L-Tryptophan in Depression and Strain

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Summary

This paper is a report by the author of his Ph.D. Thesis.

When the organism is put under exertion (load), changes in the organism result (strain). The strain is counteracted by forces mobilized in the body (stress). Stress is a physiological phenomenon. If people become ill from any type of exertion, this will, by definition be the result of strain. The (biological) changes observed in ill people can be classified as:

1) specific to the clinical picture, or
2) strain phenomenon, or
3) stress phenomenon, or
4) incidental finding.

The psychic expression of strain is the mood disorder anxiety, even when the mood disorder depression causes the strain.

L-tryptophan, an amino acid, is converted in the body into (a) serotonin (5-hydroxy-tryptamine, 5-HT), a neurotransmitter of the raphe neuronen in the brain, and into (b) nicotinic acid ribonucleotide, a B vitamin. According to the literature one has assumed that in the organism:

1) a shortage of serotonin in the cerebral synapses would be the key to the development of (vital, primary, endogenous) depression;
2) a disturbance of the nicotinic acid ribonucleotide synthesis would decrease the cerebral synthesis of 5-HT because pyrrolase, the first enzyme of the nicotinic acid ribonucleotide synthesis, would be overactivated by hypercorticism.

This thesis shows that:

1) activation of the cerebral serotonergic neurons has a favourable action in case of strain (hyperventilation syndrome);
2) disturbance of the nicotinic acid ribonucleotide synthesis occurs particularly with anxiety and less with depression;
3) disturbance of the nicotinic acid ribonucleotide synthesis is not based on activation of the pyrrolase, but instead on disturbance of vitamin B6-dependent enzymes in the nicotinic acid ribonucleotide synthesis.

From the data it is concluded that: 1) the cerebral serotonergic neurons play a b.v.

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role in the regulation of stress, but not so in depression; 2) the disturbance of the nicotinic acid ribonucleotide synthesis is a strain phenomenon in depressive patients as well. Summarizing, the disturbance of the L-tryptophan metabolism to nicotinic acid ribonucleotide can be measured by means of the excretion of xanthurenic acid after oral challenge with L-tryptophan. Such a disturbance is indicative of strain and can be corrected by pyridoxine supplementation. If the L-tryptophan metabolism is substantial for the 5-HT synthesis in the cerebrum, an L-tryptophan disturbance can become a load and thus produce strain.

Preface

This book is the Ph.D. Thesis, successfully submitted by Dr. Hoes at the Catholic University of Nijmegen on 4th September 1981. The supervising professor was Dr. S.J. Nijdam, professor of clinical psychiatry at the Medical Faculty of the University of Nijmegen and the co-supervisor was Dr. E. van der Kleyn, head of the Clinical Pharmacy department of the Sint Radboud Ziekenhuis, the Academic Hospital at Nijmegen.

This thesis comprises an introduction and 13 articles, published or accepted for publication by diverse scientific journals; eight of these articles have been written in English, whereas the other five and the introduction are in Dutch.

The introduction indicates the objects of the 13 articles, discusses the problems which are examined and worked out in the 13 articles and contains a precise formulation of the problems examined in 11 questions. After a summarizing survey of the articles, the answers to the questions are given. The introduction concludes with practical instructions to perform the loading test, the excretion of xanthurenic acid in the urine, for 24 hours after intake of 5 g L-tryptophan at 10 p.m. Anxiety is defined as **the** indication for the test.

The book itself concludes with a summary written in English. This summary includes the 11 questions posed with the answers as formulated on the basis of the investigations.

On the invitation of the editor-in-chief of this journal, Dr. A. Hoffer, the author gives a concise survey of the contents of this thesis below, the first one submitted in The Netherlands with an orthomolecular approach to psychiatric problems.

THEORETICAL CONSIDERATIONS

Depression, Anxiety, Load, Strain and Stress

The book starts with the definition of depression as a symptom of mood disorder, as a syndrome or a nosological unity. As a mood disorder, depression is put on a par with anxiety; as a symptom, depression is always present in the syndrome or the nosological unity.

The mood disorder anxiety may be considered the psychic characteristic of adap-tational problems (Hinkle, 1974; Linford Rees, 1976). In adaptational phenomena a load which has an effect on the organism is clearly distinguished. This load strains the organism; the strain manifests itself in numerous changes within the organism. Through one or several changes adaptational processes are activated, which form a counter-force or stress in the organism. These will at least compensate for those changes of strain, which have functioned as a signal to activate the stress. Selye (1976) has defined stress as "the non-specific response of the body to any demand". Activation of this response by the load, however, occurs via strain, i.e. via certain phenomena of strain which function as a signal to activate the stress. Depression, too, will form a load for the organism, lead to strain in that organism and thus induce stress (Klerman, 1979). In a patient with the syndrome or nosological unity of depression, load due to this depression or vice versa will be concerned. This load will lead to strain. Only the strain is pathogenic, stress actually is a beneficial response, i.e. stress phenomena are, by definition, never pathogenic; a load may only lead to pathology via the induced strain. When a stress response is not attuned to the strain that induced it, it becomes a load.

In the adaptational process strain is pathogenic and the mood disorder anxiety may be considered the psychic manifestation of strain (Jenkins, 1979). So this anxiety is also pathogenic. A strain that develops in a

certain person, for any reason, e.g. an infection, may trigger a depression. Hence, a depression leads to strain and anxiety; strain and its anxiety may lead to depression.

The Significance of Biological Changes in Patients with a Disease

According to classical medicine, diseases are based upon specific affections of intracellular processes (Hoes, 1980). As explained in the previous chapter, a patient will show signs of strain because the disease or other environmental factors, such as the situational context, form a load for him. Because he shows strain he will develop stress as a compensation.

If biological changes are noted in a patient, they may be ascribed to one of the four following possibilities:

a) either they belong to the pathogenis or pathophysiology of the disease;
b) or they form part of the phenomenon of strain;
c) or they belong to the activated adapta-tional processes, tension, stress;
d) or they are purely coincidental and have nothing to do with disease, strain or stress.

The Role of the Metabolism of L-tryptophan in Depression

The biological determinants of depression as a syndrome (primary depression, vital depression) or symptoms of a disease (endogenous depression) have been thoroughly investigated.

On the basis of these investigations it has been suggested that a disorder of the L-tryptophan metabolism would be of pathogenic significance for the depression.

L-tryptophan is an aromatic amino acid. It is the mother substance of the neurotransmitter serotonin. Serotonin is specifically used as a neurotransmitter by the neurones of the raphe nuclei in cerebro (Ungerstedt, 1971). A serotonin deficiency is considered by certain authors of great significance in the pathogenesis of the syndrome or disorder of depression (Curzon, 1969; van Praag, 1976). Furthermore L-tryptophan is essential for the production of the B vitamin, nicotinic acid ribonucleotide (Bogert et al., 1973). This synthesis consumes the main part of the absorbed amount of L-tryptophan (750 mg of approximately 1 g). The first enzyme of this synthesis, pyrrolase, also limits the rate of biosynthesis.

The activity may particularly be increased by induction of the apo-enzyme production through corticosteroids and by increased supply of substrate (L-tryptophan). Since an increase of the blood levels of adrenal cortex hormones leads to a three to six-fold increase of the total activity of pyrrolase (Wolf, 1974), a few authors suggested that this mechanism would lead to a decrease of the plasma levels of L-tryptophan (Curzon, 1969).

This has been proven in experimental animals. If this were also the case in man, it would mean that less L-tryptophan from the blood would be supplied to the brain. It is highly acceptable that the synthesis of serotonin in cerebro directly depends upon the supply of L-tryptophan from the blood (Fernstrom and Lytle, 1976). An excess of corticosteroid would hence decrease the plasma levels of L-tryptophan by activation of the pyrrolase, and thus reduce the cerebral serotonin synthesis. This might trigger a depression. Certain authors assume that this is the pathogenesis of the depression. However, it is unlikely that there is a direct relation between a serotonin shortage and the existence of a depression. The serotonin hypothesis is not backed up sufficiently for the following reasons:

a) not all patients who are treated with reserpine show a depression; however reserpine does cause a serotonin depletion in the serotonin containing neurones (van Praag, 1976)
b) subjects undergoing a serotonin depletion, by inhibition of the serotonin synthesis with parachlorophenylalanine, do not develop a depression, but unrest, uncertainty and anxiety (Mendels and Frazer, 1974)
c) suppletion of L-tryptophan or 5-hydroxy-tryptophan to depressive patients only renders a significant improvement of depression in a limited number of studies (d'Elia et al., 1978; Abrams, 1978)
d) the conversion of serotonin to 5-hydroxyindolacetic acid, measured as conversion rate in the liquor, is only reduced in a restricted number of

depressive patients (Asberg et al., 1976; van Praag, 1974).

Furthermore it appears from the consequences of the activity increase of pyr-rolase that Cortisol, hence a hyper-activation of stress processes (bad!) would lead to serotonin decrease. This would only be compatible with a depression-provoking role for serotonin depletion, hence corresponding with a provocation of depression by strain.

On the basis of above considerations it may be concluded that the disorders in the L-tryptophan metabolism are caused by a hypercorticism. The L-tryptophan metabolism may at most provoke a depression via a decrease of the serotonin levels in cerebro.
This results in the following questions.

THE QUESTIONS (Q) AND THE ANSWERS (A)

The crux of this thesis is whether the disturbance of L-tryptophan metabolism in the depressive patient is of primary importance for depression or strain. This cardinal issue has been split up into the following questions:

Q. 1. Can a disturbed metabolism of L-tryptophan be demonstrated in depressive patients? If so can the depression be influenced therapeutically by remedying this disorder?

A. 1. In depressive patients, a disturbance in the L-tryptophan metabolism has been measured in the excretion of xanthurenic acid with urine for 24 hours, after intake of 5g L-tryptophan at 10 p.m. The administration of 125 mg pyridoxine t.i.d. remedies the disturbed excretion of xanthurenic acid and the depression within 4 weeks (paper I).

Q. 2. Will the effect of administering 125 mg pyridoxine t.i.d. be increased by adding 2 g L-tryptophan at night?

A. 2. When for a period of 4 weeks 2 g L-tryptophan at night are added to 125 mg pyridoxine given t.i.d. the xanthurenic acid excretion returns to normal. The same is true when only pyridoxine is administered. The depression as measured with the Zung test, shows significant improvement under pyridoxine alone ($p < 0.0125$) and a highly significant improvement under the combination ($p < 0.00005$)) (t-test) (paper I).

Q. 3. Can any biological signs of acute strain be demonstrated in depressive patients and how do such variables respond to the administration of 125 mg pyridoxine t.i.d. combined or not with 2 g L-tryptophan at night?

A. 3. Parameters used to record acute strain were the breakdown products of dopamine i.e. homovanillic acid, and those of (nor) adrenaline i.e. vanillylic mandelic acid as measured in 24 hours urine; these values did not increase. The administration of 125 mg pyridoxine t.i.d. did not induce a significant change in this excretion after 4 weeks, whereas 125 mg pyridoxine t.i.d. associated with 2 g L-tryptophan at night did not induce a significant decrease in homovanillic acid ($p < 0.0025$) and vanillylic mandelic acid ($p < 0.025$) (t-test) (paper II).

Q. 4. Is there any significant difference in the excretion of xanthurenic acid with 24 hours urine after intake of 5 g L-tryptophan between patients with an endogenous (primary) or reactive (secondary) depression on the one hand, and those with anxiety as a predominant psychic disturbance, on the other?

A. 4. Excretion of xanthurenic acid with the urine for 24 hours after a challenge with 5 g L-tryptophan in normal subjects acting as controls, at a rate of 68.8 ± 19.0 umol/24 hours (paper IX) is significantly (t-test) different in patients with primary depression ($p = 0.04$), secondary depression ($p = 0.05$) and highly significant in patients with anxiety ($p = 0.01$).

Q. 5. Does the administration of 125 mg pyridoxine t.i.d. along with 2 g L-tryptophan at night, have a therapeutic result in patients with a disorder produced by chronic strain (hyperventilation syndrome)? Can this effect be predicted by detecting a disturbance in the excretion of

xanthurenic acid with 24 hours urine after intake of 5 g L-tryptophan?

A. 5. The administration of 125 mg pyri-doxine t.i.d. and 2 g L-tryptophan at night has a therapeutic effect on a number of patients with a disorder induced by chronic strain (hyperventilation syndrome). This effect can be predicted when the L-tryptophan metabolism measured as the excretion of xanthurenic acid with urine, 24 hours after the intake of 5 g L-tryptophan, is disturbed (papers IV,V,VI).

Q. 6. Is 125 mg pyridoxine t.i.d. a dosage that may be expected to produce a measurable increase in the pyridoxine content in the organism within a period of 24 hours?

A. 6 Proceeding from kinetic studies, one may expect that 125 mg pyridoxine t.i.d. produce a measurable increase in pyridoxine in the body for 24 hours (paper VIII).

Q. 7. Do patients with a primary depression show a change in absorption, distribution or elimination of L-tryp-tophan after the intake of 5 g of this compound?

A. 7. The kinetics of L-tryptophan in the plasma of depressive patients after intake of 5 g L-tryptophan is in no way different from that of healthy subjects acting as controls (paper IX).

Q. 8. Do patients with a primary depression show a difference in the therapeutic response to depression or anxiety when they are treated with 125 mg pyridoxine t.i.d. along with 3 g L-tryptophan at night — a combination that is supposed to develop a serotonergically activating effect?

A. 8. Patients with a primary depression do not respond differently with symptoms of either depression or anxiety to treatment with 125 mg pyridoxine t.i.d. and 3 g L-tryptophan after 2 or 4 weeks (paper X).

Q. 9. Do patients with a primary depression show a difference in the therapeutic response to depression or anxiety, when they are treated with maprotiline 100 mg at night, a noradrenergic activating compound?

A. 9. Patients with a primary depression do not respond differently with symptoms of either depression or anxiety to a treatment with maprotiline for 2 weeks or 4 weeks (paper X).

Q.10. Is there a difference in the therapeutic response to depression and anxiety after treatment with pyridoxine and L-tryptophan (question 8) or with maprotiline (question 9)?

A. 10. There is no different response for depression or anxiety after a probably serotonergically activating therapy (answer 8) or after a nor-adrenergically activating therapy (answer 9).

Q.ll. Does the excretion of xanthurenic acid with the urine for 24 hours following the intake of 5 g L-tryptophan in depressive patients represent a measure for depression or strain? What disturbance can be expected? How can they be cured?

A. 11. The excretion of xanthurenic acid with the urine within 24 hours following the intake of 5 g L-tryptophan in depressive patients is a measure for strain (paper XII). In this experimental design the excretion of xanthurenic acid can be increased or — if the deviation is very severe — it can be lowered. The normal values (m± 2 s) are 30.8-106.8 umol/24 hours (paper IX). Pyridoxine 125 mg t.i.d. restores the disturbance within 2 weeks of treatment (papers **I** and **X).**

SUMMARIZING SURVEY OF THE ARTICLES

I. ***Hoes, M.J.A.J.M.*** *(1979): Pyridoxine, L-tryptophan and Zinc Sulphate for Depressive Patients; I. Therapeutic Effect Related to Increased Xanthurenic Acid Excretion and Serum Copper Values. Tijdschrift voor Psy-chiatrie 21, 302-312 (own investigation, polyclinic psychiatry, Nijmegen in Dutch).*

It was observed that the excretion of xanthurenic acid (XA) is disturbed in de-pressive patients after oral intake of L-tryptophan. Hence a reduction of

5-hydroxyindole acetic acid (5-HIAA) might be expected in these patients.
However, the excretion of 5-HIAA in the urine did not deviate. This may be explained by the fact that the enterochromaffin system, a major producer of serotonin in the body, has already absorbed the L-trypto-phan required, before the latter is removed via the enterohepatic vessels (Levine, 1974).

The pyridoxine therapy (125 mg 3 times daily) corrects the deviation in the XA-excretion. A therapy with pyridoxine (125 mg 3 times daily) and L-tryptophan (2 g in the evening) or with pyridoxine (125 mg 3 times daily) and zinc sulphate (50 mg 3 times daily) renders comparable results. However, with regard to the depression there are clear differences between the therapeutic effects. The combination of pyridoxine and L-tryptophan has a better antidepressive effect than pyridoxine alone. One of the possible explanations could be that the patients would have shown an L-tryptophan deficiency in their circulation, with a pathophysiological significance for the depression.

II. ***Hoes, M.J.A.J.M.*** *(1979): Pyridoxine, L-tryptophan and Zinc Sulphate for Depressive Patients; II. Effect on the Excretion of Homovanillic Acid and Vanillylmandelic Acid as Stress Parameters. Tijdschrift voor Psychiatrie 21, 312-321 (In Dutch).*

In the depressive patients of the previous study the effect of the three therapies on a few clinical and biochemical parameters, anxiety, occipital pain and hyperventilation were measured. As biochemical parameter the excretion in the urine of homovanillic acid (HVA) and vanillylmandelic acid (VMA) was measured; these are decomposition products of the catecholaminergic neurotransmitters dopamine and (nor) adrenaline respectively which are synthesized in the sympathetic nervous system and adrenal marrow. The excretion of these two decomposition products may be used as a parameter for strain in the acute phase (Levi, 1972). The clinical picture responded favorably to each of these therapies, without any difference in effect between the therapies being found. If, therefore, it is decided that the observed symptoms belong to the depressive symptomatology and must not be considered separate strain phenomena, it must still be explained why all patients suffered from these symptoms to such an extent, whereas these somatic symptoms do not form a regular part of depressive symptomatology as described by Nelson and Charney (1981).

It therefore seems plausible to consider these symptoms strain phenomena. The excretion of HVA and VMA was not higher before the treatment than in normal subjects. This corresponds with the prolonged existence of the depression and the accompanying transience of the acute strain. In other words, it is an argument for the hypothesis that the strain in these patients, measured as anxiety, headache and hyperventilation, is caused by the depression. The reason for this is that no more biological changes of acute strain can be observed any more. However, it is striking that precisely under the pyridoxine and L-trypto-phan therapy there is a marked reduction of the excretion of HVA and VMA. Two conclusions may be drawn from this. Firstly, the catecholamines are used more effectively under the pyridoxine and L-tryptophan therapy. Furthermore, this reduction of the conversion of catecholaminergic neurotransmitters occurs precisely under the most successful antidepressive therapy. This is an additional argument for the hypothesis that the strain in these persons has been caused by the depression.
These two studies, however, did not make clear whether the disordered XA-excretion formed part of the depression or strain. III. *Hoes, M.J.AJ.M. (1979): The Clinical Significance of Xanthurenic Acid Excretion in Psychiatric Patients. ActaPsychiatricaBelgica 79, 638-646 (own investigation,* Tiel) The increase of the XA-excretion in depressive patients is ascribed to an increased secretion of glucocorticoids by the adrenals. Since an increased secretion of glucocorticoids is typical of a condition of strain, Selye's biological stress syndrome (1976), the correlation of a disordered XA-excretion with an increased secretion of 17-hydroxy cortico steroids (17 OHCS) (Curzon, 1969; Rubin, 1967) suggests that strain and not depression determines the disturbance in the XA-excretion of these depressive

patients. The increased secretion of 17 OHCS is indeed only found in depressive patients who are very anxious or psychotic (Sachar, 1967). Since anxiety is the main psychopathological correlate of strain (Selye, 1980) and since the hypercorticism disturbs the XA-excretion it is expected that particularly anxious patients and not patients with a clear depression will show a disturbed XA-excretion. This has been confirmed in this study of patients with depression, anxiety, alcoholism and a control group of psychiatric patients. Only alcoholics showed a higher excretion of XA during the withdrawal phase. Both a vitamin deficiency and the anxiety which is quite normal in alcoholics during the withdrawal phase (Keller, 1977) may have contributed to this.

IV. ***Hoes, M.J.A.J.M.*** *(1980): Serotonin and the Hyperventilation Syndrome. Tijdschrift voor Geneesmiddelenon-derzoek 4, 726 (In Dutch).*

Up to now it was clear that the disturbed XA-excretion was not linked to the mood disorder, but rather to anxiety. Therefore a separate investigation into the significance of the L-tryptophan metabolism in conditions of anxiety seemed useful. L-tryptophan is converted into serotonin. In this article it is shown how the cerebral serotonergic systems may play an important part in the pathophysiology of the hyperventilation syndrome. This is a topical example of a syndrome caused by strain.

Serotonin appears to play an essential role in the control of respiration, muscular tension and level of anxiety which has been demonstrated in animal studies. And a major part of the hyperventilation syndrome is formed precisely by disorders in these functions.

If the L-tryptophan metabolism has a pathogenic or pathophysiological relation which is important for the strain, these two predictions with regard to the hyperventilation syndrome should hold true. Firstly an underactivity of the serotoninergic transmission should play an important part in the pathophysiology of the syndrome. Therefore activation of serotoninergic systems has been tried as a therapy. Secondly a correction of possible deviations in the XA-excretion should be therapeutically effective.

If L-tryptophan is withdrawn from the circulation by an increased activity of pyrro-lase the cerebral production of serotonin is disturbed via a decrease of plasma level of tryptophan. Therefore a therapy with pyridoxine to which L-tryptophan is added, should be tried out, in order to correct a possible deficiency of L-tryptophan.

V. ***Hoes, M.J.A.J.M., Colla, P., Folgering, H.*** *(1980): Clomipramine Treat ment of Hyperventilation Syndrome. Pharmakopsychiatrie-Neuro-Psychopharmacologie 13,15-19 (own investi gation, Nijmegen).*

First it was tested whether activation of the serotonergic transmission rendered a favorable therapeutic effect. The six patients treated were a negative selection, since they had all unsuccessfully completed at least one-and-a-half years of behavioral therapy aimed at their hyperventilation syndrome. Clomipramine is the most active serotonergic agonist momentarily available for clinical use. The unanimously satisfactory result which was maintained during one-and-a-half years of follow-up, is in favour of the therapy (Anafranil^{R}, 25 mg 3 times daily). Because clomipramine is a serotonin agonist, it must not be directly concluded that the serotoninergic hypoactivity is the crucial moment in the pathophysiology of the hyperventilation syndrome. All tricyclic substances with a tertiary amino group at their end are demethylated in the organism, the tertiary amine group being converted into a secondary one (Bickel, 1976). This provides them with even more noradrenergic activating properties. It is possible that the noradrenergic activity of the metabolite of clomipramine, desmethyl-clomipramine, has contributed to the therapeutic effect.

VI. ***Hoes, M.J.A.J.M., Colla, P., Folgering, H.*** *(1981): Hyperventilation Syn drome, Treatment with L-tryptophan and Pyridoxine; Predictive Values of Xanthurenic Acid Excretion. Journal Orthomolecular Psychiatry 10,1, 7-15 (own investigation, Nijmegen).*

Patients with the hyperventilation syndrome show a disturbed renal excretion of XA, which confirms that this disturbance is not specific for depressions. Treatment with pyridoxine and L-tryptophan normalized

the decreased as well as the increased XA excretion and yielded favourable clinical results. From the latter it could be concluded that the serotonergic transmission was indeed disturbed and that this is the pathophysiological basis of the hyperventilation syndrome. Pyridoxine particularly improved the synthesis of serotonine (Dakshinamurti et al., 1976) whereas L-tryptophan inhibits the uptake of tyrosine in the catecholaminergic cells (Fernstrom and Lytle, 1976). This activates the serotonergic transmission and deactivates the catecholaminergic transmission. These results yield an argument against a possible noradrenergic effect of clomipramine on the hyperventilation syndrome.

VII. ***Hoes, M.J.A.J.M.*** *(1980): TheL-tryp-tophan and Pyridoxine Metabolism in Depressive Patients: a Serotonin Hypothesis for the Corticosteroid Regulation and Adaptation. Tijdschrift voor Geneesmiddelenonderzoek 3, 685-694 (In Dutch).* Up to now there was every appearance that the serotonin hypothesis of depression demonstrated too little expressiveness. Moreover the metabolism of L-tryptophan appeared to play a part in the cerebral serotonergic metabolism.

The metabolism of L-tryptophan in the organism, measured as XA-excretion, is disturbed in a condition of strain. However, if activation of the serotonergic transmission renders a marked improvement in a condition of strain, the cerebral serotonergic transmission should be important for the regulation of adaptational processes. As a guide for further research a model was designed for the serotonergic hypothesis of the regulation of adaptation.

This was based on the role that serotonergic systems play in the regulation of numerous homeostatic functions, such as sleep (Jouvet, 1972), temperature (Myers, 1978) and particularly in the negative feedback of the corticosteroids on the ACTH-secretion. In a condition of strain the glucocorticoid plasma levels are increased. These substances induce the enzyme pyrrolase, due to which more L-tryptophan is withdrawn from the circulation. If the cerebral serotonin synthesis becomes insufficient because of this, the negative feedback of the glucocorticosteroids on the ACTH-secretion is disturbed furthermore; the regulation of numerous homeostatic functions may be disturbed. The cancellation of the negative feedback will result in a positive feedback in the glucocorticoid-L-tryptophan metabolism on the level of pyrrolase, due to which the plasma level of L-tryptophan will decrease even further and many homeostatic functions of the patients will become and remain permanently disturbed.

This model can explain why certain depressive patients show a reduced 5-HIAA level in the liquor, why the XA-disturbance is associated with hypercorticism, why a pyridoxine and L-tryptophan therapy has a positive effect on depressive patients with an L-tryptophan deficiency.

VIII. ***O'Reilly, W.J., Guelen, P.J.M., Hoes, M.J.A., Van den Kleijn, E.*** *(1980): The Determination of Pyridoxine and Congeners in Biological Fluids of Man after High Dose Therapy by Means of High Performance Liquid Chromatography. Journal of Chromatography Biomedical Applications 183, 492-498 (own investigation, Tiel).* At the beginning of the study it was opted for megadoses of vitamin B6. A dose of 125 mg 3 times daily is considerably higher than the daily amount of 2 mg which is generally accepted as physiologically required (Bogert et al., 1973).

Often loading doses of vitamins are administered to correct deficient conditions. The kinetics of pyridoxine were not clear either, so that it was not known whether a single daily dose would be sufficient. Therefore half a tablet at 125 mg was given 3 times a day. A high performance liquid chromatographic method was developed at the laboratory for clinical pharmacy of the Radboud-ziekenhuis, in order to study the kinetics of vitamin B6, pyridoxine, as well as its metabolites. About one hour after intake the absorption is already maximal.

This trial shows that the 125 mg dose 3 times daily is sufficient to maintain peak levels of pyridoxine in the organism. Indeed 24 hours after intake of the 750 mg load the plasma concentration of pyridoxine is still elevated, and also the level of its metabolite,

pyridoxinic acid. Furthermore, only 35 percent of excretion products is measured during the 24 hours after intake. This may either indicate that certain active or non-active metabolites of pyridoxine are still unknown or that an oral dose of pyridoxine is not completely absorbed from the gastrointestinal tract.

IX. *Hoes, M.J.A.J.M., Kreutzer, E.K.J., Sijben, N.* *(1981): Xanthurenic Acid Excretion in Urine after Oral Intake of 5 g of L-tryptophan by Healthy Volunteers: Standardization of the reference values. Journal Clinical Chemistry Biochemistry 19, 259-264 (own investigation, Tiel).* A major variable in the studies of this dissertation is the measurement of the renal excretion of xanthurenic acid after L-tryp-tophan load. Precise normal values were lacking, because the reference values were derived from a collection of 648 samples of 24-hours' urine of neurological and psychiatric patients. Loading with 5 g L-tryptophan guaranteed a complete saturation of the enzyme capacity of the NiA synthesis, but many variables were not yet investigated with relation to the XA-excretion. For this study healthy volunteers were selected in whom particularly anxiety and depression were lacking. In these patients it was deter-mined that the study should preferably start in the evening, that urine had to be collected over a period of 24 hours and that the test, repeated by the subjects within a week, leads to reproducible results. In contrast to the psychiatric patients of study HI, a significant correlation between the urine volume, urine flow and XA-excretion was found in these subjects. The test did not depend on sex, age, height, body weight, urine pH, excretion of creatinin and of 17 OH corticosteroids. This means that the XA-measurement is a method that is hardly not disturbed by the daily changes of, for instance, diet. Of the original 648 data, 327 were within the average m ± 2. S.D. of the new reference values.

X. *Hoes, M.J.A.J.M., Sijben, N.* *(1981):The Clinical Significance of a Disordered Excretion of Xanthurenic Acid in Depressive Patients. Psycho-pharmacology, accepted (own investigation, Tiel).*

Using the XA-excretion as an instrument, it was then assessed whether there was any connection between the excretion of xanthurenic acid and that of 17 OH corticosteroids. Furthermore a relation as expected between the degree of anxiety, as a psychopathological coincident of strain, the excretion of 17-hydroxy-steroids. No direct relation was expected between the depression and the excretion of xanthurenic acid or of 17-hydroxy-steroids, because depression dependent on stress leads to an increased 17 OH-corticosteroids excretion. The degree of anxiety or depression was measured by means of rating scales. Except for the lack of any relation between the depression and the excretion of XA or 17 OHCS, none of the above suppositions was confirmed. The response to either an activating treatment of the serotonergic transmission (pyridoxine and L-tryptophan) or a noradrenergic activating therapy (Maprotiline) was assessed by means of clinical and biological parameters. It could be expected that the decrease of anxiety would correspond to changes in the excretion of XA or 17 OHCS and that the degrees of anxiety and depression would diminish simultaneously.

The only correlation found, was that the normalization of the XA-excretion after two weeks under each of both therapies correlated significantly with a decrease of both anxiety and depression after four weeks treatment.

Although many factors, such as patient selection or severity of the depression could have had an effect on the result, this study confirmed that the therapy with pyridoxine and L-tryptophan yielded a comparable decrease of depression and anxiety as the noradrenergic activating therapy did. This brings the specificity of the monoaminergic deviations in depression under discussion and at least requires further investigation.

XL *Hoes, M.J.A.J.M., Loeffen, T., Vree, T.B.* *(1981): Kinetics of L-tryptophan in Depressive Patients. A*

Possible Correlation between the Plasma Concentrations of L-trypto-phan and Some Psychiatric Rating Scales. Psychopharmacology, 75, 350-353 In the same patients as in publication X a pharmacokinetic study of the plasma level of L-tryptophan was carried out during the performance of the L-tryptophan load. For this purpose a high performance liquid chromatographic method was set up in the Laboratory of Clinical Pharmacy of the Radboud Ziekenhuis, in order to determine the L-tryptophan concentration. If an increased XA-excretion leads to a decrease of the plasma level of L-tryptophan, this will be found in the results.

And it was not so, neither for the sober and blank L-tryptophan value the first time, nor for the measurements during the load. Furthermore it would be expected that the kinetics of L-tryptophan would show another distribution or elimination phase, if the patients were suffering from a shortage of L-tryptophan. This supposition was not confirmed, either. Moreover, no difference was observed between the plasma levels of L-tryptophan at the start or after two and/or four weeks therapy with either pyridoxine and L-tryptophan or maprotiline. This is even more interesting since the patients from one group received not less than three times the physiological daily dose of L-tryptophan for four weeks. The elimination of L-tryptophan occurs rapidly, no accumulation was observed. In this study no correlation was found between the plasma concentration of L-tryptophan and the anxiety or agitation score, as a measure for the strain. A negative correlation would be expected.

XII. *Hoes, M.J.A.J.M.* *(1982): The Excretion of Xanthurenic Acid in 24 Hours Urine After Oral Intake of 5 g L-tryptophan: a Measure of the Strain in the Organism. Selye's Guide of Stress research, Van Nostrand Reinhold, Toronto, Vol. Ill, accepted (own investigation,* Tiel). Up to now it has been assumed on the basis of literature data that the blank values of the XA-excretion are about zero, i.e. near to the detection limits of the measuring method. In order to methodically deepen out the XA-determination, the blank values of the XA-excretion are investigated. It appeared that the XA-excretion without load with L-tryptophan was high; in depressive patients, however, it was lower than in the control subjects. This may presumably be ascribed to the daily activities, because after recovery the XA-excretion was noted in the control subjects as well as in the patients.

Furthermore, there is a clear difference between the absolute excretion values of the control persons and those of the patients. That is the reason why the XA-excretion should always be measured after load.

XIII. *Hoes, M.J.A.J.M.* *(1981): The Clinical Psychiatric Significance of a Disturbed XA-excretion in 24 hours' Urine After Oral Intake of 5 g L-tryptophan. Bulletin commissie bio-chemisch onderzoek sectie geestelijke gezondheidszorg, Nationale Gezond-heidsraad, 81-1, 4-9 (in Dutch).* In this publication the investigations as performed were finally summarized with regard to practical usefulness. True that not all results of the total investigation were positive as to the expectations and suppositions, but it still may be concluded that a disturbed XA-excretion after oral load with tryptophan has a few correlates. Psycho-pathologically, it is associated with anxiety and presumably also with agitation, so that these two symptoms are an indication to perform an XA-test. As a sign of strain an increased secretion of corticosteroids is physiologically likely.

From a biochemical point of view, there is an absolute or relative deficiency of vitamin B6 in the nutrition, so that a pyridoxine therapy is indicated. The particular significance of this test lies in the fact that a disturbed XA-test may indicate a disordered cerebral serotonin synthesis. Because cerebral serotonin is important for the control of homeostatic processes, a disturbed XA-test may point to a disordered homeostasis and precisely a disordered homeostasis c.q. adaptation may be improved by a pyridoxine therapy.

PRACTICAL USE OF THE L-TRYPTOPHAN LOADING TEST

1. The excretion of xanthurenic acid in the urine (dark pot) during 24 hours after oral intake of 5 g L-tryptophan at 10 p. m. by healthy volunteers amounts to 68.8 ± 19.0 umole/24 h (m ± s).
2. Especially in anxiety the XA-excretion is disturbed. Hence the L-tryptophan load test is indicated in anxiety*.
3. The disturbance of the xanthurenic acid excretion is effected by induction of the rate limiting enzyme pyrrolase and by an absolute or relative (with relation to nutrition) deficiency of pyridoxine. Since both mechanisms may be triggered by an enhanced excretion of adrenal cortex hormones, a disordered XA-excretion in an anxious patient is a measure for the strain in the organism. 4. Pyridoxine 125 mg 3 times daily corrects the disordered clinical condition and XA-excretion within 4 weeks.

If the results are unsatisfactory the pyridoxine therapy is continued and L-tryptophan 2 to 3 g in the evening is added; after 4 weeks the medication is discontinued, irrespective of the results.

* Note: A patient is considered sufficiently anxious to have an L-tryptophan loading test performed when two conditions are complied with:
l.the patient says he is anxious;
2.at least half of the following symptoms are clearly present: tension; fear; panic; mental disintegration; tremorousness; tremors; physical pain; tiredness; restlessness; palpitations; dizziness; fainting; dyspnoea; paraesthesia; nausea and vomiting; polla-kisuria; sweating; flushing, insomnia, nightmares (according to Zung, 1971)

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