THE known biochemical abnormalities in Parkinson's disease consist of a decrease of melanin pigment in the substantia nigra and a decrease of some biogenic amines in the substantia nigra and the corpus striatum. These 2 defects might be interrelated, as suggested by the fact that in both melanocytes and sympathetic cells tyrosine is hydroxylated to dihydroxyphenylalanine, a common precursor in the synthesis of both melanin and catecholamines. Furthermore, both melanocytes and sympathetic cells originate from the neural crest. It was suggested earlier that the interrelations between melanogenesis and extrapyramidal disease might be of fundamental importance. It was noted that chronic exposure to at least 2 chemicals, manganese and phenothiazine compounds, may induce extrapyramidal manifestations. Manganese was shown to accumulate in the various melanin granules analyzed, a property that is shared by phenothiazines. In addition, metals such as manganese interact in vitro with phenothiazines to give semiquinone-free radicals, similar to those present in normal melanin.

In the present work an effort was made to ameliorate the known biochemical abnormalities in patients with Parkinson's disease. Initially the effect of melanocyte-stimulating hormone was investigated. This agent increases melanin deposition at least in the integumental melanocytes, and it was hoped that it might similarly affect the pigmented cells of the brain. Furthermore, this peptide has increased the amplitude of evoked monosynaptic potentials in the spinal cord of the cat. It became apparent, however, that the Parkinsonian state was reversibly aggravated by the administration of this hormone. A serviceable working hypothesis compatible with this finding might be that the hormone was shifting dihydroxyphenylalanine (DOPA), the precursor of melanins and biogenic amines, from the brain to the integument. Therefore, it was considered desirable to investigate the therapeutic potential of DOPA, particularly since the early reports of short-lived improvement were disputed by later studies. Administration of higher doses than previously reported effected a striking, sustained improvement in several patients. In some of the patients depression of the circulating granulocytes and marked vacuolization of the corresponding bone-marrow cell developed. Similar hematologic complications associated with either phenylalanine deficiency or chloramphenicol toxicity have been reversed by phenylalanine. Excesses of this amino acid have also increased the dopamine concentration in rat brain, and low dopamine concentrations have been linked with the pathogenesis of Parkinsonism in human beings. Therefore, this amino acid was also administered. The present paper summarizes these findings and discusses their relation to the therapy of Parkinsonism.

**Materials and Methods**

**Clinical Material**

Seventeen patients with Parkinsonism were admitted to this study. All had been referred to us by their physicians, after treatment with several stand-
ard medications for relief of this disorder, including 2 who had been subjected to cryopalillidectomy. All were studied as inpatients in the metabolic wards for several to many months. Some of the patients with Parkinsonism had previously participated in therapeutic studies of their disease. Three without Parkinsonism were included as controls. The patients were made aware of the nature and consequences, but not of the timing, of the regimens.

**Drugs and Dosages**

Beta melanocyte-stimulating hormone,* with activity of $2 \times 10^6$ units (Shizuwa-Lerner) per gram, (10 mg. per vial), was administered intramuscularly in 2 equal doses dissolved in 1 ml. of 16 per cent gelatin. The doses were slowly increased but did not exceed 40 mg. per day. The periods of administration were bracketed by periods of injection of the gelatin as a placebo twice daily.

D,L-phenylalanine and D,L-dihydroxyphenylalanine (DOPA) were studied because of the great expense of the L-compounds. These amino acids, obtained from the Nutritional Biochemicals Corporation, were made up in pink capsules containing 100, 200 or 500 mg. The same capsules filled with lactose served as placebo. As a rule, the total number of capsules was kept constant during the evaluation of both the placebo and the amino acid.

In all studies these agents were started at a small dose that was gradually increased while the placebo was simultaneously decreased.

**Laboratory Tests**

A comprehensive battery of tests selected to detect evidence of drug toxicity was carried out at various intervals. The hematocrit, hemoglobin, total and differential white-cell counts, Coombs test and platelet counts originally performed every two weeks were done twice a week after abnormalities first became apparent. The total peripheral blood granulocytes per cubic millimeter of blood were calculated by multiplication of white-cell count by the percentage of segmented and band neutrophils. Bone marrow aspirates collected in 1 per cent EDTA in saline solution were examined in 12 patients. They were immediately smeared and stained by the Wright–Giemsa method to avoid degenerative artifacts. Differential vacuole counts were made on 500 to 800 myeloid cells of 13 aspirates from 8 patients. All vacuoles were counted in thin areas of the smears and in places where there was minimal lipid deposition.

Tests of hepatic and renal function were carried out before therapy and subsequently at two-week intervals. The liver-function studies included serum protein determination with albumin-globulin ratio, serum electrophoresis, serum alkaline phosphatase, bilirubin, cholesterol, cephalin flocculation, serum glutamic oxalacetic transaminase and the bromsulfalein test. The kidney-function tests included urin-

*Kindly supplied by Armour Laboratories, Chicago, Illinois.

analysis, creatinine clearance, urea clearance, determination of twenty-four-hour urinary protein and occasional phosphate clearance. Weekly analyses of whole-blood manganese, serum copper and serum iron were obtained in most cases. Blood glucose, serum electrolytes, serum calcium, phosphorus, uric acid, protein-bound iodine and urinary 5-hydroxyindoleacetic acid, ferric chloride test for indoles and twenty-four-hour urinary glucose by glucose oxidase and Benedict's reaction were determined intermittently. Serum amino acid analyses before and during phenylalanine and dihydroxyphenylalanine administration are in progress.

**Clinical Evaluation**

Visits and periodic physical examinations were conducted twice a day in all cases. Handwriting, the number of steps required to walk 10 meters and the observed facility to sit down or stand up, to pick up an object from the floor and to draw a straight line were tested periodically. Cogwheel phenomenon, rigidity, tremor, festination, dysarthria, salivation, muscle strength and mental state were evaluated regularly, with the patients on the placebo and on the compounds tested. Cinematographic records were obtained both before and during therapy in several cases.

**RESULTS**

**Clinical**

As shown in Table 1, the melanocyte-stimulating hormone was given to 6 patients. Other drugs had been withdrawn in 5 of these, and the neurological manifestations had reached a plateau. In the other patient (Case 6) this was impossible due to emergence of dysphagia. All patients initially had abdominal cramps and diarrhea, which disappeared after a few days in all but 1 (Case 4), in whom the hormone was stopped. Increased pigmentation of the skin gradually developed, most noticeably over the arms and face.

The progressive increments of the hormone induced an increase in manifestations of Parkinsonism: tremor appeared or became aggravated whereas muscular strength, posture, gait and associated movements became further impaired. Salivation emerged in 1 case, but in none was rigidity changed to an appreciable degree.

In the repeated trials of D,L-DOPA (Cases 1, 6, 7, 8, 9, 10, 16 and 17) of the 16 patients showed either complete, sustained disappearance or marked amelioration of their individual manifestations of Parkinsonism. These included tremor, cogwheel phenomenon, rigidity, loss of associated movements, muscular weakness, festination, salivation and loss of facial expression. The dose required for improvement was possibly a function of the body mass (Table 1). As the dose of D,L-DOPA was gradually increased, the improvement was first noted in the rigidity, and only at higher levels was there a decrease or disappearance of tremor. The reduction in
tremor was reflected in the electrocardiograms taken under identical conditions before and during therapy with d,l-DOPA. The improvement in the handwriting of one patient is shown in Figure 1. In another patient (Case 9), mental confusion associated with garrulity was markedly improved on this drug. This was particularly striking because every standard agent for relief of Parkinsonism tried by several physicians had either aggravated this patient's mental confusion or induced visual and auditory hallucinations. Simultaneously with motor improvement and disappearance of tremor, euphoria associated with exaggerated facial expression and gesticulation on talking developed in another patient (Case 7). These manifestations disappeared whenever the d,l-DOPA was discontinued and the full syndrome re-emerged.

In Case 6 DOPA controlled dysphagia, tremor and weakness, none of which had been significantly affected by full doses of trihexyphenidyl hydrochloride (Artane), ethopropazine (Parsidol), promethazine (Phenergan) or benztropine (Cogentin). Intermittent athetoid movements of the tongue were seen in this case only on DOPA. Moderate athetoid movements of all 4 extremities were exhibited by another (Case 17). Cases 10 and 16 had been subjected to cryopallidectomy elsewhere. Euphoria was not a common finding, but the sedation and "drugged" sensation associated with most therapy against Parkinson's disease was notably absent. In 8 patients (Cases 2 and 11), although sustained improvement was induced by DOPA, significant degrees of either tremor or rigidity remained. By sharp contrast, 4 (Cases 3, 4, 12 and 15) with early unilateral disease remained essentially unimproved. Case 13 became pale, apathetic and immobile on 2 trials, and DOPA was therefore not continued. In 1 (Case 14) intercurrent fever caused us to stop the drug.

In a sixty-two-year-old man with cerebral atrophiesclerosis and bilateral Parkinsonism transient left-sided hemiplegia developed after a total of 6.1 gm. of d,l-DOPA over three days, and further therapy with this amino acid was discontinued. The control patients (Cases 18 and 19) had no discernible mental or physical consequence during administration of d,l-DOPA. Athetoid movements were observed only in patients with Parkinson's disease and only when the therapeutic effect was impressive.

Eight of the 16 patients with Parkinsonism who had received d,l-dihydroxyphenylalanine (DOPA) were subsequently given its precursor compound d,l-phenylalanine. By contrast, none of these 8
patients had any discernible improvement in their Parkinson's disease on D,L-phenylalanine, and the majority were adversely affected. Case 1 received 4 gm. of D,L-phenylalanine at the time she was enjoying marked improvement from D,L-DOPA. On this combination tremor, rigidity, weakness and drowsiness developed, so that the phenylalanine was discontinued. Gradual readministration of this amino acid when she was not receiving DOPA induced only a moderate aggravation of the clinical manifestations, even at a dosage level of 12.6 gm. per day. By contrast she was under sustained full control of her disease with 4.0 gm. of DOPA per day. Among the remaining 7 patients 1 (Case 11) had aminesia for the first time after receiving phenylalanine, 1 (Case 14) showed no significant changes, and in the remainder minimal to moderate aggravation of the rigidity and tremor developed. Two controls, 1 with congenital hydrocephalus (Case 19) and the other with rheumatoid arthritis (Case 20), received respectively 4.8 and 8.0 gm. of D,L-phenylalanine daily for about a week, without physical or mental changes.

**Toxicologic Effects**

Nausea, faintness and occasional vomiting did occur during DOPA administration, but only with increments larger than 0.5 gm. per dose. These symptoms were transitory as a rule and were not encountered with increments of less than 0.2 gm. per dose. The hematologic changes are discussed below.

**Laboratory Data**

The patients with Parkinsonism generally had low serum phosphorus concentrations that were unaffected by the drugs used in this study. The mean and standard deviation of 200 serum phosphorus determinations on these patients was 3.0 ± 0.5 mg. per 100 ml., with a range of 1.8 to 4.1 (normal, 3.0 to 4.5 mg. per 100 ml. by the Tausky and Shorr method). The serum calcium, alkaline phosphatase and twenty-four-hour urinary phosphorus were all normal and unaffected by these drugs.

In 2 patients who were on long term therapy with D,L-DOPA the blood manganese level decreased as shown in Figure 2. The lower plateau was reached in both cases after a period of DOPA administration approximating the life-span of the erythrocyte.

The remainder of the laboratory examinations were contributory only in that the urines of patients on DOPA became black on standing and showed a positive Benedict but a negative glucose oxidase reaction.

In 4 of 16 patients (Cases 1, 4, 6 and 7) granulocytopenia developed during the course of treatment with D,L-DOPA. Two episodes were rapid, and 2 gradual, the latter occurring over several months. The total granulocytes decreased to 1800 to 2300 per cubic millimeter and rose to normal, or nearly normal, between one week and six months after cessation of DOPA. There was no direct correlation between duration of treatment and total dose of D,L-DOPA administered, although all cases occurred after more than 200 gm. had been consumed. There was no direct evidence of a sensitization type of reaction. Occasional atypical lymphomonocytoid cells were seen in the peripheral blood of D,L-DOPA-treated patients, especially in those in whom granulocytopenia developed.

Quantitative and differential counting of vacuoles in cells of the myeloid series confirmed the first impressions that they were increased in numbers in bone marrows of patients treated with D,L-DOPA. The vacuoles were mostly cytoplasmic, although some overlay nuclei, and were increased in number in the more immature forms of the myeloid series (Fig. 3). The number of vacuoles per cell were also increased in blast forms, promyelocytes and myelo-
cytes in 4 patients who were receiving, or had recently received, d,l-DOPA. Two of these patients had concomitant granulocytopenia, and 1 had previously been granulocytopenic. Only occasional vacuoles were seen in erythrocyte cells, and these were not quantified.

**DISCUSSION**

The sustained beneficial effects of DOPA observed here are in sharp contrast to some previous reports. This difference can be ascribed to the larger, sustained doses used during the present investigation. Although small doses of DOPA can reduce rigidity, the larger amounts used here are necessary to eliminate both rigidity and tremor. Some of the most striking results were obtained in patients who had advanced disease for which they had been subjected to intensive conventional medical or surgical therapy before this study. The 4 patients who did not respond significantly to the full regimen exhibited a relatively mild unilateral type of Parkinson’s disease.

The mechanisms by which the effects described above were brought about remain obscure. The finding of decreased concentrations of dopamine in the brain in Parkinson’s disease might have some bearing on the improvement noted in our patients. DOPA passes through the blood-brain barrier, leading to an increase in dopamine concentration in the brain. It is of interest that the onset of improvement when sufficient DOPA was given was rapid (within two or three hours), whereas the re-establishment of the base-line state with abrupt termination of the drug, after prolonged therapy, was much longer (four to fourteen days). Long-term therapy with DOPA may well have some effect on the catecholamine storage granules that have been described in certain neuron cells. If increased dopamine was the only mechanism by which improvement was brought about, one would expect effects in the same direction to follow the administration of an earlier precursor, phenylalanine. This was certainly not the case. Since the conversion of phenylalanine to DOPA requires hydroxylation, it is logical to suggest that defective hydroxylation of this or other aromatic amino acids might emerge as a biochemical error in this disease.

The mechanisms by which athetoid movements were induced have not been elucidated. These movements were observed only in patients with Parkinson’s disease and only when the therapeutic effect of DOPA was marked.

The mode of action of melanocyte-stimulating hormone in aggravating Parkinsonism also is not clear. The stimulation of the skin melanocytes was definite, and this could have reduced DOPA available for brain metabolism. Chlorpromazine has been reported to increase skin pigmentation as well as to produce extrapyramidal symptoms. Although this explanation remained speculative no further effort was made to substantiate it in view of the patients’ discomfort.

The diminution of the concentration of whole-blood manganese might be worthy of comment. The time that elapsed until a new plateau was reached after administration of DOPA had approximated the life-span of 1 generation of erythrocytes. This was compatible with two earlier demonstrations: that manganese becomes incorporated in a manganoporphyrin of human erythrocytes, and that the exact enantiomeric Figure 2 was obtained after feeding of excessive but steady amounts of manganese as shown in Figure 2 of Cotzias et al. Many amino acids have significant chelating properties and are able to facilitate metal transport into cells. The decrease in blood manganese could result from the redistribution of this metal by DOPA. Further investigations of this hypothesis are planned. Other essential metals have been implicated in the syndrome of Parkinsonism as well as in the metabolism of some biogenic amines. The low serum phosphorus levels encountered here, coupled with the normal calcium levels, indicate that not only manganese but also magnesium must be studied in the present context.

Administration of d,l-DOPA resulted in granulocytopenia in a sizable percentage of the patients studied. In none did infection occur, and all episodes of granulocytopenia were reversed. In 3 of the patients the drug was stopped, and in the fourth, granulocytes rose despite continued therapy. Noted in association with the granulocytopenia was extensive vacuolization of immature cells of the myeloid series. Although there is no direct evidence linking the 2 findings it is reasonable to assume that they are related. The vacuoles seen in the bone-marrow elements are similar to those noted in the erythroid and myeloid cells of patients with phenylalanine deficiency and chloramphenicol-induced erythroid suppression.

The sum of the evidence presented indicates that DOPA is an effective agent for certain cases of Par-
kinsonism and worthy of further investigation. The hematologic complications were relatively mild since they consisted of only a mild granulocytopenia and morphologic changes in the bone marrow. Still caution must be exercised in the study of p,l-DOPA. A similar long-term investigation with L-DOPA seems highly warranted as soon as it becomes economically feasible.

SUMMARY AND CONCLUSIONS

Some compounds were selected for study because of their possible effects on abnormal melanogenesis and catacholamine metabolism that occur in Parkinson's disease. These compounds included melanocyte-stimulating hormone, p,l-phenylalanine and p,l-dihydroxyphenylalanine (DOPA).

Melanocyte-stimulating hormone (20 to 40 mg.), given intramuscularly to 6 patients, resulted in an aggravation of their tremor but no significant effect on their rigidity. Oral administration of p,l-phenylalanine (1.6 to 12.6 gm) exacerbated both tremor and rigidity in 7 out of 8 patients with Parkinson's disease.

Of the 16 patients receiving p,l-DOPA (3 to 16 gm per day by mouth) 8 showed either complete or marked sustained improvement of several individual manifestations of Parkinsonism. Rigidity decreased or disappeared at relatively lower doses whereas only at higher levels of DOPA was there a decrease or disappearance of tremor. Two additional patients were improved but to a lesser degree by this amino acid. A significant side effect of administration of p,l-DOPA was a transient granulocytopenia encountered in 4 cases. This was associated with extensive vacuolization of the more immature cells in the myeloid series of the bone marrow. Another side effect was the reversible induction of atiethoid movements, which has been observed thus far only in patients with Parkinson's disease and only when the therapeutic effect was significant.

Although p,l-DOPA emerges as an effective therapeutic agent, the hematologic complications indicate that caution is required in further studies of this compound.

We are indebted to Mr. Samuel T. Miller and Miss Judith Edwards, who performed the neuron-activation analyses for manganese, to the nurses, under the supervision of Martha Hill, R.N., for support and to Charles J. Goldman, Ph.D., for the expert fabrication and supply of the active chemicals and their respective placbos in the forms studied here.

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