry in the clinical evaluation of parkinsonism."
Arch Phys. Med. & Rehab. 55: 246, 1974.

13. MENA I., HORIUCHI K., LOPEZ G.
"Factors enhancing entrance of manganese into the brain: Iron deficiency and age."
J. Nuc. Med. 15: 516, 1974.

14 MENA I.
"The role of manganese in human disease."
Annals of Clinical & Laboratory Science 4:487, 1974

15. MENA I., COTZIAS G.C.
"Protein intake and treatment of parkinsonism with levodopa."
New Eng. J. of Med. (in press)

16. COTZIAS, G.C.
"Levodopa, manganese, and degenerations of the brain."
The Harvey Lectures, Academic Press, Series 68, 117-147,
1974

H. Staff.

Dr. Ismael Mena, principal investigator, spent from October thru October 1973 at Brookhaven National Laboratory conducting work with Dr. George Cotzias. In November 1973 he became Professor of Radiological Sciences and Chief of Division of Nuclear Medicine at the UCLA-Harbor General Hospital Campus in Torrance, California.

Dr. George Cotzias finished his period as a co-principal investigator in October 1973 and continued as a consultant to this program. He considered that a research program among three centers, the Catholic University, Santiago, the University of California at Los Angeles and Brookhaven National Laboratory in New York coordinated by the Pan American Health Organization in Washington would be a very complex project to run. He suggested therefore that the project be continued on the basis of the existing collaboration between the Catholic University in Santiago and the University of California at Los Angeles with the sponsorship of PAHO. He became then a consultant to this program.

Staff (Professional Personnel EMPLOYED)

Name	Title	Pd. Apptment.	Time%/Hours
At Brookhaven National Laboratory, Upton, N.Y.:			
Miss Kathleen Burke	Technical Assoc.	Oct.1, 1970 Jul.1, 1971	100%/48 hrs.
Ismael Mena, M.D.	Principal Invest.	Oct.22, 1971 Oct.15, 1973	100%/48 hrs.
In CHILE at Catholic University:			
A. Foradori, M.D.	Research Assoc.	Oct.1, 1973 Jul.31, 1974	100%/48 hrs.
Miss Kazuko Horiuchi	Chemist	Oct.1, 1970 Oct.1, 1973	100%/48 hrs.

Name	Title	Pd. Apptment.	Time%/Hours
Jaime Court, M.D.	Research Neurologist	Oct.1, 1970 Jul.31, 1974	40%/19 hrs.
Luis Aranda, M.D.	Neuro-physiologist	Oct.1, 1970 Jan.1, 1974	60%/28 hrs.
Ines M. Leyton	Secretary	Oct.1, 1970 Oct.1, 1971	100%/48 hrs.
Veronica Kramer	Secretary	Oct.1, 1971 Jan.1, 1973	100%/48 hrs.
Josefina Vial	Secretary	Jan.1, 1973 Jul.31, 1974	100%/48 hrs.
Virginia Risopatron	Technician	Oct.1, 1970 Jul.31, 1974	100%/48 hrs.
Carmen G. Velasquez	Technician	Apr.10, 1972 Jul.31, 1974	100%/48 hrs.
Mr. Gilberto Lopez	Biochemist	Oct.1, 1970 Jul.31, 1974	100%/48 hrs.

S U M M A R Y
FINAL REPORT

Program Director:

Humberto Torloni, M.D.

Period Covered:
From: 1 Oct. 1970 Through: 31 July 1974

Name of Organization:

PAN AMERICAN HEALTH ORGANIZATION

Title of Project:

MANGANESE POISONING : A METABOLIC DISORDER

Our report for years 01-07 of this grant defined the clinical and metabolic aspects of manganese poisoning. This work terminated with the discovery that levodopa was able to correct the symptoms of extra-pyramidal damage induced by manganese.

Susceptibility to manganese poisoning was linked among other factors to increased intestinal absorption of manganese in anemic individuals. While manganese ore dust is inhaled it is eventually degluted thus the route of entry becomes the gastrointestinal tract. A positive correlation was demonstrated for iron and manganese intestinal absorption. Parallel studies of manganese poisoning and Parkinson's disease have shown chemical and metabolic similarities and dissimilarities. The most important differences related to the side effects of levodopa in parkinsonians that were notably absent in manganism. The most important side effects were involuntary choreo-athetoid movements, pyschotic phenomena, and intermittent loss of effects of levodopa. The major goals for this period became a) observation on long term treatment of manganic patients and induction of side effects, b) elucidation of mechanism for intermittent loss of control and involuntary movements induced by levodopa, c) experimental studies pertaining to interaction of manganese and dopa, permeability of blood brain barrier to manganese and susceptibility to manganese poisoning.

A. Observations on long term treatment with levodopa.

Eight manganic patients were treated for periods up to 5 years with levodopa, the symptoms of rigidity, postural reflexes, hyperkinesia and loss of facial expression responded markedly in a sustained way. There was no effect of dysarthria. The effects on dystonia were marked with loss of effect after periods of 2-3 months of treatment or whenever stressful situations occurred. There was a notable absence of involuntary movement and psychotic episodes in these patients.

B. Growth hormone release and electroencephalographic findings during sleep in manganic patients.

Untreated manganic patients showed diminished concentration of growth hormone during sleep 1.8 ± 0.3 ng/ml from 5.9 ± 1.6 ng/ml observed in normal individuals $p < 0.02$. Also in untreated manganic patients there was a significant diminution of the rapid eye movement periods evaluated by electroencephalography. While 11 normal individuals spent $13.9 \pm 5.8\%$ of their total period of sleep in REM sleep, 6 manganic patients showed a diminution of the period of REM sleep to 5.4 ± 2. On optimal doses of levodopa, the same individuals had a percentage of total sleep spent in REM phase of $8.2 \pm 2.6\%$.

C. Protein intake and intermittent refractoriness to levodopa.

High protein intake enhances the intermittent refractoriness to levodopa (off-on phenomenon) of unstable parkinsonian patients but not of patients with manganese poisoning. On high protein diet, parkinsonian patients showed a marked impairment of postprandial function that was stabilized when the patients received a 10 g of protein per day diet. The protein intake effects were blocked when a peripheral decarboxylase inhibitor M-486 was used. In 5 manganic patients similar dietary maneuvers failed to produce morning or afternoon changes in the response to levodopa.

Furthermore, measurements of total body decarboxylation of levodopa ^{14}C showed no significant differences between parkinsonian and manganic patients with and without the peripheral decarboxylase inhibitor M-486.

In iron deficient rats the binding capacity of manganese by transferrin is increased approximately 100% as is the entrance of manganese into the brain. This appears coupled with transport of manganese to the blood brain barrier by transferring and would link therefore increased manganese transport capacity to increased entrance of ^{54}Mn to the central nervous system. Manganese intake appeared also to have influence on the concentrations of dopamine and noradrenalin in newborn in the brains of newborn rats. There was a diminution of noradrenalin and dopamine in brains of newborn rats from mothers fed manganese deficit diets during gestation and also a significant decrease of dopamine concentration in brains of newborns from mothers that received 10 mg of manganese per day during gestation.

With replacement of lead with manganese in gasoline, manganese is becoming a new environmental air pollutant. There is need to define susceptible populations and among these two important ones appears to be newborns and infants in view of their minimal natural intake and lack of protective barriers.