

# RES MEDICA

Journal of the Royal Medical Society



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### Abstract

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Studies of cerebral metabolism in depressive illness are necessary in each of the above situations. In 1, 2 and 3 above they are necessary to justify the assumptions relating general and cerebral metabolism, whilst in case 4 the information is not obtainable by any other technique.

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ISSN: 2051-7580 (Online) ISSN: 0482-3206 (Print)

Res Medica is published by the Royal Medical Society, 5/5 Bristo Square, Edinburgh, EH8 9AL

Res Medica, Winter 1967-68, 6(1): 21-25

doi: [10.2218/resmedica.v6i1.830](https://doi.org/10.2218/resmedica.v6i1.830)

# A BIOCHEMICAL APPROACH TO DEPRESSIVE ILLNESS

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From a lecture to the Royal Medical Society reporting work carried out by the Unit for  
Research in Brain Metabolism (Medical Research Council)

We also have a fourth possibility, namely that the changes in metabolism may be taking place only in the brain although the same metabolic process is to be found also in other tissues. If this situation exists it may be impossible to infer changes in cerebral tissue from changes in metabolites in blood and urine.

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## TECHNIQUES FOR THE STUDY OF CEREBRAL METABOLISM

Advances in the direct biochemical study of diseases affecting the brain have been made using two main types of technique:

1. The analysis of brain tissue obtained at post mortem or as biopsy material during neurosurgical operation. This technique has been particularly valuable when the biochemical abnormality involves structural components of neural tissue, e.g. in the cerebral lipoidoses.
2. The establishment of animal models of the disease allowing careful control of variables and biochemical studies at varying stages of the disease. This approach has provided valuable evidence, e.g. in the vitamin deficiency states.

The suggestion that depressive illness may be associated with biochemical or metabolic disturbance is not new but added impetus has been given to this approach in recent years by the success of physical methods of treatment, e.g. electroconvulsive therapy and the antidepressant drugs. Studies of electrolyte distribution, adrenal steroid hormones and the metabolism of the biogenic amines have occupied most attention, and excellent reviews of changes reported in depression are available by Durell and Schildkraut, and Coppen (1967).

*Biochemical studies in depression.* The majority of the published studies have been carried out in relation to "whole body" metabolism, using blood and urinary estimation of metabolites. Whilst we would agree that depressive illness is a disease of the whole person we would argue that the most important and significant disturbances involve the higher nervous functions. Thus studies of general body metabolism will be of relevance only if certain special conditions are fulfilled:

1. If it is known that the whole body changes in metabolism are paralleled by cerebral changes;
- or 2. If the metabolites measured in blood and urine have brain as their only source and are not produced in any other tissue;
- or 3. If changes in blood chemistry are found the effects of which are known from previous studies, e.g. hypoxia or hypoglycaemia.

Whilst tests of the biochemical functions of many organs are in regular clinical use, e.g. liver function tests, renal function tests, the design of studies of biochemical functions of the brain in living man are limited by its inaccessibility. Logically it would seem that there are only two practical approaches to the study of the biochemical functioning of the living brain, the estimation of arteriovenous differences of metabolites and the estimation of metabolites released from brain into the cerebrospinal fluid.

In depressive illness the biochemical approach to the brain is further limited for our patients do not die from the disease process, though they may die because of it, e.g. by suicide. In addition we are unable to devise an acceptable animal model of the disease. We must look, therefore, to studies in the living patient to provide us with our information. My colleagues and I in the Unit for Research in Brain Metabolism decided to investigate the possibility of using the release of a cerebral metabolite into the cerebrospinal fluid for the purpose of examining the possibility of a disturbance of amine metabolism in depressive illness.

*The Amine Hypothesis* — a specific hypothesis relating depressive illness to changes in the cerebral metabolism of the biogenic amines.

A number of biogenic amines are found in brain tissue including histamine, 5-hydroxytryptamine, noradrenaline and dopamine. Whilst there is a rough parallel between the distribution of the amines in brain in that they tend to be concentrated in central structures and basal ganglia, there are also differences in the distribution of the individual amines which suggest specific relationships to certain physiological systems. Studies of the distribution of the amines have been facilitated by the application of the technique of fluorescence microscopy (Fuxe et al (1965)) by which the amine-containing cells and fibre tracts can be visualized in histological preparations. The suggestion that the amines function as transmitter substances at central synapses has received support from the fluorescence microscopy studies. Such studies have suggested a possible role of dopamine as a transmitter in the extrapyramidal system and changes in dopamine levels in parts of this system are reported in Parkinsonism.

5-Hydroxytryptamine is concentrated in parts of the limbic system, e.g. the hypothalamus, amygdala and hippocampus, whilst noradrena-

line is concentrated in hypothalamus and mid brain, possibly in relationship to systems involved in central autonomic control.

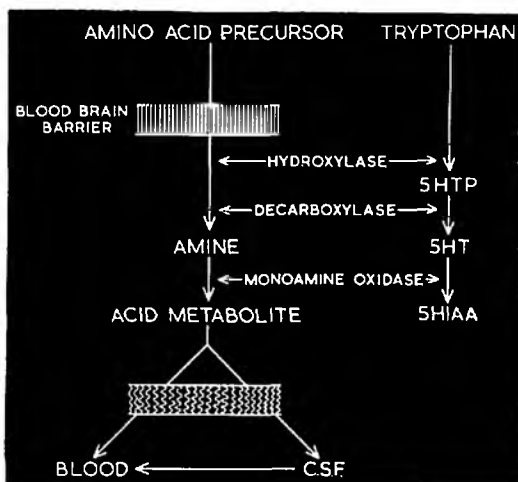
The evidence for a disturbance of amine metabolism is presented by Durell and Schildkraut (1964) and Coppen (1967), and is based on the known actions of drugs which either precipitate or are used to treat depressive illness. In general terms drugs which can precipitate depression in susceptible individuals, e.g. reserpine, reduce the concentrations of 5-H.T. and noradrenaline, whilst the antidepressant drugs either increase brain amine levels (monoamine oxidase inhibitors) or potentiate the effects of the released amines (tricyclic group of drugs). Thus a general hypothesis can be advanced to relate changes in amine metabolism to depressive illness.

#### WORKING HYPOTHESIS

"That depression will occur whenever the levels of biogenic amines are reduced at reactive sites in Brain".

This hypothesis makes no attempt to distinguish between the relative importance of changes in noradrenaline, dopamine and 5-H.T. metabolism. Our first attempts to investigate this problem were, however, limited to investigation of 5-H.T. Later studies and also studies by Dencker et al (1966) have included studies of dopamine metabolism. To date studies of noradrenaline have been handicapped by the failure to detect its metabolites in cerebrospinal fluid.

The metabolism of 5-hydroxytryptamine in brain is illustrated diagrammatically in Fig 1.



The amino acid tryptophan is actively transported from blood into brain tissue where it is hydroxylated to 5-hydroxytryptophan. The 5-hydroxytryptophan is then decarboxylated to the amine 5-hydroxytryptamine which is metabolised to 5-hydroxyindol-3-acetic acid (5-HIAA) after release. The 5-HIAA is then cleared into blood directly or via the cerebrospinal fluid. Since 5-HIAA does not pass in the reverse direction from blood to cerebrospinal fluid we argued that the levels of 5-HIAA in the fluid would reflect the levels of the acid in the brain and that this in turn would reflect the turnover of parent amine 5-hydroxytryptamine.

levels of amine metabolites and brain and to investigate the mechanisms for the removal of the acids from CSF.

#### HUMAN STUDIES

The results of such a study are shown in Table 1. (Ashcroft et al (1966)). They appear to confirm the hypothesis of a disturbance of cerebral 5-HT metabolism in depressive illness, the low levels of 5-HIAA in the lumbar cerebrospinal fluid of the depressed patients being consistent with a defect in the release or synthesis of the amine. Further studies revealed a correlation between the severity of the de-

#### CONCENTRATIONS OF 5-HYDROXYINDOLE COMPOUNDS IN CEREBROSPINAL FLUID

Type of Patient	No.	Site and Method of C.S.F. Sampling	Concentration of 5-hydroxyindoles + (ng. per ml. $\pm$ S.D.)
Neurosurgical .....	6	Ventricular drainage	88.1 ( $\pm$ 22.6)
Neurological (organic disease of the central nervous system):			
Neurological Unit .....	28	Lumbar air-encephalography	33.3 ( $\pm$ 11.2)
Neurological Unit .....	7	Lumbar puncture	17.4 ( $\pm$ 5.3)
Psychiatric Unit .....	8	Lumbar puncture	20.0 ( $\pm$ 4.7)
Alcoholic .....	6	Lumbar puncture	19.8 ( $\pm$ 2.9)
Depressed:			
On imipramine .....	8	Lumbar puncture	8.8 ( $\pm$ 2.4)
On no specific antidepressant .....	24	Lumbar puncture	11.1 ( $\pm$ 3.9)
Hypomanic .....	4	Lumbar puncture	18.7 ( $\pm$ 5.4)
Schizophrenic:			
Acute .....	7	Lumbar puncture	10.9 ( $\pm$ 2.3)
Chronic .....	7	Lumbar puncture	16.4 ( $\pm$ 2.9)

\* Concentration of 5-hydroxyindoles expressed as 5-H.I.A.A.

(Reprinted from THE LANCET, November 12, 1966, pp. 1049-1052).

The identification of the acid in human cerebrospinal fluid gave preliminary results indicating a difference between the levels in patients with depressive illness and a group of nondepressed patients with neurological disease. Ashcroft and Sharman (1960) suggested that this approach might be a fruitful one. Subsequent studies have been directed to further investigation of these problems and have been of two types:

1. Studies of CSF levels in man;
2. Animal studies designed to examine the relationship between cerebrospinal fluid

pression and 5-HIAA levels and also a return towards normal of the levels with remission from the illness irrespective of the type of treatment (ECT or imipramine).

Confirmation of these results was provided in a study by Dencker et al (1966) who also extended the findings by measuring homovanillic acid, the metabolite of dopamine. Whilst they found the levels of 5-HIAA to be reduced the levels of HVA were within normal limits in depressed patients.

Closer inspection of the results in Table 1 reveals an alternative explanation of the low

levels of 5-HIAA in depression which must be considered before we can accept them as indicating an alteration of cerebral metabolism. In nondepressed subjects there is a gradient in levels from ventricular fluid to lumbar CSF. Fluid obtained at air encephalography which may be considered as a mixture of ventricular fluid displaced by injected air with CSF from other levels has a concentration of 5-HIAA part way between that of lumbar and ventricular fluid. These results suggest that the 5-HIAA is added to the ventricular fluid and removed as the fluid passes down the cerebrospinal axis. Thus low levels in lumbar cerebrospinal fluid could represent either less addition at ventricular levels or a greater degree of removal as the CSF passes down to the lumbar region.

#### ANIMAL EXPERIMENTS

The relationship between the concentration of 5 HIAA in C.S.F. obtained from different levels of the cerebrospinal axis and the metabolism of the parent amine in brain was thus revealed as a complex one and it seemed unlikely that the problems could be resolved in clinical studies. Animal studies were thus utilised and have provided the vital links in the chain which may now allow us to move forward again with studies in man.

##### 1. Relationship between Cerebrospinal fluid and brain levels.

Studies were made using a technique allowing the sampling of CSF from the lateral ventricles and cisterna of the dog and the following results were obtained.

- (1) The levels of homovanillic acid in the lateral ventricular fluid reflects the levels of the acid in the underlying brain tissue, i.e., the caudate nucleus. Levels of 5-HIAA are higher than might be expected if caudate nucleus were the only source of the acid and it suggested that structures in the inferior horn of the lateral ventricle, viz. the amygdala and hippocampus, may contribute to the levels. (Guldborg et al (1966)).
  - (2) The levels of 5-hydroxyindol - 3 ylacetic acid in the cisternal fluid reflect concentrations in mid-brain and hind-brain. (Eccleston et al (1967)).
2. Active transport of acid metabolites from brain and CSF to blood.

Experimental studies by Pappenheimer et al (1961) and Davson et al (1962) have demonstrated the presence of a transport mechanism capable of actively transporting certain organic acids, e.g., diodrast and para amino hippuric acid from cerebrospinal fluid into blood. This mechanism appears similar to the renal tubular mechanism which transports organic acid, and the suggestion is made that it is a property of the choroid plexus. We reasoned that the gradient in levels of 5-HIAA noted in our patients between ventricular and lumbar fluid could have resulted from the action of such a transport mechanism and animal experiments were designed to test this hypothesis.

(a) A comparison of ventricular and cisternal CSF levels of 5-HIAA and HVA in the dog revealed a similar gradient to that observed in man.

Pretreatment of the animals with probenidol, a drug which blocks the renal transport mechanism for organic acids, was shown to reduce significantly the gradient levels of 5-HIAA and HVA between ventricles and cisterna with a marked relative rise in cisternal acid levels. Guldborg et al (1967). Such a result is consistent with the presence of an active transport mechanism for the organic acids situated in the CSF pathway.

(b) A technique was developed for the perfusion of the cerebral ventricles in the conscious dog similar to that used in the goat by Pappenheimer (1961). Such a method (Ashcroft et al (1967)) was used to study the clearance of 5-HIAA and HVA and inulin from the CSF, the results confirming the presence of an active transport mechanism for the acids and localising it in the fourth ventricle. Neff et al (1964) have provided evidence for a similar transport mechanism for 5-HIAA from brain tissue to blood.

#### CONCLUSIONS

The studies described in brief above represent an attempt to develop a technique for the study of the cerebral metabolism of 5-hydroxytryptamine in patients with depressive illness. The progressive evaluation of the technique has required the use of animal experimental procedures to examine the relationship between CSF and brain in addition to studies in man.

Our simple model (Fig. 1) must be amended in light of these studies to show a variation in 5-HIAA levels with the site of CSF sampling

and to include the concept of active transport rather than the passive diffusion of acid metabolites from brain and CSF into blood.

The results of the CSF studies in depressed patients showed lowered levels of 5-HIAA but normal HVA levels in lumbar fluid. Such a selective lowering of 5-HIAA levels argues against an increased rate of transport of the acid from CSF as disturbance of such a mechanism would be expected to lower concentrations of both HVA and 5-HIAA. We must conclude, therefore, that the findings appear to indicate a diminished release or synthesis of the amine 5-hydroxytryptamine in the brain of the depressed patient and would suggest that the most likely defect is in the hydroxylation of tryptophan.

The results, however, give us only a glimpse of the problem and the investigations have left us with more questions to answer than when we started, e.g. are the symptoms of depressive illness causally related to the change in amine

metabolism? Do different depressive clinical syndromes have different associated biochemical findings? Can the biochemical findings be shown to have any predictive value in terms of treatment and prognosis?

#### Acknowledgments.

In this paper I have concentrated on the technical aspects of an investigation which also has important ethical considerations. Our patients' permission for the investigations was sought after a full explanation of the nature of the tests and we would like here to acknowledge their ready co-operation. I wish to emphasise that this investigation was carried out by a team of workers.

In addition to those referred to in the text I wish to extend thanks to the medical and nursing staffs of the Royal Edinburgh Hospital and particularly to Dr. Elizabeth Robertson and Professor W. L. M. Perry.

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## Duel Purpose

"Hence, notwithstanding a most keen and illiberal persecution, which the new doctrine sustained from Cullen and his adherents, the disciples of Brown, from their numbers, shortly became so formidable to their adversaries that the latter, upon all occasions, and in all public societies, were perpetually annoyed and molested by them. The palaestra, where their contests were maintained with the greatest violence, was the Royal Medical Society. Here the partisans of both doctrines used to assemble weekly; and the debates on each side were conducted with such vehemence and intemperance, that they very commonly terminated in one member calling out another to the field. Such encounters had so frequently disgraced the Society, that, in order to prevent the growing evil, it became necessary to pass a law, by which it was enacted, that any member, who challenged another, in consequence of what had been said in the public debates, should be expelled the Society."

—from a Biographical Account of Dr. John Brown by William Cullen Brown (1804): John Brown was President of the R.M.S. three times, and during his third term of office published the "Elementa Medicinæ", expounding his system of medicine which raised a great deal of controversy in Edinburgh, but had more success on the Continent.