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ON THE OCCURRENCE OF HOMOVANILLIC ACID AND 5-HYDROXYINDOL-3-YLACETIC ACID IN THE VENTRICULAR CEREBRO-SPINAL FLUID OF PATIENTS SUFFERING FROM PARKINSONISM.

Submitted by

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APOLOGIA.

In medical terminology the word 'diagnosis' means the determination of the nature of a diseased condition. In the past it was sufficient to identify a disease by a constellation of symptoms and signs and this is still of pre-eminent importance - but it is becoming increasingly common to use ancillary aids to diagnosis as these are developed by modern scientific method. It is always difficult to predict the possible applications of 'pure' investigative research but we suggest that the work described in the following paper may prove to be relevant to the diagnosis of Parkinsonism, using biochemical estimations in the cerebro-spinal fluid of homovanillic acid and 5-hydroxyindol-3-ylacetic acid, metabolites of the cerebral amines dopamine and 5-hydroxytryptamine respectively.

This work, which constituted part of a thesis submitted for the degree of Ph.D., was carried out in the Departments of Pharmacology and Surgical Neurology. I am grateful to Professor W.L.M.Perry and Professor F.J. Gillingham for their help and encouragement and to Mr. J. W. Turner who collected the samples of ventricular cerebro-spinal fluid.

INTRODUCTION.

The syndrome of Parkinson's disease has been associated with changes in concentrations of certain cerebral amines, in particular that of dopamine. Estimations of the dopamine content of various brain regions, post-mortem, of patients who had suffered from Parkinson's disease and post-encephalitic Parkinsonism have shown that the concentration of this amine was most markedly decreased in the basal ganglia (Ehringer and Hornykiewicz, 1960; Hornykiewicz, 1962). Very low concentrations of dopamine were found in the caudate nucleus, putamen and globus pallidus, particularly so in the post-encephalitic cases. The substantia nigra of Parkinsonian patients has also been shown to contain sub-normal amounts of dopamine (Hornykiewicz, 1963). The same worker also observed a moderate decrease in the noradrenaline content of the hypothalamus, and the 5-hydroxytryptamine concentration was reported to be lower than normal in several brain regions of Parkinsonian patients (Bernheimer, Birkmayer and Hornykiewicz, 1961). The concentrations of noradrenaline and 5-hydroxytryptamine were, however, never so markedly decreased as that of dopamine.

The suggestion that Parkinsonism is associated with an error in dopamine metabolism was substantiated by the observation that Parkinsonian patients excreted less dopamine in the urine than normal controls (Barbeau, Murphy and Sourkes, 1961; Bischoff and Torres, 1962).

Studies on some of the metabolites from the amines

have also been reported in Parkinsonism. Homovanillic acid (HVA), the main metabolite from dopamine, has been shown to occur in the corpus striatum, the globus pallidus and the substantia nigra of the brain from Parkinsonian patients in markedly decreased concentrations (Bernheimer and Hornykiewicz, 1964). This acid metabolite has also been found to be present in significantly lower concentrations in lumbar C.S.F. from patients with Parkinsonism than from a control group. (Bernheimer, Birkmayer and Hornykiewicz, 1966).

The urinary outputs of HVA as well as of 5-hydroxy-indol-3-ylacetic acid (5-H1AA), the acid metabolite from 5-hydroxytryptamine, have been estimated in Parkinsonian patients. The excretion of HVA was reported to be normal (Greer and Williams, 1963). Barbeau and Jasmin (1961) found that the excretion of 5-H1AA was lower than normal, but this finding was not confirmed by Resnick, Gray, Koch and Timberlake (1962).

A study has been made, with the results reported below, of the concentrations of HVA and 5-H1AA in the ventricular C.S.F. of patients who were undergoing stereotaxic surgery for the relief of Parkinsinism. The patients making up the control group were undergoing cerebral ventricular catheterisation as a diagnostic or therapeutic procedure and they had no clinical evidence of disease of the extrapyramidal system. The biochemical findings are discussed in relation to various clinical observations.

Materials and Methods.

Patients with Parkinsonism.

The subjects of the study were patients admitted to Western General Hospital, Neurosurgical Unit (S.N.3), Edinburgh, who were undergoing stereotaxic surgery for the relief of Parkinsonism (Gillingham, Watson, Donaldson and Naughton, 1960). There was no selection of these patients beyond the criteria for selection of the Parkinsonian patient for operation. A few results were, however, excluded from the final analysis, and this problem is discussed later in conjunction with the difficulties in sampling of the ventricular C.S.F. and some technical aspects associated with the biochemical analysis of the C.S.F. samples. Thirty-seven patients suffering from Parkinsonism have been included in this survey. Clinically it was found difficult to differentiate between the various types of Parkinsonism, but most of the cases were thought to be suffering from idiopathic Parkinson's disease while a few were of the post-encephalitic type. Arteriosclerosis can be a contributing factor in the development of Parkinsonism, but none of the patients showed obvious clinical evidence of arteriosclerosis.

In about one-third of all the patients, all drugs, except paracetamol for the relief of headaches, were stopped. The drugs were discontinued before or on arrival of the patient at the hospital and the minimum period between stopping the drugs and the start of the

study was 48 hours. This discontinuation of drugs was adhered to for all the cases admitted for surgery during a certain period of time. In the remaining two-thirds of the patients the medical treatment of Parkinsonism was continued over the period of study. The drug treatment consisted of one of the following anticholinergic drugs: Benzhexol (Artane), Benztropine (Cogentine) or Orphenadrine (Disipal).

In addition to one of the anticholinergic drugs, 4 patients were being treated with antidepressants, 2 patients with amitriptyline and 2 with imipramine. One patient was also treated with probenecid in addition to benztropine. The patients continuing drugs were allowed sedative drugs at night as well as a variety of mild analgesics. The only drugs used at operation were local anaesthetics for anaesthetising the skin. No specific dietary restrictions were instituted.

The patients were assessed carefully preoperatively with regard to certain of their symptoms, in particular, the extrapyramidal symptomatology of tremor, rigidity and akinesia. The degree of tremor and rigidity was graded clinically (0 to ++++) for both sides of the body and akinesia, defined as "difficulty to initiate voluntary movements", recorded as present or absent. Age and sex of the patient was recorded, and, in most cases, an attempt was made to find out the duration of the illness to the nearest year. The patients were also questioned closely as to the occurrence of encephalitis or "severe flu" in the past history. In only 2 cases did we obtain

a definite history of encephalitis ; in a further case there was a past history of "severe flu" and in the fourth case there was a history of the onset of Parkinsonism following desensitization for an allergy. These four cases have arbitrarily been defined as suffering from post-encephalitic Parkinsonism. A clinician was responsible for the clinical assessments of the patients and the person responsible for the biochemical analyses was unaware of the clinical facts until the end of the study.

Patients suffering from dystonias other than Parkinsonism.

A small group of patients (5 cases) suffering from dystonias of types different from that of Parkinsonism was also included as a pilot study. The diagnosis in 2 of these was: "torsion dystonia" ; 2 cases were diagnosed as: "spasmodic torticollis" and the fifth case as: "familial tremor". This group has not been included in the final analysis of the main study.

Patients in the "control group".

These patients were undergoing cerebral ventricular catheterisation, usually as a diagnostic procedure for ventriculography and, less commonly, as a therapeutic procedure for the drainage of C.S.F. as in hydrocephalus or to perform frontal lobectomy as in the psychiatric cases. Seventeen cases were included in the final series, 11 from the same hospital as the Parkinsonian patients and 6 from the Head Injury Unit situated in the Royal Infirmary of Edinburgh. Most of the cases were suffering

from organic disease, 5 cases of cerebral tumours, 3 cases of subarachnoid haemorrhage, 3 cases of hydrocephalus and 3 cases of head injuries. There were 3 patients with psychiatric disease, (Table 3). The only criterion for selection of the type of case to be included was the absence of any clinical evidence of disease of the extrapyramidal system.

Originally, the control-group consisted of 21 cases, but 4 cases had to be excluded for the following reasons. Examinations of the x-ray photographs for the position of the catheter through which the C.S.F. was obtained, in 3 cases, showed that the tip of the catheter was found to be in the aqueduct or the 4th ventricle or the region of the cisterna magna. There is normally a marked concentration difference for HVA and 5-HIAA between lateral ventricular and cisternal C.S.F. in the dog (Guldberg, Ashcroft and Crawford, 1966) and this appeared also to be the case in man. One case had to be excluded because the C.S.F. sample contained haemolyzed blood and the blood pigments were found to interfere with the estimations of HVA. Similarly, the xanthochromia of the C.S.F. from two of the patients with subarachnoid haemorrhage, made it necessary to exclude these samples from the HVA estimates.

It was not practically possible to stop drug treatment in the control patients.

Collection of ventricular C.S.F. samples.

All the C.S.F. samples were collected into chemically clean, graduated, glass stoppered test tubes. The volume

of C.S.F. withdrawn at any one time was 4 ml. The samples were stored at -20°C. until the analyses could be performed, usually, on the following day. If traumatic bleeding into the C.S.F. had occurred, the sample was centrifuged and the cell free supernatant decanted into a clean test tube.

C.S.F. sampling in Parkinsonian cases.

(1) 4 ml. of C.S.F. was withdrawn from the lateral ventricle following the introduction of the catheter at "stage 1" of the operation (sample A1). This was an important base-line sample.

In addition, it was decided to investigate in a few cases the effect on the concentration of HVA and 5-H1AA of repeated sampling, by withdrawing in rapid succession another two samples each of 4 ml. of C.S.F. (samples A2 and A3).

(2) On the day of therapeutic operation, "stage 2" of the operation which was generally 2 days after stage 1, a 4 ml. sample of C.S.F. was again withdrawn at the onset of the operation (sample B1). A further sample was withdrawn about 0,5 hr. later (sample B2) after the intraventricular injection of the radio-opaque material "Myodil". Another sample of the C.S.F. (sample B3) was withdrawn after destruction of the target areas. The target areas were commonly the ventrolateral nucleus of the thalamus and/or the globus pallidus and/or internal capsular fibre connections.

(3) In several patients the ventricular catheter was left in situ for another 3 days and the final sample

of 4 ml. of ventricular fluid withdrawn before removal of the catheter, "stage 3" of the operation. This sample, (C1), was most important for the study of possible biochemical changes occurring in the C.S.F. following the operational therapy.

In each case a record was kept of the target area for the operation and the clinical assessment of the therapeutic result of the operation.

At the end of the study, all the x-rays were viewed to determine the position of the catheter from which the samples were taken. The burr-holefor the ventricular catheter was always made on the right side of the skull. In most cases the catheter was found to be placed in the right lateral ventricle, but sometimes it was found to be situated in the left lateral ventricle and on occasions in the third ventricle or at the foramen of Munro.

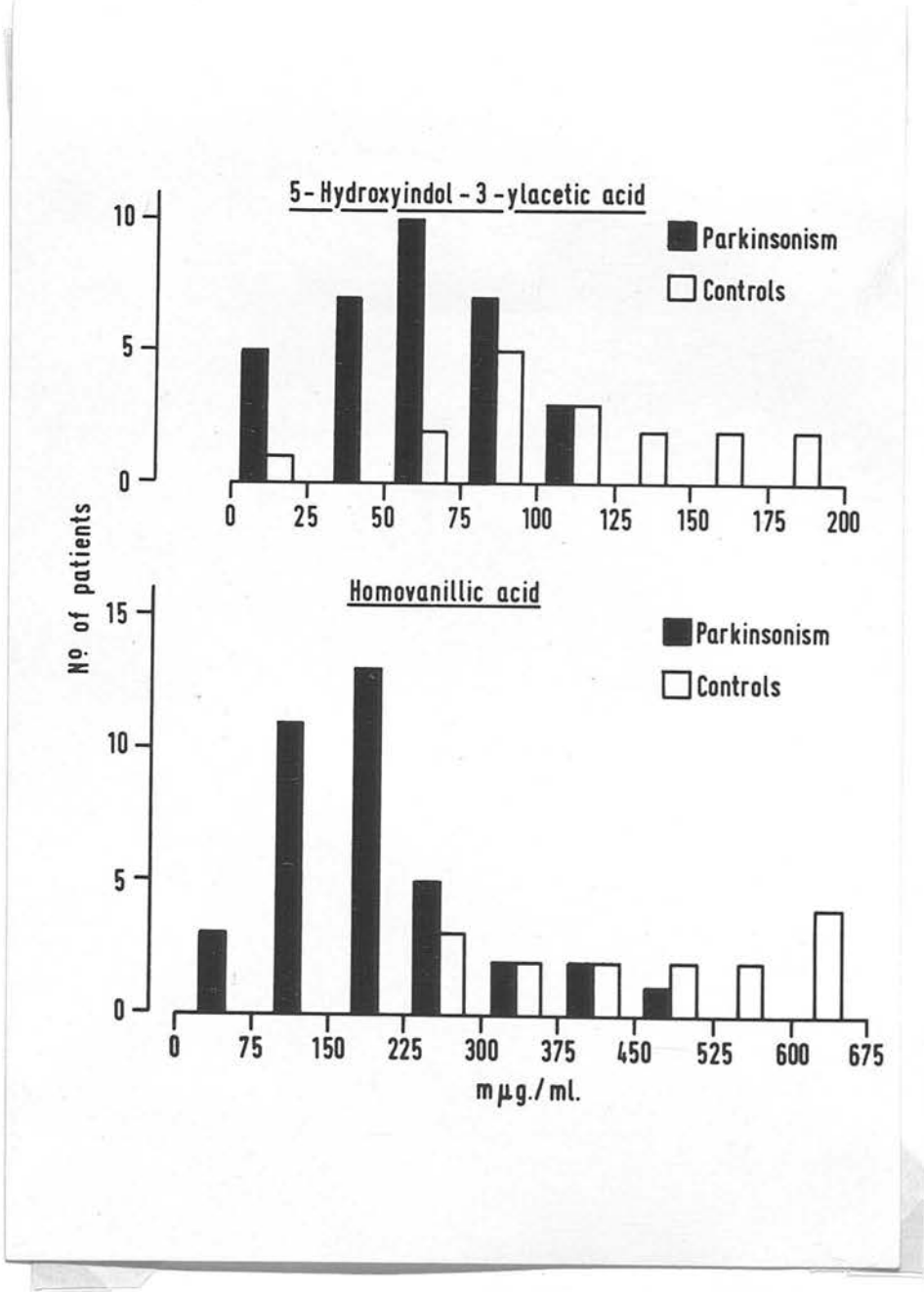
C.S.F. sampling from the "control" cases.

In this group, comprising the non-Parkinsonian cases with no clinical evidence of disease of the extrapyramidal system, 4 ml. of C.S.F. was collected from the lateral ventricle in exactly the same way as for the Parkinsonian cases (samples A1). C.S.F. was withdrawn immediately following the introduction of the catheter, and in no case was any C.S.F. withdrawn just previous to the sample. Generally, only a single sample was obtained from each control patient except in 3 psychiatric patients undergoing the operation of frontal lobectomy, when C.S.F. samples were collected during the operation.

Estimation of HVA and 5-H1AA in ventricular C.S.F.

The estimations of the phenolic acids were carried out on 2,0 ml. samples of C.S.F. A technically simple procedure was adapted from existing methods to permit the estimation of the two acids, HVA and 5-H1AA, in a single sample of the lateral ventricular C.S.F. This procedure was analogous to that for ventricular C.S.F. from the dog and the details of the procedure have been described elsewhere (Guldberg,1967). The C.S.F. was acidified to pH<2with concentrated HCl, saturated with sodium chloride and the acids extracted into 2 vol.ethyl acetate, by shaking the mixture for 5 min. The phases were separated by centrifugation for 5 min. The ethyl acetate extraction was repeated and the acids in the pooled ethyl acetate extracts were back-extracted into 2,2 ml. 0,1M "tris" buffer, pH 8,6. Each acid was estimated fluorimetrically in separate aliquots of 1 ml. of this extract. HVA was estimated by the method described by Andén, Roos and Werdinius (1963) and 5-H1AA by the method of Ashcroft and Sharman (1962). Recoveries of 200 mμg. of HVA and 5-H1AA taken through the entire procedure were of the order of 95% for HVA and 85% for 5-H1AA. The estimates of the acids in the C.S.F. samples have not been corrected for recoveries. It was discovered that if the C.S.F. sample for analysis contained appreciable amounts of blood pigments, these would interfere with the fluorimetric estimation of HVA. The results from such samples had to be discarded.

Fig. 1. Frequency distribution for the homovanillic acid (HVA) and 5-hydroxyindol-3-ylacetic acid (5-H1AA) levels of Parkinsonian and control patients.



RESULTS.

HVA and 5-H1AA in the C.S.F. from Parkinsonian patients.

We estimated the mean concentrations of HVA and 5-H1AA in the ventricular C.S.F. of patients suffering from Parkinsonism to be 186 ± 105 (S.D.) and 59 ± 27 mμg./ml. C.S.F.) respectively, (table 2). These results have been calculated from the estimated concentrations of the acids in the preoperative (base line) samples of C.S.F. In more than two-thirds of all the cases these samples were obtained at stage I of the operation and in each case were the first 4 ml. of C.S.F. withdrawn from the patient. The remaining patients did not undergo stage I of the operation and the estimations were done on sample B1 of stage 2. Samples A1 and B1 were comparable in that both were the first samples of C.S.F. withdrawn in the morning albeit two days apart, and that there had been no change in the treatment in the intervening period. There was, indeed, no significant difference ($p>0,1$) between the mean concentrations of HVA and 5-HIAA in sample A1 and B1 in a small number of patients (8 cases) where this comparison was made.

The ranges for the concentrations of the two acids in Parkinsonian patients were large. This was particularly marked for HVA, range 20-500 mμg./ml., and much less for 5-HIAA : 20-120 mμg./ml., (table 2). Figure 1 shows the histograms of the frequency distribution for the acids in the C.S.F. of all the patients and it can be noted that for HVA there was a skewed distribution

for the higher concentrations while the distribution for 5-HIAA was relatively symmetrical. We have some evidence to believe that the estimates of HVA for the 3 patients showing the highest concentrations, i.e. > 375 mμg./ml. C.S.F., were erroneously high. The results quoted were estimates done on sample A1 and these showed an unusually poor correlation with the estimates of sample B1, all the sample B1 estimates being less than 300 mμg./ml. No apparent reason could be found for these discrepancies.

It was observed that in two patients who gave a definite history of encephalitis the acid levels were extremely low. Another two patients, possibly of the post-encephalitic type of Parkinsonism, have been included in the small group of post-encephalitic Parkinsonism. The mean concentrations (mμg./ml. C.S.F.) for these patients are HVA 64 ± 33 and 40 ± 18 for 5-HIAA. It appeared, therefore, that this type of Parkinsonism could lead to particularly low levels of HVA in the C.S.F.

HVA and 5-HIAA of control group.

The mean concentration of HVA in the non-Parkinsonian patients was 447 ± 153 mμg./ml. and that for 5-HIAA : 111 ± 50 mμg./ml. These estimates were based on 15 patients for the HVA and 17 patients for the 5-HIAA (table 3). The range of concentrations for HVA was 230-670 mμg./ml. and that for 5-HIAA : 20-200 mμg./ml.

The histograms for the frequency distribution (fig.1) of the acid concentrations in the control patients showed no definite peaks round the mean concentrations.

Table 1. Concentrations (mμg./ml.) of homovanillic acid (HVA) and of 5-hydroxyindol-3-ylacetic acid (5-H1AA) in ventricular C.S.F. of Parkinsonian patients.

The patients are listed in order of diminishing HVA concentrations.

Explanatory notes -

"Duration of illness" in years.

"Tremor and rigidity" - graded 0 to ++++ according to severity.
upper line - right side
lower line - left side

"Grade" column - grade 1, unilateral Parkinsonism with tremor and/or rigidity.
grade 2, bilateral Parkinsonism with tremor and/or rigidity
grade 3, grade 2 with akinesia also present.

"Drugs" - drugs administered have been referred to in main text.

"Position of catheter" at time of sampling -
R.V., right lateral ventricle
L.V., left lateral ventricle
III.V., third ventricle

"Side of operation" - L, left
R, right.

"Type of operation" - T.P., destruction of ventrolateral nucleus of thalamus and globus pallidus.
T., destruction of ventrolateral nucleus of thalamus.
P., destruction of globus pallidus.
M., Mundinger operation.
C., Capsular lesion.

† beside patient's number indicates history of encephalitis.

" ___ " in table indicates that no information was available about this parameter in this particular case.

Table 1

Patient	Age	Sex	Duration of illness	Tremor	Rigidity	Grade	Drugs	Position of catheter	Side of operation	Type of operation	HVA	5-H1AA
1p	62	♀	9	+++ / +++	++ / ++	3	No	L.V.	R.	T.P.	500	120
2p	65	♂	3	+++ / +	∞	2	No	R.V.	L.	M.	400	100
3p	41	♂	4	0 / ++	0 / ++	1	Yes	R.V.	R.	T.P.	400	80
4p	51	♂	7	++ / +++	++ / +++	3	Yes	R.V.	R.	T.	360	80
5p	65	♀	5	++ / +	++ / +	3	No	L.V.	L.	T.	300	50
6p	54	♀	5	0 / ++++	0 / ++++	1	Yes	III.V.	R.	-	290	100
7p	60	♂	-	+ / ++++	+ / +++	3	Yes	R.V.	R.	T.P.	250	70
8p	68	♂	-	++ / 0	++ / 0	1	No	L.V.	L.	M.	250	80
9p	48	♂	16	++ / +	+++ / +++	3	Yes	III.V.	R.	-	250	55
10p	60	♂	3	+ / +++	+++ / ++++	3	No	L.V.	R.	T.P.	240	60
11p	54	♀	4	+ / +++	- / -	2	Yes	R.V.	R.	T.P.	200	60
12p	51	♀	5	+++ / ++	++ / ++	3	Yes	R.V.	L.	T.P.	190	75
13p	62	♀	5	++++ / 0	+++ / 0	1	Yes	R.V.	L.	T.P.	190	30
14p	59	♂	10	0 / +	+++ / ++++	3	Yes	III.V.	R.	T.	190	20
15p	54	♀	15	+ / +++	+ / 0	3	No	III.V.	R.	T.P.	190	40
16p	42	♀	9	+++ / 0	+++ / 0	1	Yes	R.V.	L.	T.P.	185	-
17p	57	♂	9	- / +	- / +++	3	Yes	R.V.	R.	-	185	90
18p	60	♂	10	+ / +	- / -	3	No	R.V.	L.	T.	185	90
19p	55	♀	6	+ / +++	+ / ++++	1	Yes	R.V.	R.	T.P.	185	70
20p	58	♂	3	0 / ++	0 / ++++	1	No	R.V.	R.	T.P.	175	65
21p	53	♀	7	+ / ++	0 / +	3	No	R.V.	R.	T.P.	160	50
22p	49	♂	6	- / +	- / +++	2	Yes	R.V.	R.	T.P.	150	-
23p	49	♂	8	0 / 0	0 / +++	3	Yes	R.V.	R.	P.	150	-
24p	50	♂	8	++ / ++	+ / ++	2	Yes	R.V.	R.	T.P.	135	-
25p	50	♂	14	+ / 0	+++ / +	3	Yes	III.V.	L.	T.P.	130	40
26p	62	♂	3	+ / +++	- / ++	2	No	III.V.	R.	T.P.	120	70
27p	65	♂	9	++++ / ++++	++++ / ++++	3	No	R.V.	R.	T.P.	120	65
28p	62	♀	10	+ / +	++ / +	2	Yes	III.V.	L.	T.P.	120	20

Table 1 continued.

Patient	Age	Sex	Duration of illness	Tremor	Rigidity	Grade	Drugs	Position of catheter	Side of operation	Type of operation	HVA	5-HIAA
29p	67	♀	8	++++ +++	++ ++++	3	Yes	III.V.	R.	T.P.	120	80
30p	59	♂	10	0 -	+++ -	3	No	R.V.	L.	T.	100	-
31p+	49	♂	4	0 ++++	0 +++	1	Yes	R.V.	R.	T.	95	65
32p+	51	♂	3	++ 0	++ 0	1	Yes	III.V.	L.	T.P.	90	20
33p+	35	♂	10	++ +	+ +	2	Yes	L.V.	L.	T.	90	35
34p	54	♀	10	+ ++	++ ++++	2	Yes	L.V.	R.	T.P.	90	35
35p+	53	♀	11	0 +	0 +	3	No	L.V.	L.	C.	40	40
36p+	59	♂	24	0 ++++	+ +	1	No	R.V.	R.	M	30	20
37p	45	♂	5	+++ +	++++ -	3	Yes	L.V.	L.	T.P.	20	20

Table 2. showing the mean concentrations(mμg./ml.) of HVA and 5-H1AA in patients with Parkinsonism and in control patients.

Acids	Parkinsonism	Controls
Homovanillic acid	186 ± 105 (37)*	447 ± 153 (15)
5-hydroxyindol-3-ylacetic acid	59 ± 27 (32)	111 ± 50 (17)

The standard error of the difference between the two means:

HVA 43,01 5-H1AA 12,97

Observed differences between the means :

HVA 261 5-H1AA 52

For HVA : observed difference approximately 6 times greater than standard error of difference i.e. highly significant.

For 5-H1AA : observed difference is approximately 4 times greater than standard error of difference i.e. highly significant.

* Mean concentration ± Standard Duration (No. of patients).

The experimental difficulty in obtaining a sufficient number of control patients and the fact that the patients included had various pathological lesions of the brain, which, per se, could alter the C.S.F. acid concentrations, made the control group not entirely satisfactory. In addition, most of the patients of the control group were of a younger age-group than the Parkinsonian patients. The difficulty in restricting drug treatment before sampling C.S.F. in these patients, made changes in acid concentrations induced by drugs a possibility. It was striking how one patient, 6c (table 3), who was undergoing frontal lobectomy for a schizoid state showed persistently high levels for HVA, about 570 mμg./ml. C.S.F., but extremely low levels, 20 mμg./ml. for 5-H1AA. This is unlikely to be drug induced and it would be interesting if the low 5-H1AA in the C.S.F. was associated with the patient's mental state.

Comparison between Parkinsonian patients and "control" patients.

The mean concentrations of HVA and 5-H1AA in the ventricular C.S.F. of patients suffering from Parkinsonism were 40% and 53% respectively of those of the "control" group. For HVA the observed difference between the means of the two groups was approximately 6 times greater than the standard error of the differences and for 5-H1AA 4 times greater. The difference between the two groups of patients are, therefore, highly significant in respect of both acids, ($p < 0,001$).

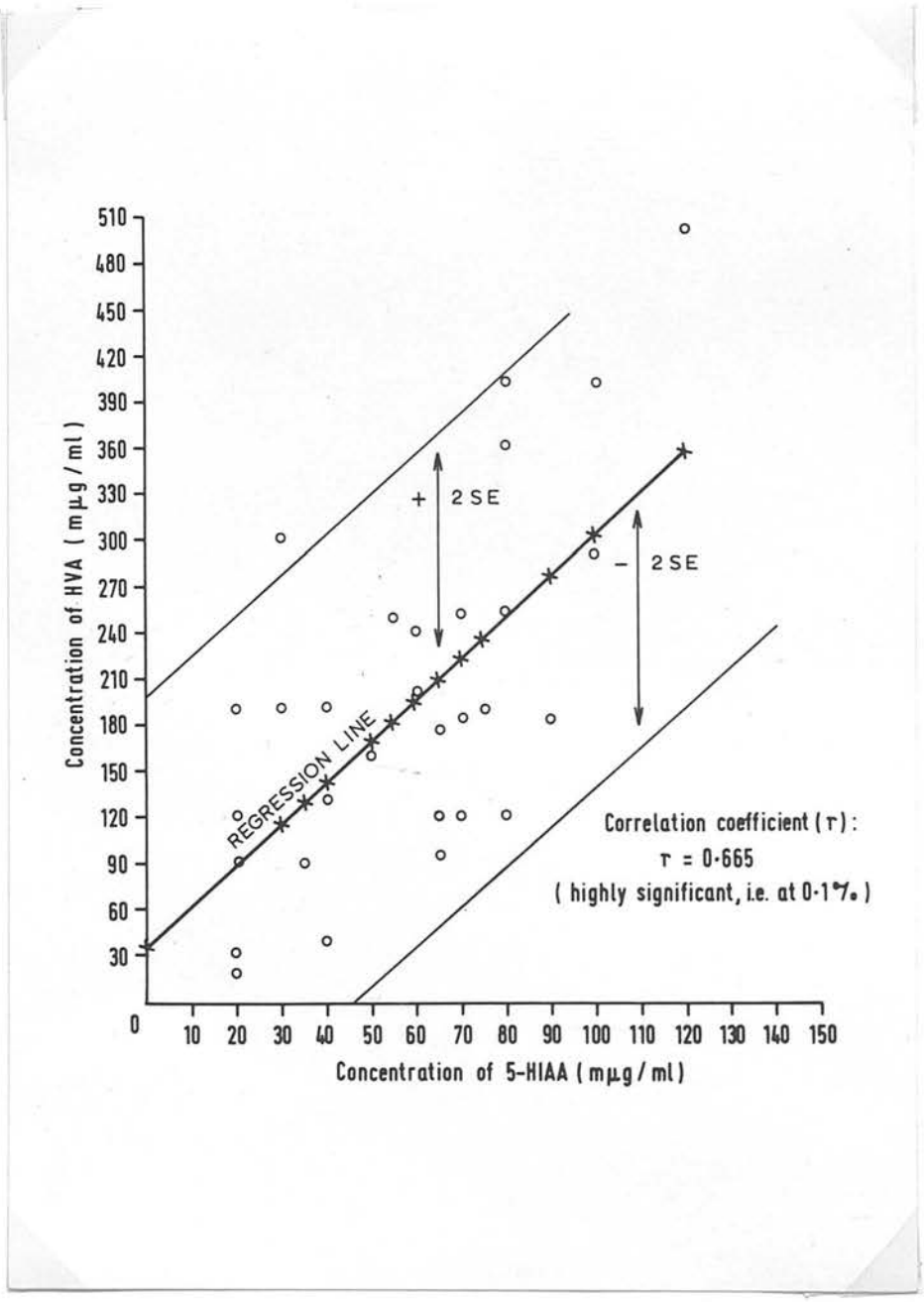
Table 3. showing control group and acid concentrations.

Patient No.	Diagnosis	Concentrations (mμg./ml C.S.F.) HVA	5-H1AA
1c	Hodgkin's disease	670	120
2c	Cerebellar medulloblastoma	600	170
3c	Subarachnoid haemorrhage	-	110
4c	Cerebral abscess	320	190
5c	Subarachnoid haemorrhage	-	200
6c	Schizoid state	570	20
7c	Subarachnoid haemorrhage	530	60
8c	Astrocytoma	600	90
9c	Cerebellar haemangio blastoma	500	145
10c	Head injury	410	80
11c	Anxiety state	240	80
12c	Hydrocephalus	470	140
13c	Head injury	630	80
14c	Head injury	400	160
15c	Aqueduct stenosis	300	100
16c	Anxiety, phobias	230	85
17c	Hydrocephalus	240	60

Fig. 2. Regression line for the concentrations of 5-hydroxyindol-3-ylacetic acid (5-H1AA) versus homovanillic acid (HVA) in Parkinsonian patients

o - patients.

x - calculated points for "line of best fit".



HVA and 5-H1AA C.S.F. concentrations in patients with dystonias other than Parkinsonism.

We have included a small study on the concentrations of HVA and 5-H1AA in the ventricular C.S.F. of 5 patients who were undergoing stereotaxic surgery for the treatment of dystonias, but who were not suffering from Parkinsonism, as defined clinically. The results are shown in table 4. Four of these patients appeared to have concentrations of HVA and 5-H1AA in the C.S.F. of the order of the upper part of the range for the concentrations of the acids in Parkinsonism. One case of spasmodic torticollis, 4d, table 4, showed acid concentrations within the range of those of the control group.

We have not attempted any analysis of these results because of the small number of patients studied so far.

Correlation between HVA and 5-H1AA concentrations in the C.S.F.

We have analysed the correlation between the concentrations of HVA and 5-H1AA within the patients suffering from Parkinsonism, as well as the control cases. For the analysis, in the former group we had 32 patients who had both HVA and 5-H1AA estimated in the base-line samples, while we had 15 patients constituting the latter group, (table 5).

The correlation coefficient ($r = 0,665$) for HVA and 5-H1AA in the Parkinsonian cases was highly significant ($p < 0,001$). The regression line, "line of the best fit", has been drawn in figure 2, which also demonstrates that for 31 of the patients, the points representing the

Table 4. The concentrations of HVA and 5-H1AA in C.S.F. in Dystonias other than Parkinsonism.

	Diagnosis	Acid concentration (mμg./ml. C.S.F.)	
		HVA	5-H1AA
1d	Torsion dystonia	275	50
2d	Torsion dystonia	180	75
3d	Familial tremor	180	80
4d	Spasmodic torticollis	770	125
5d	Spasmodic torticollis	240	60

Table 5. Mean concentrations of HVA and 5-H1AA (mμg./ml.) in Parkinsonism and control group and the correlation coefficient between the two acids for each group of patients.

	HVA	5-H1AA	Correlation coefficient (r) between acids
Parkinsonism	192 ± 110 (32)*	59 ± 27 (32)	0,665
Control	447 ± 148 (15)	105 ± 44 (15)	0,02

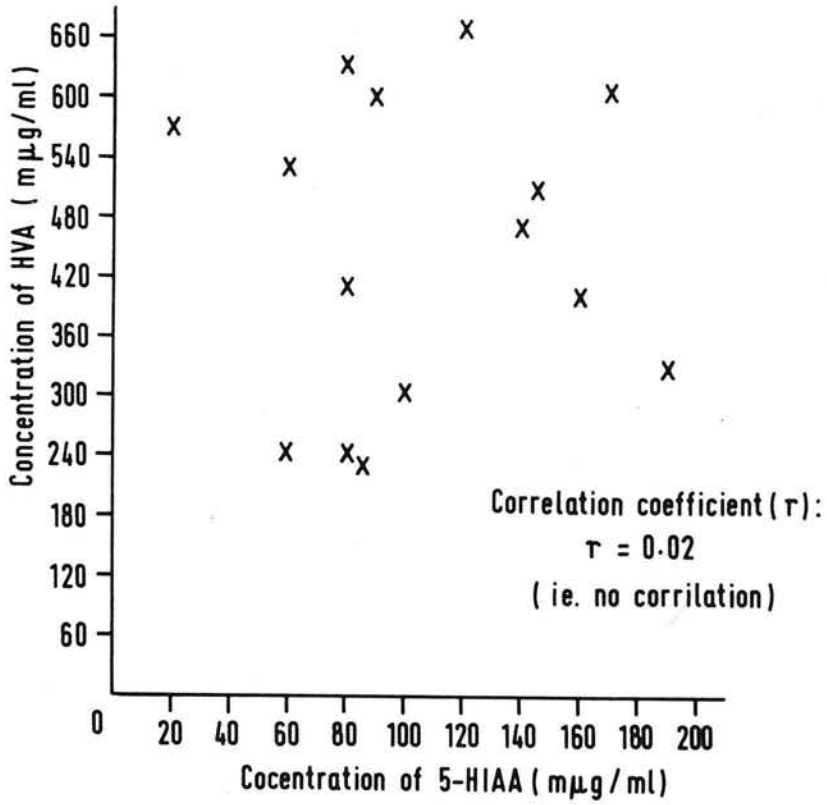
* Mean concentration ± standard deviation (No.of patients).

Significance test for r using t test:

Parkinsonism: t = 4,87. This is highly significant, i.e. at 0,1%

There was no correlation (r = 0,02) between HVA and 5-H1AA in the patients of the control group.

Fig. 3. Correlation between concentrations of 5-hydroxyindol-3-ylacetic acid (5-H1AA) and homovanillic acid (HVA) in control patients.



correlation between the HVA and 5-H1AA fall within ± 2 Standard Errors of the regression line.

In the control group we found no correlation between HVA and 5-H1AA in the C.S.F. (r = 0,02). The wide scatter of the points is evident from fig. 3.

Sampling of C.S.F.

(I) Repeated sampling of C.S.F. at short intervals.

When 3 samples of ventricular C.S.F. (the volume of each sample, as usual, 4 ml.) were withdrawn in rapid succession from some patients, there was usually a tendency for the acid concentrations to fall in the second and third samples. This was more evident in the case of HVA (table 6) where the mean concentration of the first sample (sample A1) was 174 ± 139 and that of the third sample (sample A3) was 108 ± 24 mμg./ml. while the corresponding results for 5-H1AA were 53 ± 35 and 50 ± 32 mμg./ml. respectively. The fall in the concentration of HVA with repeated sampling appeared to be more marked when the initial concentration was high.

It appeared, therefore, that when comparing the results from various samples, it was important that the C.S.F. was withdrawn under comparable conditions.

(II) Site of the catheter.

The position of the sampling catheter was checked carefully, since there is evidence of marked concentration gradients for the acids within the C.S.F. compartments (Guldberg, Ashcroft and Crawford, 1966); and Ashcroft,

Table 6. The effect on the acid concentrations of withdrawing three 4 ml. samples of C.S.F., the samples being withdrawn in rapid succession.

Sample	HVA	5-H1AA
A1	174 ± 139 (9)*	53 ± 35 (9)
A2	143 ± 131 (9)	58 ± 35 (9)
A3	108 ± 24 (9)	50 ± 32 (9)

* Mean concentration (mμg./ml. C.S.F) ± S.D. (No.of patients).

Crawford, Eccleston, Sharman, MacDougall, Stanton, Binns, 1966).

Unilateral Parkinsonism could provide a situation in which might occur the possibility of different acid concentrations in the two lateral ventricles. If the passage of substances did not take place freely between the two ventricles and only one side of the brain was affected, then a lower acid concentration might be expected on the affected side as compared to the unaffected side. In 6 patients with unilateral Parkinsonism in which the catheter was situated on the side of the lesion, the mean acid concentrations were: 189 $\pm$ 128 mµg./ml. for HVA and 63 $\pm$ 25 mµg./ml. for 5-H1AA, (table 7). We have only 2 patients where the catheter was found to be on the opposite side to the lesion and the acid concentrations were for HVA 188 mµg./ml. and for 5-H1AA 30 mµg./ml. Although we need more results to give an unequivocal answer, it would appear not to matter which side the catheter was situated, possibly, because the acids equilibrated readily between the two compartments.

In two cases of unilateral Parkinsonism where the catheter was situated in the third ventricle, the acid concentrations were not appreciably different from the lateral ventricular concentrations (table 7). Moreover, in the whole group of Parkinsonian patients (37 cases), the catheter was situated in the third ventricle in 9 cases, but the mean acid concentration for those, HVA: 167 $\pm$ 68 and 5-H1AA: 50 $\pm$ 29 mµg./ml., were not

significantly different from the mean of lateral ventricular C.S.F. concentration. ($p > 0,1$).

Relationship between acid concentrations and some clinical data.

Table 1 gives some clinical data along with the concentrations of HVA and 5-H1AA in the ventricular C.S.F. of 37 patients of Parkinsonism studied. The patients have been listed in order of decreasing concentrations of HVA. We have analysed these results in some detail.

(1) Age and sex.

The patients were divided into three age-groups, 40-49 years, 50-59 years and 60-69 years. The mean concentrations of the acids for these groups are shown in table 8 and the highest age group showed the highest mean concentrations for both acids, but these were found not to be significantly different from those of the other groups ($p > 0,1$).

There was no significant difference in the mean acid concentrations for males and females suffering from Parkinsonism (table 9).

(II) Duration of illness.

In our series, none of the patients had suffered from Parkinsonism for less than 2 years and the patients have been divided into these groups: 2-5 years, 6-9 years and 10 or more years' duration of illness. Such a division gave fairly equal numbers of patients in each group, (table 10).

There were no significant differences in acid

Table 7. The relationship in unilateral Parkinsonism between acid concentrations and the site of the catheter from which the C.S.F. sample was withdrawn.

	Concentration (mμg./ml.)					
Site of catheter	HVA			5-H1AA		
Same side as lesion	189	±	128 (6)*	63	± 25	(6)*
Opposite side to lesion	190 and		185 (2)	30		(1)
Third ventricle	290 and		90 (2)	100 and	20	(2)

* Mean concentration ± S.D. (No. of patients).

Table 8. Mean concentrations of HVA and 5-H1AA in the C.S.F. in patients with Parkinsonism in different age groups.

Age groups	Mean concentrations (mμg./ml.C.S.F.) HVA	5-H1AA
40 - 49 years	179 ± 121 (7)*	55 ± 26 (4)
50 - 59 years	161 ± 82 (17)	54 ± 26 (15)
60 - 69 years	233 ± 120 (12)	70 ± 28 (12)

* Mean concentration ± S.D. (No. of patients).

Comparison of groups 50-59 and 60-69 years for both acids gave no significant difference between the groups($p > 0,1$).

Table 9. Showing mean concentrations of HVA and 5-H1AA in males and females suffering from Parkinsonism.

Sex	HVA	5-H1AA
Males	177 ± 103 (24)*	57 ± 27 (20)
Females	203 ± 113 (13)	63 ± 28 (12)

* Mean concentrations inmμg./ml. C.S.F. ± S.D.(No. of patients).

Statistically, no significant differences between males and females, ($p > 0,1$).

Table 10. Giving the mean concentrations for HVA and 5-H1AA(mμg./ml. C.S.F.) in groups of Parkinsonian patients with different duration of illness.

Duration of illness	HVA	5-H1AA
2 - 5 years	209 ± 116 (13)*	61 ± 26 (13)
6 - 9 years	212 ± 109 (13)	78 ± 18 (9)
10 and more years	129 ± 68 (11)	40 ± 21 (10)

* Mean concentration ± S.D. (No. of patients).

Statistical analysis for :

HVA between groups 6-9 and 10 + years, using Student's t test, $p < 0,05$, hence the difference is significant.

5-H1AA between groups 6-9 years and 10 + years $0,01 > p > 0,001$, the difference is highly significant.

There was no significant difference between the groups 2-5 and 6-9 years in respect of either of the acids.

Table 11. Showing the mean concentrations (mμg./ml.) of HVA and 5-H1AA in Parkinsonian patients treated medically for their condition and on no drug treatment.

	HVA	5-H1AA
Patients on no drugs	207 ± 135 (13)*	65 ± 28 (12)
Patients on drugs	174 ± 88 (24)	56 ± 26 (20)

* Mean concentration ± S.D. (No. of patients).

$p > 0,1$ in respect of both acids for the differences between the groups.

Table 12. Showing the relationship between mean acid concentrations and degree of tremor, rigidity or akinesia - ranked groups - of Parkinsonian patients.

Clinical Assessment		HVA	5-H1AA
Tremor 1)	0 and +	138 ± 52 (8)*	50 ± 32 (6)
	+++ and ++++	202 ± 121 (19)	66 ± 27 (17)
Tremor 2)	0 and +	185 ± 107 (8)	59 ± 30 (6)
	+++ and ++++	196 ± 157 (9)	63 ± 30 (9)
Rigidity 1)	0 and +	174 ± 141 (5)	49 ± 31 (5)
	+++ and ++++	175 ± 81 (18)	59 ± 25 (15)
Rigidity 2)	0 and +	157 ± 117 (8)	46 ± 31 (6)
	+++ and ++++	177 ± 94 (10)	65 ± 14 (9)
Akinesia	Present	194 ± 111 (19)	61 ± 26 (17)
	Absent	178 ± 105 (18)	57 ± 28 (15)

* Mean concentration (mμg./ml.) ± S.D. (No. of patients).

1) Tremor or rigidity of contralateral side to lateral ventricular sample.

2) Tremor or rigidity (ranking: 0 to ++++) of either side of the body.

There are no statistically significant differences in the mean acid concentrations in respect of severity of clinical symptoms.

Table 13. Showing mean concentrations of HVA and 5-H1AA in groups of patients pre- and post-operatively.

	Groups	Concentration (mμg./ml.) in ventricular C.S.F. HVA	5-H1AA
Pre-operative acid concentrations 1)	p+	184 ± 120 (24) *	62 ± 29 (20)
	0++	217 ± 117 (15)	63 ± 29 (13)
Post-operative acid concentrations (immediate) 2)	p+	130 ± 88 (24)	54 ± 35 (20)
Post-operative acid concentrations (3 days later) 3)	0++	210 ± 111 (15)	65 ± 38 (13)

* Acid concentration ± S.D. (No. of patients).

1) Samples A1 or A2.

2) Sample B3 (after coagulation of target area).

3) Sample C1 from stage III of operation.

\+ The differences (Groups p) were not statistically significant ($p > 0,1$).

++ The differences (Groups 0) were not significantly different ($p > 0,1$).

Patients in group p were sampled pre-operatively and post-operatively, immediately after the operation.

Patients in group 0 were sampled pre-operatively and post-operatively, 3 days later.

Table 14. showing the mean concentrations of HVA in the caudate nucleus and lateral ventricular C.S.F. of man and dog.

Species	Concentration µg./g.wet tissue -HVA of caudate nucleus.	Concentration µg./ml.C.S.F. -HVA of ventricular C.S.F.	Ratio concentration C.N. to ventricular C.S.F.
Man	3,38*	0,45+	7,5
Dog	13,8 +	2,2 +	6,3

* Bernheimer and Hornykiewicz, 1965.

\+ Own results.

concentrations between the groups 2-5 and 6-9 years for either of the acids ($p > 0,1$). However, the difference for the HVA concentration in groups 6-9 and 10 or more years was significant ($p < 0,05$) and the difference was highly significant for 5-H1AA, ($p < 0,01$).

There did not appear to be any obvious correlation between duration of illness and the age of the patients.

(III) Drugs.

We did not obtain evidence that the medical treatment of Parkinsonism affected the acid concentrations since there was not a significant difference ($p > 0,1$) between the patients who had been on treatment and those in whom all treatment had been stopped, (table 11).

(IV) Relationship between symptomatology and acid concentrations.

We attempted a correlation between biochemical findings and severity of tremor and rigidity and the presence or absence of akinesia by comparing the mean concentrations of HVA and 5-H1AA in the C.S.F. of groups of patients, the groups having been determined by a separate rank order for tremor, rigidity and akinesia. Groups of patients with minimal tremor and groups with minimal rigidity were compared to the corresponding groups of severely affected patients, and groups of patients who had no evidence of akinesia compared to those with akinesia present. The results, shown in table 12, suggested that the concentrations of both acids tended to be higher in the groups of more severely

affected patients. For tremor and rigidity the differences appeared to become more evident if one compared the acid concentrations in the lateral ventricle to the contralateral limb signs rather than the overall assessment for both sides of the body. However, when the differences between the paired groups were subjected to statistical analysis, none of the differences were found to be statistically significant, ($p > 0,1$ for all the paired differences).

(V) Acid concentrations pre- and post-operatively.

The effect on the acid concentrations of C.S.F. by the surgical treatment of coagulation of certain target areas was studied by analysing the differences in concentrations of HVA and 5-H1AA pre- and post-operatively. We studied the effect on the acid concentrations both immediately following the coagulative destruction of target areas and 3 days later. The results are summarised in table 13, and there was clearly no difference in the mean concentrations for either acids when the pre-operative acid levels were compared to the acid levels 3 days after the definite operation, the difference between the means for HVA was 7 mμg./ml. and for 5-H1AA: 2 mμg./ml. However, there appeared to be a fall in the concentrations of both acids immediately following the coagulation and for HVA the difference was not quite significant ($p = 0,1$) and for 5-H1AA not significant ($p > 0,1$).

DISCUSSION

The study of cerebral metabolism in man has obvious difficulties. Most of the biochemical investigations on the cerebral amine metabolism in Parkinsonism have hitherto been based either on measurements of some amine and its acid metabolite content, post-mortem, of brain structures or on urinary excretion studies of these substances. For the purpose of biochemical investigations, the scope of human post-mortem work is limited and it is unlikely that urinary estimations of these compounds reflect directly the levels in the brain.

An approach which shows promising results, is the use of C.S.F. for the study of cerebral amine metabolism. It is, usually, feasible to obtain lumbar C.S.F. from patients, but the relationship between lumbar C.S.F. and brain concentrations of metabolites has still to be established (Ashcroft et al. 1966). We have relied upon analysis of ventricular C.S.F., drawing inferences about the cerebral metabolism based upon work in dogs which showed that changes in the acid metabolites in the ventricular C.S.F. of the dog reflect changes in the concentrations of the acids and parent amines in brain, particularly of the caudate nucleus (Guldberg, 1967 and Guldberg and Yates, in preparation).

The results reported here showed that the mean concentrations of HVA and 5-HIAA in the ventricular C.S.F. of patients suffering from Parkinsonism was 186 ± 105 (S.D.) and 59 ± 27 mμg./ml. C.S.F. respectively,

(table 2). When these results are compared to the control group (HVA mean 447 ± 153 and 5-H1AA mean 111 ± 50 mμg./ml.), it can be seen that there was a 2,4 fold reduction in HVA and a 1,9 fold reduction in 5-H1AA concentration in Parkinsonism as compared to our control series. These differences are, statistically, highly significant in respect of both acids. The findings are in keeping with previously published data on the concentration of HVA in the caudate nucleus of post-mortem "control" subjects, 3,38 μg./g. tissue (Bernheimer and Hornykiewicz, 1965), and of patients with idiopathic Parkinson's disease, 1,19 μg./g. tissue (Bernheimer, Birkmayer, Hornykiewicz, Jellinger and Seitelberger, 1965); this ratio being 2,8. There are no published data for the concentrations of brain 5-H1AA in Parkinsonism, but our results showing a reduction of the levels in the C.S.F. were not unexpected since it has been reported that the concentration of 5-hydroxytryptamine in the brain is reduced (Bernheimer et al. 1961).

We found a positive correlation ($r = 0,665$) between the concentrations of HVA and 5-H1AA in the Parkinsonian patients; the results implying that the trend in the reduction of the HVA and 5-H1AA concentrations in the C.S.F. was in the same direction for a significant number of patients ($p < 0,001$). There was, however, no correlation between the concentrations of HVA and 5-H1AA in our control series, ($r = 0,02$). The reason for this is not understood, but it may be related to the fact that the control series consisted of a group of patients with

a wide variety of pathological lesions, (table 3), some of which may be affecting dopamine and 5-hydroxytryptamine metabolism, or the egress of the metabolites, differentially. Also, the drug treatment of these patients prior to sampling was not controlled. We obtained some evidence that drug treatment, especially anticholinergic drugs for the treatment of Parkinsonism, did not affect the acid levels in the Parkinsonian patients,(table 11), but some of the control patients received phenothiazines which are known to affect the levels of HVA, with minimal effect on 5-H1AA. This does, of course, make the validity of the results on our control patients doubtful. However, the mean concentration of HVA in human ventricular C.S.F. of about 450 mμg./ml. for the controls is, probably, a reasonable estimate since in man the ratio between the mean concentration of HVA of the caudate nucleus and the ventricular C.S.F. can be estimated as 7,5 (table 14), and this compares with that of about 6,5 for the dog. We cannot make similar calculations for 5-H1AA because there are no published results on concentrations of 5-H1AA in the relevant brain regions for man. The mean concentration of 5-H1AA in the ventricular C.S.F. of the control patients (111 mμg./ml.) is higher than that published by Ashcroft et al. (1966) for ventricular-drainage samples (88,1 mμg./ml.). This is not surprising, however, since we know from our own dog experiments (Guldberg, 1967), and also for man in the case of HVA (table 6), that the concentrations of acid metabolites in the C.S.F. fall with frequent, repeated sampling of

ventricular C.S.F.

The significance of the biochemical findings of low concentrations of HVA and 5-HIAA, as well as their parent amines, dopamine and 5-hydroxytryptamine, in the brain of Parkinsonian patients is not known. They may be related to a diminished biosynthesis of the amines, but there are no published data on the metabolic turnover in the brain of dopamine and 5-hydroxytryptamine in Parkinsonism. The enzyme dopa decarboxylase does not appear to be affected in Parkinsonian brains (Bernheimer and Hornykiewicz, 1962), so it is unlikely that the conversion of dopa to dopamine, or of 5-hydroxytryptophan to 5-hydroxytryptamine, is impaired, to account for the postulate of a diminished biosynthesis rate. Furthermore, there are, as yet, no published data suggesting a difference in tyrosine β-hydroxylase activity between the brains of Parkinsonian patients and controls, which could, if that was the case, support the hypothesis of an altered turnover of dopamine in Parkinsonism.

Another mechanism which is very important in determining the steady-state concentrations of dopamine and 5-hydroxytryptamine in the brain is the availability of binding sites for the amines. If there was a diminished ability of the Parkinsonian brain to store the amines, this would result in decreased amine concentrations in the brain. It is unlikely, however, that the storage mechanisms are at fault since this would lead to high, or possibly normal, acid levels, and not reduced acid concentrations as found in Parkinsonism.

The biochemical findings in Parkinsonism could be explicable in terms of a reduction, by cell destruction, in the number of functioning neurones, the synthesis in these neurones remaining constant. The pathological changes in Parkinsonism are somewhat diffuse and variable and also they vary between the different forms of the disease (Denny-Brown, 1960), apparently, the cell-loss being most severe and diffuse in cases of post-encephalitic Parkinsonism. Bernheimer and Hornykiewicz (1965) found the brain concentrations of HVA, as well as dopamine, to be particularly low in cases of post-encephalitic Parkinsonism. We have observed a similar trend in the C.S.F. concentrations in the small number of cases of this type which we have studied. It is apparent that the biochemical findings in Parkinsonism are not peculiar to that disease, and hence do not support the view of a specific error of metabolism, since a condition like senile dementia is also associated with low HVA concentrations in the basal ganglia (Gottfries, Rosengren and Rosengren, 1965). Pathologically this condition is characterised by loss of neurones, and it would have been interesting to know if neurone loss correlated with the fall in HVA concentrations. We found that some dystonias other than Parkinsonism were associated with low HVA and 5-HIAA concentrations in the C.S.F. but the relationship between the neuropathology of the various types of dystonias is not clear, and no comparison between neuropathological and biochemical findings can be made.

Parkinsonism is a progressive disease, and we obtained some evidence that in the group of patients whose illness had been present 10 or more years, the concentrations of HVA and 5-H1AA were even lower than in those patients in whom the illness had been present for a shorter period, (table 10). The levels of HVA and 5-H1AA did not correlate with age or sex of the patients.

The most consistent neuropathological finding in Parkinsonism is the degeneration of the melanin-containing nerve cells of the pars compacta of the substantia nigra, (Jung and Hassler, 1960). Support has been given, by the use of fluorescence microscopy, for the existence of nigro-striatal dopamine-containing fibres (Dahlström and Fuxe, 1964). The cell bodies of these fibres are situated in the substantia nigra, mainly in the pars compacta, and electrolytic lesions in the substantia nigra resulted in loss of dopamine in the caudate nucleus, (Andén, Carlsson, Dahlström, Fuxe, Hillarp and Larsson, 1964). Our findings in Parkinsonism of the low HVA concentrations in the ventricular C.S.F. are explicable in terms of a fall in the concentration of the HVA of the caudate nucleus which resulted from the degeneration of dopaminergic cell bodies in the substantia nigra. The cause of the degeneration is unknown. We are not, as yet, certain to what extent brain regions other than the caudate nucleus contribute to the lateral ventricular C.S.F. levels of HVA. For 5-H1AA it is unlikely that the caudate nucleus, containing relatively little 5-H1AA, can account solely for the levels in lateral ventricular

C.S.F. In Parkinsonism the observations of lowered 5-H1AA levels in the C.S.F. may be a manisfestation of the more diffuse neuropathological destruction associated with the disease.

The mechanisms operating in the production of the extrapyramidal symptomatology of Parkinsonism - tremor, rigidity and akinesia - are ill understood. Circumstantial evidence lends support to the hypothesis that dopamine plays a functional role in the extrapyramidal system. This evidence has largely been based on the regional distribution of dopamine in the brain, the highest concentrations occurring in the basal ganglia, the localisation of the amine by fluorescence microscopy and the definite effects of drugs like reserpine and chlorpromazine on amine metabolism, drugs which may induce Parkinsonian-like states in man. The biochemical findings in Parkinsonism have added impetus to the work in this field.

The beneficial effect of l-dopa - the precursor of dopamine - on the akinesia and rigidity of Parkinsonism has been established by several workers (Gerstenbrand and Pateisky, 1962; Hirschmann and Mayer, 1964; McGeer and Zeldowicz, 1964). We found, however, that there was no general correlation between HVA or 5-H1AA concentrations in the C.S.F. and the severity of the rigidity or the presence or absence of akinesia. Poirier and Sourkes (1965) found that, in monkeys, destruction of the most dorsomedial fibres of the cerebral peduncle and the corresponding rubro-tegmento-spinal and nigrostriatal

tracts caused a Parkinson-like tremor as well as hypokinesia. This was associated with decreases in the concentrations of both dopamine and 5-hydroxytryptamine in the striatum. More recently, similar studies have shown that there was also a fall in the striatal HVA; 5-H1AA was not measured (Sharman, Poirier, Murphy and Sourkes, 1967). In our series there was no significant correlation between the severity of tremor and the concentrations of HVA or 5-H1AA in the C.S.F. The estimates did not appear to be affected by withdrawing the C.S.F. from the ventricle ipsilateral or contra-lateral to the patients' chief symptoms.

We also attempted to assess the effect on the acid concentrations in the C.S.F. of the stereotaxic operation for Parkinsonism. Destruction of the ventrolateral nucleus of the thalamus and/or globus pallidus did not appear to result in any alterations in the mean concentrations of HVA and 5-H1AA in the C.S.F.

The lack of any general correlation between the symptomatology of Parkinsonism and the concentration of HVA and 5-H1AA in the ventricular C.S.F. as well as the lack of any effect of the operation on the acid levels, is difficult to explain. Indeed, in some patients severely affected by tremor, rigidity and akinesia the concentrations of HVA and 5-H1AA were not outside the range of concentrations for the control patients. It is interesting to note that the most important drugs used in the medical management of Parkinsonism are, essentially, anticholinergic.

It seems possible, therefore, that the symptomatology might arise from an imbalance between the activities of the two opposing neural systems involving acetylcholine on the one hand and dopamine and, possibly, 5 hydrotryptamine on the other. There is no direct experimental evidence for this hypothesis. We do, of course, need to know more about acetylcholine of the basal ganglia in Parkinsonism, and we are hoping it might be possible to approach this problem from the point of view of C.S.F. studies on acetylcholine metabolism. Further investigations are also needed in the laboratory animal to try to establish to what extent brain structures not immediately adjacent to the lateral ventricles contribute to the acid levels of the C.S.F. Surgical destruction of the globus pallidus did not affect the acid C.S.F. levels in the Parkinsonian patients, and it may be that this is so because there is no relationship between the two sites as regards the acid concentrations. So far the evidence merely points to a definite relationship, in dogs, between the HVA concentrations in the caudate nucleus and the lateral ventricular C.S.F. In a further study of Parkinsonian patients we are assessing the relationship between the concentrations of HVA and 5-HIAA in the lumbar C.S.F. and the ventricular C.S.F. If a definite relationship exists, it would be helpful in reaching a long term assessment of the changes in concentrations, particularly the changes following stereotaxic surgery, obviously because one could then do lumbar estimations.

SUMMARY

We have some evidence that the concentrations of HVA and 5-H1AA in the C.S.F. of the lateral ventricles may reflect the brain metabolism of the parent cerebral amines, dopamine and 5-hydroxytryptamine. There appears to be a definite relationship between the concentrations of HVA in the lateral ventricular C.S.F. and the caudate nucleus of dogs and man. (Guldberg, 1967). An approach based on this knowledge has been used in the analysis of ventricular C.S.F. samples of patients undergoing the stereotaxic operation for the relief of Parkinsonism. The concentrations of HVA and 5-H1AA were determined in the ventricular C.S.F. of Parkinsonian patients and a control group. There was a significant difference in the mean levels of both metabolites in the 2 groups of patients. The lower levels of the acid metabolites found in the group of Parkinsonian patients has been discussed in the light of our knowledge on the biochemistry of Parkinsonism. We have suggested that the biochemical findings could be explained on the basis of the neuropathological destructions occurring in the disease process and that they are not necessarily indicative of a primary error of the amine metabolism. Within the group of Parkinsonian patients, lower acid levels were recorded in the group of patients with symptoms of more than 10 years and also in the small number of post-encephalitic patients. In Parkinsonism there was no clear evidence of any association between

the mean levels of the metabolites and the following factors: age, sex, presence or absence of drug treatment and the degree of the clinical symptoms of tremor, rigidity or akinesia. The lack of correlation between clinical symptomatology and the acid levels is particularly interesting and we have discussed in some detail the most apparent reasons for this. It is hoped that further work might clarify some of the problems.

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