NEUROCHEMICAL ASPECTS OF CHILDHOOD AUTISM

(Neurochemische aspecten van kinderlijk autisme)

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RUDOLF BOUDEWIJN MINDERAA

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Promotiecommissie:

Promotoren: Prof. Dr. J. A. R. Sanders-Woudstra
Prof. Dr. D. J. Cohen

Overige leden: Prof. Dr. H. K. A. Visser
Prof. Dr. B. Leijnse

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To Marianne,
Alexander and
Robert

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CHAPTER I

INTRODUCTION AND AIMS OF THE STUDY

1.1 INTRODUCTION

The syndrome of infantile autism was first described by Leo Kanner in 1943 in his famous paper "Autistic disturbances of affective contact" (Kanner L., 1943). Kanner considered the core problem of the 11 children he originately described:"the inability to relate themselves in the ordinary way to people and situations from the beginning of life". Infantile autism is a behavioral syndrome. It is generally believed that it is etiologically heterogeneous. If one considers the sine qua non of the syndrome of infantile autism to be the presence of a severe and unique distortion of social relating and communication, the uniqueness of the syndrome suggests one underlying pathophysiological mechanism. Originally Kanner understood the peculiar indifference to social contact and the lack of communicative intent as arising sui generis in the autistic child itself and suggested an inherited predisposition (Kanner L., 1943, 1944). Later he thought the social and communicative deficits were attributed to psychopathological influences in the family environment (Kanner L., 1949). Subsequently up to the 1960's, psychodynamic theories predominated the litterature (e.g. Fraknoi and Ruttenberg, 1971). But in the early 1970's it appeared that much evidence had accumulated pointing in other directions, and reviewers (Ornitz, 1973; Cantwell et al., 1978) concluded that extensive clinical investigation had provided no compelling evidence of any unusual parental characteristics of family interactions in families of autistic children. Encouraged by the rapid advance of basic neuroscience and findings in schizophrenia, depression, and other psychiatric disorders new research approaches and theories appeared.

Neurophysiologic hypotheses were put forward in which infantile autism is seen as secondary to a disorder of arousal (Hutt et al., 1965; Hutt and Hutt, 1968), as secondary to a specific cognitive (language related) disorder (Rutter, 1978,1982) and as secondary to a disorder of sensory modulation (Ornitz and Ritvo , 1968; Reichler and Schopler, 1971; Ornitz, 1974, 1983, 1985). These hypotheses suggested an underlying area of neurophysiological dysfunction. In recent neuroanatomic hypotheses infantile autism is considered to be secondary to temporal lobe pathology

(DeLong, 1978; DeLong et al., 1981), to dysfunction of dopaminergic mesolimbic cortical and neostriatal structures (Damasio and Maurer, 1978) and to dysfunctions of brainstem mechanisms involving (vestibular) modulation of sensation and motility (Ornitz, 1974, 1978, 1983). (For a review, see Ornitz, 1983.)

Evidence from neurochemical and neuropharmacological studies of autistic children has often been of importance in development and support of these newer theories. Neurochemical studies have been undertaken in order to examine aspects of neural transmission in the central and the peripheral nervous system. Typically levels of neurotransmitters, their metabolites and associated enzymes in blood, urine, or cerebrospinal fluid were measured. In neuroendocrine studies the functioning of central monoamine neurotransmitters involved in controlling hormone release has been assessed by measuring hormone levels after administration of specific pharmacological agents.
Recently research has widened further. Genetic aspects and immunological factors are the new targets of research efforts.
Currently, infantile autism is viewed as a neuropsychiatric disorder of childhood and the scientific efforts to uncover its causes and underlying pathophysiologic mechanisms may serve as a model in modern research in child psychiatry.

1.2 AIMS OF THE STUDY

The topic of this thesis is neurochemical aspects of infantile autism. The experimental work is centered around the most robust and consistant neurochemical finding in child psychiatry, namely that group mean whole blood serotonin (5-Hydroxytryptamine, 5-HT) values are increased approximately 50% in autistic populations. Although the elevation has been observed many times, a number of basic questions are unanswered in respect of characterizations of this 5-HT elevation, and the elucidation of its causes.

In **chapter II** the behavioral syndrome of infantile autism will be described and the basic findings which support an organic etiology will be briefly outlined.
In **chapter III** the neurochemical findings will be reviewed with an accent on research related to the catecholamines and neuroendocrine functioning.
In **chapter IV** research in autism related to the serotonergic system will be extensively reviewed and critically discussed. This discussion will also include an examination of the original findings which are presented in the appendix. Findings concerning the "hyperserotonemia" of infantile autism will be critically discussed and related to other studies of the serotonergic system and further possible lines of research will be suggested.

The **appendix** is comprised of papers based on original experimental work related to "hyperserotonemia" in infantile autism.
Appendix I deals with whole blood serotonin and tryptophan values in autistic individuals and normal controls and its relationship to age, sex, platelet count and medication.
Appendix II describes again whole blood serotonin values and platelet count in autistic individuals and adresses to their inter-relationship and effects of medication and deals with the stability over time of whole blood serotonin values in unmedicated and medicated autistic individuals.
Appendix III adresses to the question whether an increased production of serotonin in the gut might be a cause of hyperserotonemia in autistic individuals by measuring the urinary excretion of 5-hydroxyindoleacetic acid, the major 5-HT metabolite.
In **Appendix IV** an aspect of platelet membrane function is assessed as being related to whole blood serotonin by measuring platelet imipramine binding in autistic subjects.
In **Appendix V** and **VI** the presence of primitive reflexes in autistic subjects and the relationship of the snout reflex and the visual rooting reflex to blood serotonin levels are described.

CHAPTER II

INFANTILE AUTISM: A NEUROPSYCHIATRIC DISORDER OF CHILDHOOD

2.1 INTRODUCTION AND CLASSIFICATION

Infantile autism is a syndrome that, since its first description by Kanner (1943, 1944), has raised many questions for the childpsychiatrist. The symptomatology differs from child to child in seriousness and character and may change in the course of time. There are no anamnestic features pointing to one origin of the illness and there are no specific bodily symptoms. These facts hamper the diagnosis (Ornitz et al., 1976) and this confusion becomes evident in differences in diagnostic terminology. The terms childhood autism, primary autism, infantile autism, atypical development, childhood schizophrenia, symbiotic psychosis and early onset psychosis, all refer to nearly the same symptomatology. The work of the American National Society for Autistic children (Ritvo and Freedman, 1978), The American Psychiatric Association (DSM-III, 1980) and Rutter and coworkers (Rutter, 1978b) brought more unity of meaning in the description and definition of this syndrome.
In clinical practice and for research purposes the criteria found in the third edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-III, 1980) are generally accepted. In the DSM-III infantile autism is brought under the general heading of the Pervasive Developmental Disorders of Childhood. These disorders are characterized by distortions, deviations and delays in the development of social, language and motor behaviors, attention, perception and reality testing. The DSM-III distinguises among 5 diagnostic categories within the group of Pervasive developmental disorders of childhood:

- Infantile autism, full syndrome present (299.00)
- Infantile autism, residual state (299.01)
- Childhood onset pervasive developmental disorder, full syndrome present (299.90)
- Childhood onset pervasive develomental disorder, residual state (299.91)
- Atypical pervasive developmental disorder (299.8X).

The criteria for these diagnostic categories are given in Table I. The DSM-III is a multiaxial system. Mental retardation can be diagnosed if present (Axis I) and a concomitant medical condition or disease can be diagnosed on Axis III.

Table I

Diagnostic and Statistical Manual of Mental Disorders (DSM-III), 1980

Diagnostic Criteria for Infantile Autism

A. Onset before 30 months of age.

B. Pervasive lack of responsiveness to other people (autism).

C. Gross deficits in language development.

D. If speech is present, peculiar speech patterns, such as immediate and delayed echolalia, metaphorical language, pronominal reversal.

E. Bizarre responses to various aspects of the environment, e.g. resistance to change, peculiar interest in or attachments to animate or inanimate objects.

F. Absence of delusions, hallucinations, loosening of associations, and incoherence as in schizophrenia.

Diagnostic Criteria for Infantile Autism, Residual State

A. Once had an illness that met the criteria for infantile autism.

B. The current clinical picture no longer meets the full criteria for infantile autism, but signs of the illness have persisted to the present, such as oddities of communication and social awkwardness.

Diagnostic Criteria for Childhood Onset Pervasive Developmental Disorder

A. Gross and sustained impairment in social relationships, e.g. lack of appropriate affective responsivity, inappropriate clinging, asociality, lack of empathy.

B. At least three of the following:
   1. Sudden excessive anxiety manifested by such symptoms as free-floating anxiety, catastrophic reactions to everyday occurrences, inability to be consoled when upset, unexplained panic attacks.
   2. Constricted or inappropriate affect, including lack of appropriate fear reactions, unexplained rage reactions, and extreme mood lability.
   3. Resistance to change in the environment (e.g. upset if dinner time is changed), or insistence on doing things in the same manner every time (e.g. putting on clothes always in the same order).
   4. Oddities of motor movement, such as peculiar posturing, peculiar hand or finger movements, or walking on tiptoe.
   5. Abnormalities of speech, such as question-like melody, monotonous voice.
   6. Hyper- or hypo-sensitivity to sensory stimuli, e.g. hyperacusis.
   7. Self-mutilation, e.g. biting or hitting self, head banging.

C. Onset of the full syndrome after 30 months of age and before 12 years of age.

D. Absence of delusions, hallucinations, incoherence, or marked loosening of associations.

Table 1 - continued

Diagnostic Criteria for Childhood Onset Pervasive Developmental Disorder, Residual State

A. Once had an illness that met the criteria for childhood onset pervasive developmental disorder.

B. The current clinical picture no longer meets the full criteria for the disorder, but signs of the illness have persisted to the present, such as oddities of communication and social awkwardness.

Atypical Pervasive Developmental Disorder

This category should be used for children with distortions in the development of multiple basic psychological functions that are involved in the development of social skills and language and that cannot be classified as either infantile autism or childhood onset pervasive developmental disorder.

2.2 DESCRIPTION OF THE SYNDROME

Infantile autism is a syndrome defined in terms of behavior, the typical symptoms of which exist before the age of thirty months. The most significant symptoms are:

- a disturbance in speech- and language development and the non-verbal communicative skills: retardation of speech development or mutism; if speech exists, deviations in syntaxis and articulation; absent or poor capability of using symbols; inability of abstract reasoning and comprehension; neologisms; echolalia; absence of communicative gestures, etc.
- a disorder in the relationship with people, objects and events: failure to develop a specific relationship with primary caretakers; absence of eye contact, a failure to play with peers and make friends, inadequate use of toys, stereotyped use of objects, a particular sensitivity for specific sequence of events, in which interruption of it may lead to panic, etc.
- a disturbance in the reaction on sensory stimuli; hyper-, hyporeactive, or both alternating: excessive interest in visual details; staring; attention for changes in light fall, excessive reactions on noises, or no reaction at all, touching, smelling, licking of inedible objects, increased pain threshold; attention for self produced sounds; excessive rubbing on surfaces; turning and rythmic moving of the body, attention

for spinning objects.

In general the motor-, socially adaptive, and cognitive development are seriously retarded, hampered or regressed and the mutual balance in the development has been disturbed. There is a serious disturbance of the ability of expression, the estimation and appreciation of emotions and in the regulation of anxiety and excitement.

Infantile autism should be distinguished from

- developmental aphasia, in which there is serious expressive, and often less serious receptive language disturbance. In comparison to autistic children, aphasic children have a relatively more intact inner language. They make use of gestures and mime and are communicatively directed on the other. Aphasic children are often hyperactive, and they find it difficult to focus their attention on one thing. They often develop secondary behavioral problems. Some of these children have autisticlike features. In a recent longitudinal study (Paul et al., 1983) on 28 children diagnosed as aphasic, half of the children evidenced some features similar to autism. The overlap of this group with autism may present a diagnostic problem.
- mental retardation. This is diagnosed on the basis of psycho-metric examination. The intellectual functioning and the adaptive abilities are well below average (IQ < 70). The social and emotional capabilities pointing to social attachment, and the development of speech and language are in agreement with the global general level of intellectual functioning. Since most autistic children are also mentally retarded, it is often necessary to distinguish between mentally retarded children with and those without autistic features. This distinction is often difficult to make. Many children are mentally retarded and present autistic characteristics, in a greater or less degree.
- childhood onset pervasive developmental disorders (DSM-III). In such children a serious disturbance in social relationships exists with peculiar inadequate reactions in social contacts. Furthermore sudden anxieties, inadequate expression of emotions and disturbed motor functioning are often present. In addition there are often deviations in cognitive and thought processes such as disconnected associations, bizarre preoccupations, and overly concrete thinking. The disturbance developes after the thirthieth month and before the age of twelve. To

what extent there are distinct, qualitative differences between this group of children and children with the diagnosis of autism is not quite clear (Chess et al., 1977). In psychoses in early childhood there seems to be a less serious disturbance in the forming of social relationships and more symptom variability than in autism (Prior et al., 1975).

- degenerative organical brain diseases, such as Schilder's disease and the syndrome of Heller.
- deafness. Because autistic children often are mute or may show selective desinterest in spoken language, they are often thought to be deaf. However, deaf children are usually attached to their parents, seek their attention and affection and enjoy being held.

2.3 EPIDEMIOLOGICAL ASPECTS

Infantile autism occurs with a prevalence of 4 or 5 per 10.000 children below the age of 15. If mentally retarded children with autistic features are included, the prevalence rises to 20 per 10.000. It occurs 4 times more in boys than in girls. The families of autistic children do not differ from control groups as to socio-economic status (Wing et al., 1980).

2.4 INTELLIGENCE

About 60% of the autistic children have an IQ below 50, 20% between 50 and 70, and 20% higher than 70. Since subjects with an IQ below 70 are usually considered to be mentally retarded, a majority of autistics meet this criteria. Only a very small portion are of average or above average intelligence. Severely retarded autistic children may present a somewhat different clinical picture from those functioning on a relatively high intellectual level. Hyperkinesis, stereotyped behavior, self-injurious behavior and seizures are more frequently seen in the lower functioning children; obsessional phenomena are more frequent in those who are functioning higher. The IQ is stable, measured over a longer period of time and has a prognostic value: the lower the IQ the worse are the

prospects for the total functioning of the child (Rutter and Lockyer, 1967; DeMyer et al., 1973).

2.5 PARENTS AND FAMILY

The parents of autistics do not differ as a group in frequency of psychiatric problems or in parental care, emotional response or social contact (Cox et al., 1975; McAdoo and DeMyer, 1978; DeMyer et al., 1972). The family interactions are similar to those of normal control families (Byassee and Murrell, 1975). Psychodynamic causation does not appear therefore to be a major contributor to the etiology of infantile autism. This does not imply that psychodynamics and interactions with caretakers and environment are unimportant.

2.6 GENETIC FACTORS

All reports agree that approximately 2 percent of siblings of autistics are afflicted by infantile autism, a rate 50 times greater than in the general population (Campbell et al., 1981). Folstein et al. (1977) found concordance for autism in 36% of monozygotic twins and in 0% dizygotic twins. The concordance for cognitive disturbances was 82% in monozygotic twins and 10% in dizygotic twins. So, it was concluded from this study that a genetic factor possibly plays a role in a subgroup of autistic children and probably does not concern the whole syndrome, but only the cognitive language components.

Recently two studies of Ritvo and coworkers have appeared. In a first study they reported a concordance for autism of 95.7% in the monozygotic twins (22 out of 23) and 23.5% in dizygotic twins (4 of 17) (Ritvo et al., 1985a). They judged the results compatible with autosomal recessive inheritance, which predicts 100% concordance in monozygotic pairs and 25% in dizygotic pairs. The parents reported developmental language problems in 10.3% of their nonautistic children.

In a second study 46 families were ascertained with multiple incidences of autism (41 with 2 and 5 with 3 autistic probants) (Ritvo et al., 1985b). Of several genetic mechanisms considered only autosomal recessive

inheritance was consistent with the data and could not be rejected by classical segregation analysis with correction for the ascertainment bias introduced by using multiple incidence families.
The presence of excess male probands indicates that the final answer will be more complex and involve either the identification of additional etiologic subgroups or a sex-modified expression for the trait. Of course the results of this study might only be applicable to families having at least two siblings affected with the syndrome of autism.
It appeared recently that, in a subgroup of autistics (4-10%) the fragile-X syndrome could be diagnosed, a sex-linked hereditary disease which is transmitted by the mother, and is expressed, among other things, in mental retardation in boys (Brown et al., 1982; Levitas et al., 1983; Meryash et al., 1982). Blomquist et al. (1985) reported that fragile-X syndrome occured even in 15-20% of autistic males, however Goldfine et al. (1985) reported blood samples from 37 autistic children were negative for fragile X chromosome. In reverse, nearly 50% of the children diagnosed with a fragile-X syndrome are reported to present autistic characteristics (Levitas et al., 1983).
Although in a first study of human leukocyte antigens (HLA) and autism no statistically significant relationship between HLA and autism has been demonstrated (Stubbs and Magenis, 1980), sets of parents of autistic children were found to share significantly more often at least one HLA antigen compared to sets of parents of a comparison group (Stubbs et al., 1985). This is interpreted as of possible significance because differences rather than similarities at the HLA sites between the father and the mother stimulate blocking antibodies which protect the fetoplacental unit. In a recent study of Spence et al. (1985) thirty-four families with at least two autistic children were subjected to gene linkage analyses with 30 standard phenotypic gene markers. No statistically significant evidence for linkage with a gene marker was found.

2.7 CONCOMITANT ORGANIC DISORDERS

In a number of cases it is possible to diagnose an organic disease (see Table II). The diseases most frequently mentioned to occur concomitant

with autism are phenylketonuria and congenital rubella (Chess et al., 1977; Coleman, 1979) and fragile X syndrome (see paragraph 2.6).

Table II

Selected pathologic conditions associated with autism (Ornitz, 1983)

Prenatal Conditions

- Down's syndrome
- Congenital rubella
- Congenital cytomegalovirus
- Mid-trimester bleeding
- Toxemia

Perinatal Conditions

- Anoxia
- Breech presentation
- Low Apgar score
- High bilirubin
- Retrolental fibroplasia
- Respiratory distress syndrome

Conditions Manifest in the First Three Years

- Infantile spasms
- Other seizures
- Cerebral lipidosis
- Microcephaly
- Oculocutaneous albinism
- Histidinemia
- Addison's disease
- Moebius syndrome
- PKU
- Celiac disease
- Tuberous sclerosis
- Fragile X

Conditions Manifest in Later Years

- Congenital rubella
- Herpes simplex encephalitis
- Seizures
- Temporal lobe pathology

2.8 PRE- AND PERINATAL FACTORS

The more frequent occurence of postmaturity, haemorrhage during the pregnancy, and pre- and perinatal complications in autistic subjects are mentioned by different authors (Lobascher et al., 1970; DeMyer, 1979; O'Moore, 1972; Campbell et al., 1978c). Autistics also have significantly

more minor congenital anomalies than their siblings or normal controls (Campbell et al., 1978e; Walker, 1977).

2.9 PHYSICAL CHARACTERISTICS AND NEUROLOGICAL AND NEUROPHYSIOLOGICAL FINDINGS

Young autistic children (age 2-7 years) have been found to be shorter, as a group, than a normal population (Campbell et al., 1980). There is a greater incidence of abnormal dermatoglyphic patterns than in the general population (Walker, 1976a).

Several authors have suggested that aspecific neurological findings occur in approximately 50% of autistic subjects (Kolvin et al., 1971; Gubbay et al., 1970; DeMyer, 1979; Knobloch and Pasamanick, 1975). Physical and neurological assessment of autistic children has yielded an array of neurological findings, including hyperactive tendon reflexes, hypotonia, and spasticity (Gubbay et al., 1970). Individually, none of these symptoms are found in a majority of children. We reported that a snoutreflex and a visual rooting reflex occur in a majority of autistic children (Minderaa et al., 1985a, Appendix No V). The visual rooting reflex is seen significantly more often in mentally retarded children with than in mentally retarded children without autistic characteristics (Minderaa et al., 1985b). There is a failure of lateralization in the majority of young autistic children. They remain ambidextrous at an age when cerebral dominance is established in their siblings and other normals (Campbell et al., 1984).

The incidence of epilepsy is increased. The occurence of seizures increased during and after puberty (Deykin and Macmahon, 1979). EEG-anomalies also occur with an increased frequency after the onset of puberty (Itil et al., 1976; Small, 1975; Waldo et al., 1978). Although as yet there is no EEG finding specific to infantile autism, there is suggestion that some abnormalities are indicative of failure of cerebral lateralization. In neurophysiological investigations, making use of different parameters, such as heartfrequency, vasomotor reactions (Cohen et al., 1977b; Lake et al., 1977) and electrodermal activity (Angus, 1970; Van Engeland, 1980), possible deviations have been found. By

several investigators autistic children have been found to have increased brain stem transmission times as determined by auditory evoked responses (Sohmer and Student, 1980; Rosenblum et al., 1980; Tanguy et al., 1982; Gilberg et al., 1983b). Others found smaller amplitudes of long-latency auditory event-related brain potentials in response to novel auditory information (Courchesne et al., 1985).
On the basis of CT-scan investigations, indications have been found for deviations of the left hemisphere (Hier et al., 1978; Hier, 1979) and ventricular enlargement (Damasio et al., 1980; Campbell et al., 1982; Hoshino et al., 1984) in some autistics. Other investigators have found no abnormality compared to normals (Prior et al., 1984) or in comparison with patients with various neurological disorders (Tsai et al., 1982; Tsai et al., 1983).

2.10 TREATMENT

In general longlasting coaching of parents and family is indicated. Attention should be paid to e.g. coping-problems, daily problems in the approach of the autistic child, prevention or decrease of secundary behavioral problems of the child, looking for a psychic balance of the family and advice on questions about chosing a special school, a daycare-center, or a residential treatment center. Focussing on individual parent problems and concerns within a problem solving format is important.
An approach along the lines of behavior modification, directed towards different relevant behavioral aspects of the syndrome is considered to be the most useful (DeMyer et al., 1981).
Adjusted schooling with structured classroom training is important. The autistic children require an all-day structure and a daily program adapted to their special needs.
In serious behavioral problems medication may sometimes be useful, not-sedating antipsychotic medication having appeared the most effective (Campbell et al., 1978d; Faretra et al., 1970; Anderson et al., 1984).
Haloperidol has proved to be an effective and safe drug in many autistic childen who are hyper- or normoactive when administered in conservative doses over a relative short period of time, up to 3 months (Campbell,

1984). The main improvements are decreases in hyperactivity, stereotypies, withdrawal, fidgetiness, abnormal object relations, irritability and labile affect. Furthermore, it seems that haloperidol remains an effective drug on a long-term basis. When haloperidol is carefully administered, tardive and withdrawal dyskinesias are not frequent (Campbell, 1984). Recently, fenfluramine, a drug that increases central serotonergic functioning was reported to be effective (Ritvo et al., 1983; August et al., 1984, 1985). However, critical assessment of fenfluramine's effect in large samples of patients is required to validate the preliminary findings.

CHAPTER III

NEUROCHEMICAL ASPECTS OF INFANTILE AUTISM: CATECHOLAMINE AND NEUROENDOCRINE STUDIES

3.1 INTRODUCTION

Neurochemical research in autistic children has mainly focused on the metabolism of three monoamine transmitters, namely the catecholamines dopamine (DA) and norepinephrine (NE) and the indoleamine serotonin (5-HT).

One of the reasons for the investigation of the functioning of the dopaminergic systems is the relative therapeutic effect of neuroleptics. Although the phenothiazines and butyrophenones are far from being a cure for autism, so far they have appeared to be the most effective medication available. They influence several symptoms concerning the motor system such as hyperactivity, stereotypies, aggression and automutilation. These actions of the neuroleptics are thought to be due to their blockade of DA receptors.

Stimulants, such as amphetamines and methylphenidate, worsen autistic symptoms in most cases, leading to increased motor activity, aggression, stereotypies and a general desintegration of behavior. Stimulants release dopamine from the presynaptic terminal and block its re-uptake and degradation.

The important central and peripheral neurotransmitter, NE, has also been widely studied in autism. Although little specific neuropharmacological evidence, of the type mentioned concerning DA, has been obtained to suggest NE functioning might be altered in autism, its crucial role in the nervous system has made its study worthwile.

Central monoaminergic systems are involved in the control of hormone release. Therefore, assessment of neuroendocrine parameters might throw light on central neurotransmitter functioning.

In this chapter, we will review the studies on autistic individuals dealing with the catecholamine systems (norepinephrine and dopamine), neuroendocrine systems (cortisol, prolactin, growth hormone, luteinizing hormone, follicle-stimulating hormone and the thyroid hormones, and several hormone-releasing hormones) and neuropeptide systems.

3.2 NOREPINEPHRINE

Norepinephrine (NE) is found as a neurotransmitter in the brain as well as in the peripheral sympathetic nervous system. Furthermore, NE is excreted by the adrenal gland.
Much evidence suggest that noradrenergic systems play a major role in fundamental modulatory processes including arousal, orientation, habituation, vigilance, stress responses and memory. Most of the NE cell bodies in the brain are located in the locus coeruleus (LC), a nucleus in the dorsal tegmentum of the pons. From here they project extensively in a diffuse manner to different parts of the brain. Besides NE-LC dendrites extend to the spinal cord, affecting autonomic activity via the sympathetic nervous system. In the postganglionic sympathetic nervous sytem NE serves as a neurotransmitter.

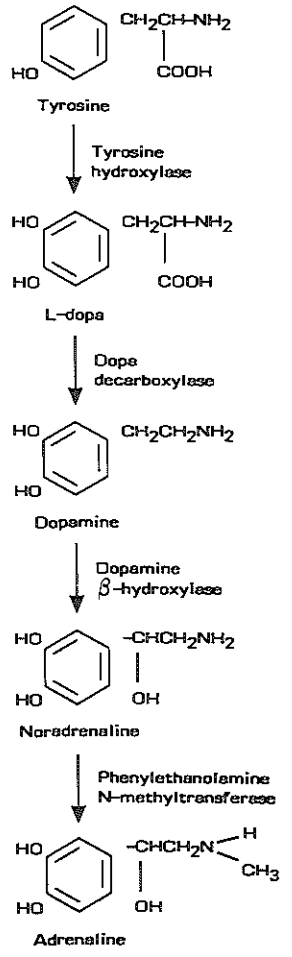
3.2.1 ASSESSMENT OF NORADRENERGIC FUNCTIONING: SPINAL FLUID MEASURES

As it is impossible in people to measure concentrations of monoamines and their metabolites in the brain directly, use is made of measurements in the cerebrospinal fluid (CSF). The amines themselves can not be measured in the CSF because, after being released into the synaptic cleft, they are retaken up or broken down immediately afterwards. Therefore in order to study the metabolism of the amines, the most important metabolites of serotonin, dopamine and noradrenaline, in the spinal fluid are measured: respectively 5-hydroxyindoleacetic acid (5-HIAA), homovanillic acid (HVA) en 3-methoxy-4-hydroxyphenylglycol (MHPG) (see Figures 1 and 2). The concentrations of these metabolites in the CSF give an impression of the turnover rate of the amines in the brain. Because of the differences between lumbar and ventricular CSF circulation, the variability over time, and the differences in concentrations between different areas of the brain which are not reflected in the CSF, one should be cautious in the interpretation (Van Praag et al., 1973; Garelis et al., 1974).
Activity of central NE neurons has been assessed by measuring levels of MHPG in the spinal fluid. MHPG levels in spinal fluid probably reflect NE activity in the brain as well as the spinal cord. Furthermore it appeared

that there is a small contribution of blood MHPG to CSF MHPG (Kopin et al., 1983).

MHPG is measured in the CSF of 6 autistic children and appeared to be 'normal', if compared to the MHPG levels of adult neuropsychiatric patients (Young et al., 1981). Gillberg et al. (1983a) found CSF MHPG levels slightly but not significantly increased in autistic individuals compared to age- and sex-matched controls. These findings suggest that central NE turnover is not grossly altered in autistic individuals.

Figure 1.
The synthesis of catecholamines from their amino-acid precursor L-tyrosine



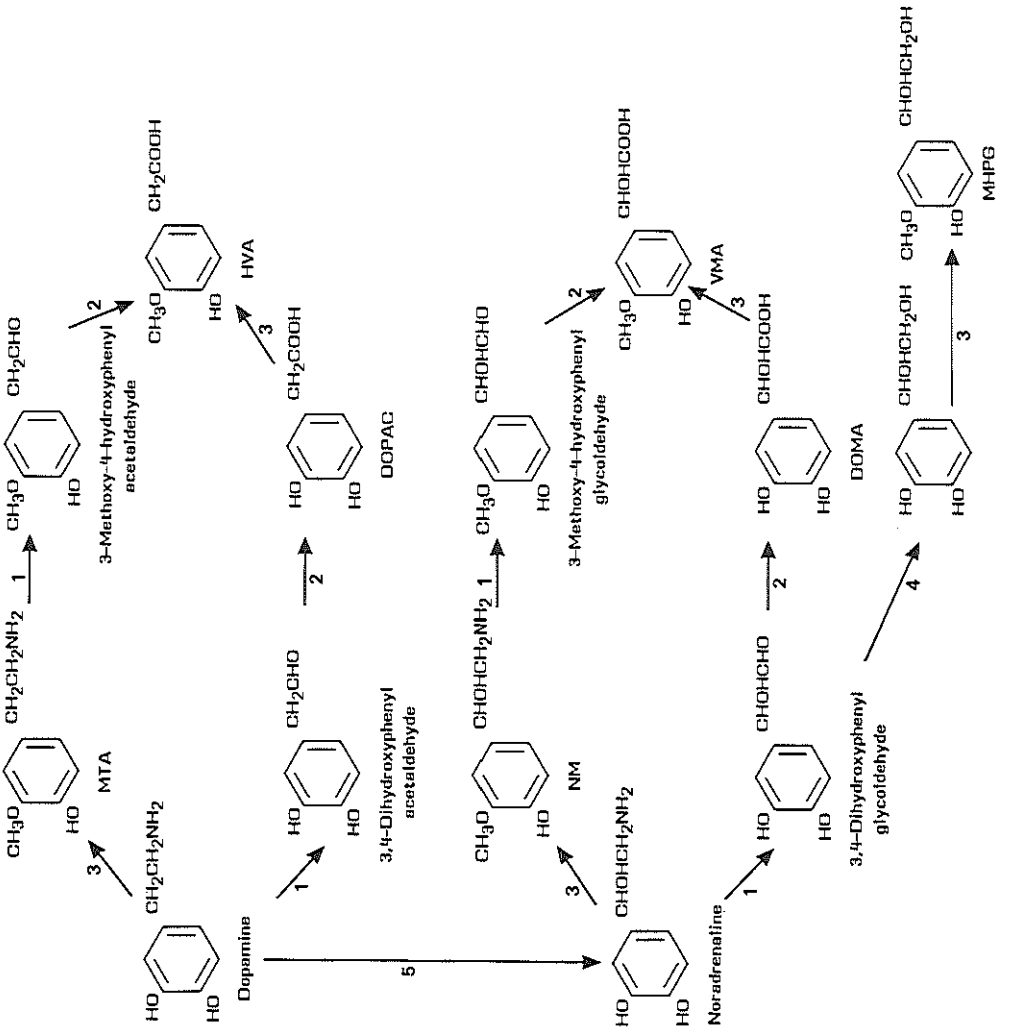
3.2.2 ASSESSMENT OF NORADRENERGIC FUNCTIONING: BLOOD MEASURES

The use of plasma norepinephrine as an index for sympathetic functioning is based on the assumption that the plasma levels are for the greater part produced by the postganglionary nerve endings. Besides, norepinephrine is also excreted by the adrenal gland (Lake et al., 1976; Taylor et al., 1978; Louis et al., 1973). Under normal physiological circumstances the sympathetic nervous system is probably the most important source of the noradrenaline of the plasma, while under stress the adrenal contribution of the plasma norepinephrine increases (Popper et al., 1977; Micallizzi and Pale, 1979). This is of importance with regard to autistic children because in clinical research vegetative disturbances are repeatedly reported, as for instance deviations in the vasomotor regulation and electrodermal activity. The remarkable responsiveness of plasma catecholamines on psychological and bodily stimuli requires that procedures to collect blood are carefully standardized in order to minimalize the effect of stimulation by the venapunction. Although norepinephrine in plasma is for the greater part produced by the sympathetic nervous system, it may reflect in an indirect way the functioning of central systems. The locus coeruleus, the important noradrenergic nucleus, influences sympathetic functions via descending noradrenergic fibers to the lateral columns of the spincal cord. This implicates that peripherally derived norepinephrine and the metabolite MHPG are probably correlated with the rate of central noradrenergic activity.

Opinions differ about the question to what extent plasma MHPG, the most important metabolite of centrally produced norepinephrine, is produced in the brain or in the peripheral systems. Variable estimates have been made concerning the actual percentage of MHPG found in plasma that originates in brain (Roth, 1983). However, as with norepinephrine, plasma MHPG seem to be a useful index for the functioning of central noradrenergic systems (Leckman et al., 1980; Roth, 1983), even if the lower percentage were correct.

Lake et al. (1977) found that in a group of 11 autistic children the norepinephrine levels in plasma were significantly higher in comparison to the control group. Young et al. (1981) found the plasma free MHPG

Figure 2. The metabolic pathways of dopamine and noradrenaline. 1: monoamine oxidase; 2: aldehyde dehydrogenase; 3: catechol-O-methyl transferase; 4: aldehyde reductase; 5: dopamine-β-hydroxylase. The abbreviations are as follows: MTA, 3-methoxytyramine; HVA, homovanillic acid; DOPAC, dihydroxyphenylacetic acid; VMA, vanillylmandelic acid; MHPG, 3-methoxy-4-hydroxyphenylglycol; NM, normetanephrine



levels in 9 autistic boys within the range which in adults is considered to be normal and in agreement with that of a control group consisting of 8 boys. Minderaa et al. (1985b) didn't find a difference in plasma MHPG levels between 17 autistics and a normal control group. These findings indicate that longterm overall release of NE is not greatly altered in autistic subjects.

Dopamine beta-hydroxylase (DBH) catalyses the conversion of dopamine into norepinephrine (see Figure 1). It is found intracellulary in free and membranebound form (in the storage vesicles of the nerve endings) and is released, together with norepinephrine, into the synaptic cleft. DBH activity is used as a possible index of sympathetic nervous system activity in man. Measurements of DBH in serum of autistic children did not yield results which can be easily interpreted. Coleman et al. (1974) found DBH activity increased in autistic subjects. However, Goldstein et al. (1976a) reported DBH serum levels were significantly lower in autistic individuals compared to normal controls. Lake et al. (1977) confirmed these results and found DBH activity significantly decreased in autistics as well as in their relatives. Belmaker et al. (1978) reported DBH activity was significantly elevated in children with functional psychoses but not in psychotic children with known organic etiology. Finally Young et al. (1980) did not find differences in DBH activity between autistics and normal controls.

Catechol-O-methyl transferase (COMT) is an enzyme that catalyses the transfer of a methyl group from S-adenosyl-methionine to a phenolic group of epinephrine, norepinephrine or other catecholamines (see Figure 2). Because of the functional similarity of COMT in synaptosomes in the brain and in erythrocytes, erythrocyte COMT activity has been examined in autistic and normal control groups. O'Brien et al. (1976) reported no difference in COMT activity of erythrocytes in autistics compared to normal controls. Walker et al. (1976b) compared institutionalized schizophrenic autistic-like children, a heterogenous group of chronical psychotic children and a group of acting-out but nonpsychotic children. The erythrocyte COMT activity was found to be significantly lower in the autistic-like schizophrenic children. Belmaker et al. (1978) reported no differences in erythrocyte COMT activity between children with functional psychosis and children with organic psychosis. Finally Giller et al.

(1980) did not find such differences between autistic children and children with the Gilles de la Tourette syndrome. These findings indicate that COMT activity in red blood cells is probably not altered in autism.

3.2.3 ASSESSMENT OF NORADRENERGIC FUNCTIONING: URINE MEASURES

Free or conjugated catecholamines in the urine are produced by peripheral systems, namely the adrenal marrow and the sympathetic nervous system. The most important metabolite of peripherally formed norepinephrine is vanillyl mandelic acid (VMA) (see Figure 2). VMA and MHPG are found in the urine in relatively large quantities (several mg excreted per day). The fact that centrally formed norepinephrine is mainly degraded to MHPG, means that urine MHPG reflects, at some degree, the brain metabolism of NE. Measurements of MHPG in 24-hours urine is a frequently used clinical method to investigate the central norepinephrine metabolism.
In a pilot study of urine MHPG in 6 autistic boys and 9 normal boys a significant decrease in the mean urinary excretion of MHPG was found in a group of autistics compared to the control group (Young et al., 1979). At the same time there was a parallel decrease of the free catecholamines in the urine in this group of autistic children. Free catecholamines (NE and epinephrine) mainly reflect noradrenergic activity, consisting of 80 or 90% noradrenaline. However, Minderaa et al. (1985b) found no difference between a group of 17 unmedicated autistics and a normal control group in the urine excretion of MHPG, norepinephrine or epinephrine.
In addition, the diurnal variation observed in normal children was seen to be similar in the autistic group, with lower values overnight compared to the late afternoon and evening values.
It might be concluded, based on CSF, blood and urine studies, that noradrenergic functioning is not greatly altered in autism. The finding in conflict with this conclusion is, above all, the apparent increased values of blood NE. However, the noradrenergic system is episodically responsive. NE levels, in contrast to MHPG and DBH measures, reflect acute sympathetic response and NE has a short chemical halflife. So, increased NE levels might reflect a dysfunction in functional responsivity of the noradreneric system, while the overall long term func-

tioning might be normal. Noradrenergic challenge tests might throw new light on these questions in a more dynamic way.

3.3 DOPAMINE

Dopamine (DA) is found as a neurotransmitter in the brain and as a precursor to NE in the peripheral sympathetic nervous system, it is also excreted by the adrenal gland. In the brain most dopamine containing neurons lie in the neostriatum, the nucleus accumbens, and the tuberculum olfactorium. The central dopaminergic systems are especially important in the control of motor function, in cognition and in the regulation of the release of hormones. DA-blockers such as haloperidol or thioridazine, have been observed to be effective in decreasing some symptoms of autistic children like stereotyped behavior. Furthermore, drugs which enhance dopaminergic functioning, like amphetamines make autistic symptoms worse.

3.3.1 ASSESSMENT OF DOPAMINERGIC FUNCTIONING: SPINAL FLUID MEASURES

As pointed out homovanillic acid (HVA) is the major metabolite of dopamine in the brain (see Figure 2). Measurements of HVA in spinal fluid have been used to assess the central dopamine turnover.
During the 1970's probenecid was widely used to get a better picture of the monoamine metabolism in the brain. Probenecid hampers membrane-transport and thus blocks the egress of the acid metabolites 5HIAA and HVA out of the spinal fluid into the blood stream. Therefore the blockade by probenecid causes a rise in the concentrations of these metabolites in the CSF and thus offers a reflection of the metabolism over a longer period of time. Moreover, after administration of probenecid, metabolite measures reflect better production instead of steady state values which are influenced by kinetic aspects of elimination processes. In autistic children the CSF concentrations of 5-HIAA (see chapter IV) and HVA are tested both without and after administration of probenecid. The values are compared to those of contrast groups because normal values in healthy

children are not available.
In an investigation in 6 autistic boys, without using probenecid, the concentrations of HVA and 5-HIAA were relatively low, clustered and within the range as found in adults (Cohen et al., 1980b). The several transmitter systems seem to play a different role in the organization of behavior, and their mutual balance may throw light on certain aspects of disturbed behavior. Therefore the ratio between the 5-HIAA and HVA values is considered after probenecid loading. In autistic children the 5-HIAA/HVA ratio is in the low range of those of adult schizophrenic and depressive patients (Cohen et al., 1978). This may partially be explained by the fact that HVA concentrations in the CSF of children are higher than in adults. Within the group of autistic children there existed a subgroup with more increased HVA concentrations, both absolutely and in comparison with the 5-HIAA concentrations. This subgroup distinguished itself by the great amount of stereotyped and repetitive behavior, and increased locomotor activity. In general the most seriously disturbed children belonged to this subgroup. Within the group of autistic children the authors searched for correlations between CSF metabolite concentrations and behavior ratings. As there exists a relation between the concentrations of the metabolites and probenecid in low probenecid levels, metabolite concentrations might be reflected in their relations to the probenecid concentrations. For 10 autistic children the HVA/probenecid ratio was negatively correlated with scores concerning social responsiveness and attention. The ratio 5-HIAA/HVA was positively correlated with these behavioral aspects. So, autistic children who functioned worse in these aspects of behavior, had higher concentrations of CSF HVA and lower concentrations of 5-HIAA.
In another study (Winsberg et al., 1980) the increases in HVA levels in spinal fluid after probenecid loading appeared to be normal in autistics. Recently, Gillberg et al. (1983a) found that the baseline levels of HVA in spinal fluid of autistic subjects appeared to be increased compared to an age- and sex-matched control group of neurologically disordered children. These findings suggest that the dopaminergic system might be overactive in autism.
HVA levels in the liquor of autistic children may be related to the remarkably more frequent occurence of this disease in boys than in girls,

as CSF HVA levels have been reported to be higher in men than in women (Leckman et al., 1980; Shaywitz et al., 1980). This may make men more vulnerable for diseases in which the dopaminergic system is involved, such as autism, chronical multiple tic syndrome and attention deficit disorder with hyperactivity. All these disorders occur more frequent in boys than in girls (Young, 1982c).
Another hypothesis could be that the HVA elevation might represent a developmental delay in the maturation of dopaminergic systems, as higher CSF HVA levels are seen in younger children (Anderson et al., 1985a) and in young animals (Shaywitz et al., 1985).

3.3.2 ASSESSMENT OF DOPAMINERGIC FUNCTIONING: BLOOD MEASURES

Most of the DA and HVA measured in peripheral body fluids is derived from the adrenal gland and the sympathetic nervous system. The relationship of these measures to central dopaminergic function is unclear. Dopamine measured in urine is estimated to be almost completely derived from peripheral sources. HVA measured in blood or urine might be of approximately 25% (Mars et al., 1980) central origin.
We know of only one study dealing with plasma HVA levels in autistic subjects (Minderaa et al., 1985c). In this study no differences have been observed between autistics and normal controls. No studies of the levels of dopamine itself in autistics have been reported.
Boullin and O'Brien (1971) measured in vitro uptake and loss of ^{14}C-dopamine by blood platelets. No differences were found between autistic subjects and normal age-matched controls.

3.3.3 ASSESSMENT OF DOPAMINERGIC FUNCTIONING: URINE MEASURES

Several investigators found increased urine HVA levels in autistic children (Lelord et al., 1978; Garreau et al., 1980; Martineau et al., 1981; Martineau et al., 1984). In one study (Garreau et al., 1980) urinary HVA excretion was correlated with the severity of autistic symptoms. Martineau et al. (1981) showed urinary HVA levels decreased

with treatment with Vitamin B6 and magnesium, while Lelord et al. (1978) reported a reduction of urinary HVA excretion after administration of Vitamin B6. However, we have found no difference between unmedicated autistics and normal controls in total urinary DA or HVA excretion (Minderaa et al., 1985c).

3.4 ADRENOCORTICOTROPIC HORMONE, CORTISOL AND RELATED COMPOUNDS

Adrenocorticotropic hormone (ACTH) is secreted by the anterior lobe of the pituitary gland and acts primarily on the adrenal cortex. It stimulates the release of cortical steroid hormones, the principal one of which is cortisol. Levels of cortisol in plasma vary with the time of the day, and are increased in the early morning. The release of ACTH is regulated by the hypothalamic corticotropin-releasing factor (CRF). Urinary levels or cortisol and of the other glucocorticoids, the 17-hydroxy corticosteroids are useful indices of adrenocortical function. Metapyrone is used for testing pituitary reserve of ACTH. The effect of metapyrone is a decreased secretion of cortisol which thereby causes an increase in ACTH release.

Another way to assess the pituitary-adrenal function is by use of the dexamethason suppression test. Dexamethason, a potent analogue of cortisol, suppresses cortisol production by inhibiting ACTH release of the pituitary.

Brambilla et al. (1969) studied three children with early onset psychosis. They found reduced levels of urinary 17-hydroxy- and 17-keto-steroids but normal plasma levels of ACTH. After stimulation of ACTH with metapyrone a blunted response was seen.

Hill et al. (1977) reported plasma levels of cortisol being decreased in six autistic subjects.

However, Hoshino et al. (1984a) and Jensen et al. (1985) reported normal 4 PM baseline cortisol levels in autistic subjects compared to mentally retardeds and normal controls, and 8 AM baseline cortisol values in autistics similar compared to those of normal adults respectively.

Maher et al. (1975) found an increased elevation of cortisol after insulin loading in 11 autistic children compared to mentally retarded

controls.
Yamazaki et al. (1975) reported a normal increase in 11-hydroxy corticosteroids after pyrogen stress, but deviant diurnal variations in levels of these compounds in autistic individuals.
A dexamethasone suppression test (DST) has been performed with autistic subjects by Hoshino et al. (1984a) and Jensen et al. (1985). The first group of investigators found no suppression of plasma cortisol after dexamethasone administration in all of six autistics with low IQ (IQ < 60). The second group performed a dexamethasone suppression test in a group of low-functioning autistic subjects (IQ $\leqslant$ 30) and found 10 out of 12 autistics did not suppress cortisol release.
In conclusion, it can be said that baseline levels of cortisol are not probably abnormal in autistic subjects, and that low functioning autistics do suppress cortisol secretion after dexamethasone loading to a lesser extent than controls.

3.5 HUMAN GROWTH HORMONE AND PROLACTIN

Human growth hormone (hGH) and prolactin (PRL) are secreted by the anterior pituitary. Release of hGH is regulated by hypothalamic secretion of growth hormone-releasing factor (GRF) and somatostatin (somatotropin release-inhibiting factor, SRIF). Hypothalamic release of dopamine stimulate secretion of GRF and, in turn, of hGH. Prolactin is controlled primarily by prolactin-inhibiting factor (PIF) and perhaps by a prolactin releasing factor (PRF). Dopamine blockers increase and dopamine agonists decrease serum prolactin, presumably by altering dopamine release by the tubero-infundibular neurons. Serotonergic mechanisms apparently play a modulatory role in prolactin secretion. Thyrotropin-releasing factor also increases prolactin levels, probably via a direct action on the pituitary.
Hoshino et al. (1983, 1984b) assessed baseline levels of plasma human growth hormone and prolactin and after loading with the precursor of serotonin, L-5-hydroxytryptophan (L-5-HTP) in six autistic children and a group of normal controls. The baseline hGH levels as well as the hGH elevation after L-5-HTP loading were similar in the autistic subjects,

compared to the normal controls. The autistics did show lower baseline levels of PRL and a blunted response of prolactin after L-5-HTP challenge. These results are interpreted as possible indicating a diminished central serotonergic functioning or an enhanced dopaminergic activity of the tuberoinfundibular system.
In contrast Minderaa et al. (1985c) found normal baseline levels of prolactin in a group of unmedicated autistics and apparently normally increased levels of prolactin in neuroleptic medicated autistic subjects.
Several investigators measured plasma prolactin levels after challenge with thyrotropin-releasing hormone (TRH). Campbell et al. (1978a) reported a normal response of prolactin to TRH in 10 psychotic children. Suwa et al. (1984) found a increased response of PRL to TRH in one out of four autistic children. Hoshino et al. (1983) however, found a blunted response of PRL to TRH in 6 autistic children.

3.6 THYROID HORMONE AND THYROID STIMULATING HORMONE

Thyroxine (T4) and triidothyronine (T3) are the hormones secreted by the thyroid gland. The thyroid is stimulated by the thyroid-stimulating hormone (TSH), a glycoprotein produced and secreted by the anterior pituitary. TSH synthesis and release is stimulated by thyroid-releasing hormone (TRH), which is synthesized in the hypothalamus and secreted into the pituitary. The precise way the release of TRH is regulated is not known, but monoamines like dopamine, serotonin, norepinephrine and opiates like β-endorphin and metenkephalin seem to play an important role in the regulation of the activity of tubero-infundibular neurones.
Kahn (1970) reported diminished values of T3 uptake in more than 70% of a group of 62 autistic subjects. However, Abbassi et al. (1978) and Cohen et al. (1980a) found normal levels of T3, T4, and thyroid stimulating hormone (TSH) in autistic children.
Several investigators have performed thyrotropin-releasing hormone (TRH) tests in autistic children. Campbell et al. (1978a) found after TRH challenge an elevated response of TSH and a delayed or blunted response of T3 in a group of 10 psychotic children. Hoshino et al. (1983) reported an increased response of TSH to TRH in 6 autistic children as well.

Finally Suwa et al. (1984) found an enhanced response of TSH to TRH in 3 out of 4 autistic children.
So rather good agreement does exist about the finding of an enhanced reaction of TSH secretion after TRH stimulation in autistic subjects.

3.7 LUTEINIZING HORMONE-RELEASING HORMONE (LH-RH)

Luteinizing hormone (LH) and follicle-stimulating hormone (FSH) are the gonadotropic hormones produced by the anterior pituitary. The hypothalamic control of these gonadotropic hormones is performed by the decapeptide luteinizing hormone-releasing hormone (LRH).
Hoshino et al. (1983) adminstered LH-RH intravenously and measured plasma release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) (LH-RH test) in 6 autistic male subjects. The elevations of both LH and FSH release was decreased after LH-RH loading.

3.8 NEUROPEPTIDES

The behavioral effects of the neuropeptides are the result of a direct action on the central nervous system, and consist of influences on memory processes, motivation, attention and arousal, pain perception, appetite and sexual behavior.
Panksepp and coworkers (Panksepp et al., 1979, 1980) demonstrated the importance of the endogenous opiate system for the establishment of social bonds, the regulation on the neurobiological level of attachment-behavior, imprinting processes, experienced contact comfort and separation distress reactions. Based on animal work they hypothesized that hyperactivity of endogenous opiate systems would contribute to a state of progressive social isolation and social withdrawal which seems similar to the symptoms of autistic children in social behavior.
So far there are few studies to test this interesting hypothesis.
Coid et al. (1983) found significantly increased plasma levels of the short chain opioid metenkephalin in a group of adult habitual self-mutilators compared to a group of former self-mutilators who were

discharged. B-endorphin and lipotropin levels were similar in these groups. However, Weizman et al. (1984) found significantly lower levels of humoral-endorphin in plasma in autistic individuals compared to normal controls.
Naxolone, a blocker of opoid systems, has been succesfully used in mentally retarded individuals with self abusive behavior (Sandman et al., 1983). So far as known, this drug has not been used with autistic subjects.
No quantitative studies about urinary excretion of peptides in autistic subjects are known. However, qualitatively deviations in urinary excretion of these substances have been described. Gillberg et al. (1982) concluded that half of the autistic patients showed different urinary excretion patterns of peptides compared to normal children and only 8% of the autistic subjects exhibited the normal pattern seen in 97% of the controls.

3.9 DISCUSSION

Up to the present time the neurochemical research on catecholamines in children suffering from infantile autism has not yielded any specific biochemical characteristic. However, there are indications that noradrenergic and dopaminergic systems in the brain are involved in the syndrome. These indications are among other things: therapeutic effects of medication influencing these transmitter systems, increased baseline HVA levels in spinal fluid and a subgroup with relatively raised HVA values in the CSF after probenecid loading, and a raised norepinephrine level in plasma. Furthermore abnormalities have been found in neuro-endocrine functioning and in the peptide excretions. The most robust finding of which is an increased release of TSH after stimulation with TRH. It is not clear whether these findings are of a primary or secondary nature. There is no clarity either about the nature and localisation of the pathogenetic substrate. It has not yet been ascertained whether there are correlations of neurochemical variables with syndromal subgroups or specific behavior characteristics, but there are indications that a subgroup with high CSF HVA concentrations is characterized by a more

serious degree of stereotypy, repetitive behavior and locomotor activity, and in general a more severely disturbed behavior. In the very first place it will be necessary to replicate the recent findings with larger groups of children. It will be of importance that factors as age, sex, level of intelligence and level of activity are put under experimental control. A great number of practical problems will play a role. Although the parents are often very willing to co-operate, it is often difficult to gain the co-operation of the children in a number of procedures, so that reliable results are not easily obtained. The burden of a spinal tap for child and family is great and requires intensive support. Medication and other therapeutic interventions often complicate the possibilities to interprete data. Scoring procedures of emotional and behavior variables should be accurately attuned to the specific problems of autistic children and are difficult to perform. Replication of findings is required, and investigations should focus for example on finding correlations of HVA values in the CSF with behavior variables measured with more objective behavior observation instruments. Research on the therapeutic and metabolic effect of medication influencing the monoamine metabolism will yield data which can be both of practical and theoretical significance. Further research is required on the relation between the dopaminergic functioning in the brain and abnormal motility and behavior phenomena. Pharmacological provocation of the system (as with stimulants and neuroleptics) can throw new light in this field as well, when it is related to clinical and metabolic variables. Moreover, it is of importance to investigate the relation between adrenergic functioning and clinical phenomena such as excitement and anxiety. In this case the system can be loaded by pharmacological means (for instance clonidine, a partial noradrenergic agonist) or by functional means, such as an intellectual task or a physical exertion. Stress-situations of every-day-life can also be used as an experimental loading. Correlations can be investigated with neurophysiological parameters. A different line of research can be the investigation of maturation changes of biogenic amines and the substances related to their metabolism. Dopaminergic activity appears to decrease during youth, noradrenergic activity appears to increase and serotonergic activity is stable or decreases. These age-related changes may have fundamental effects on behavior. The balance

of the monoamine systems and their relative rates of development, can be of importance for the development of the child (Young et al., 1982c). When the profile of neurotransmitter maturation in different tissues and bodily fluids has been determined with more certainty, it will be possible to trace an abnormal development on a molecular level if it occurs and to relate it to clinical pathology.

As autism and other neuropsychiatric syndromes such as 'attention deficit disorder' (ADD) and the syndrome of Gilles de la Tourette are 3 or 4 times more frequent in boys than in girls, differences in monoamine functioning between men and women are also of importance.

More direct information about receptor functioning can be obtained by the investigation of the binding characteristics of specific receptors with radioactive ligands.

Another approach would be to assess neurochemical parameters within families to look after deviations under genetic control.

On the basis of investigations of the basic functioning of the nervous system, and a clinical neurochemical research and by the strong progress in the neurochemical analytical techniques there are many hypotheses which are waiting to be tested. It will be the task of the future to translate this knowledge and these skills into practical and workable clinical experiments, so that new insight will be acquired into the syndrome of infantile autism.

CHAPTER IV

CHILDHOOD AUTISM AND THE SEROTONERGIC SYSTEM: "HYPERSEROTONEMIA" AND RELATED SUBJECTS

4.1 SEROTONIN

4.1.1 INTRODUCTION

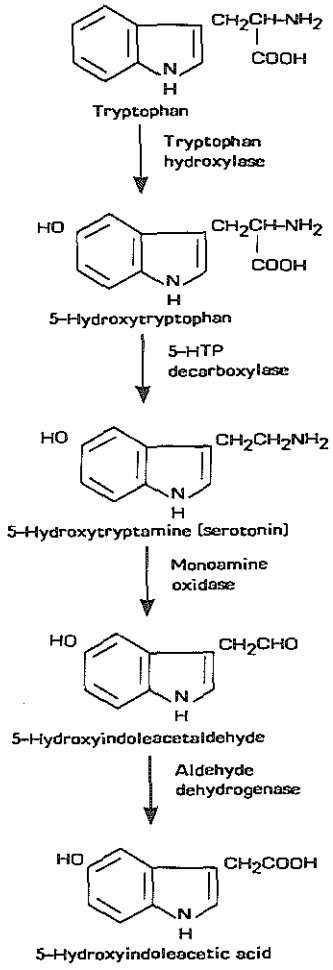
About 100 years ago a substance was discovered in serum with vasoconstrictive properties. This substance, called serotonin, was isolated about 40 years ago and characterised as 5-hydroxytryptamine (5-HT) by Page and coworkers (For review, see Page, 1968).
In the same period a substance called enteramine found in enterochromaffin cells of the gut, and known to be able to constrict smooth muscular tissue, particularly of the gut, appeared to have the same chemical structure (For review, see Erspamer, 1961).
Shortly afterwards in the early 1950s, 5-hydroxytryptamine was demonstrated to be present in the mammalian brain by Page (1968) and by Gaddum (1953).
In the human body about 90% of the 5-HT is found in the gut, 8-10% in the blood platelets and only 1-2% in the brain.
The precursor of serotonin is tryptophan (see Figure 1).

4.1.2 TRYPTOPHAN

Tryptophan is an amino acid that is primarily derived from the diet. In animals elimination of dietary tryptophan can profoundly lower the levels of brain serotonin.
A portion of the dietary tryptophan is drawn off by the intestinal flora for bacterial protein synthesis. The rest is absorbed and carried to the liver in the hepatic portal blood. The transportation of tryptophan across the mucosal membrane is an active process occurring against a concentration gradient. After a protein meal, the concentrations of amino acids in portal plasma are elevated but the increases are much smaller in venous plasma because of active uptake by the liver and other tissues. Less is known about the ways these processes are regulated and about its effects on regulation of brain tryptophan. In the liver tryptophan and other amino acids are used for protein synthesis. When the protein is broken down the component amino acids become available again.

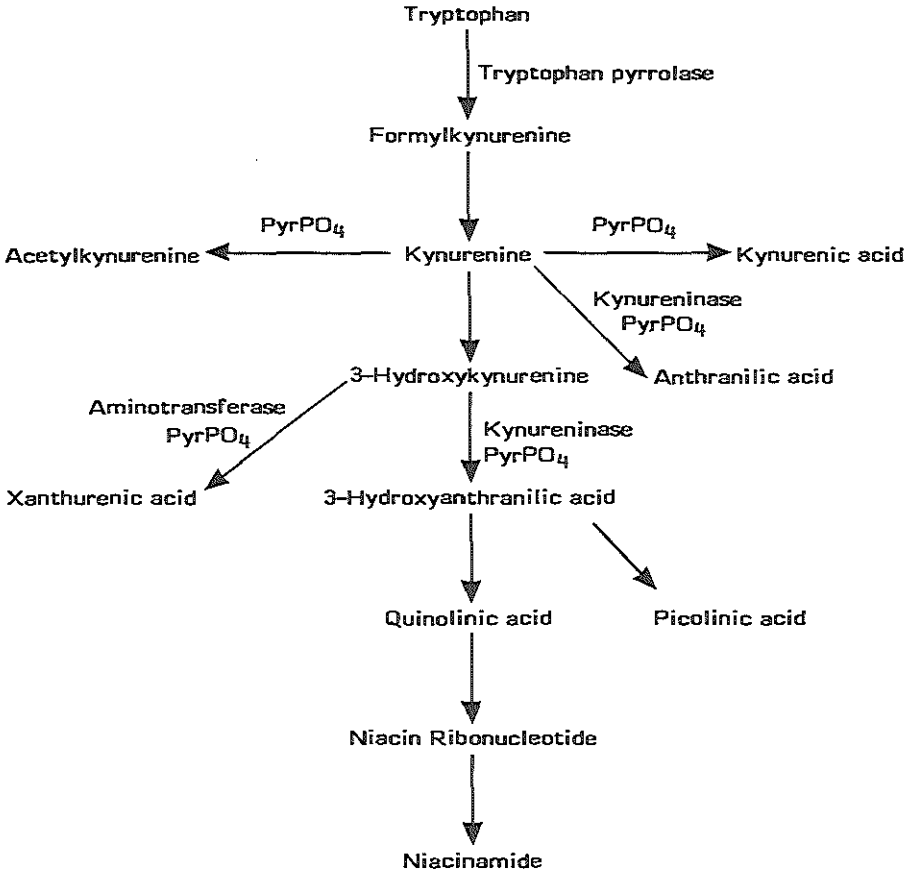
Figure 1.

The metabolic pathway of tryptophan to 5-hydroxytryptamine, tryptamine, and their acid metabolites



Another important fate of tryptophan in the liver is its catabolisation by tryptophan pyrrolase. Quantitatively most tryptophan is metabolized by this enzyme to kynurenine and then to a variety of other metabolic products (Figure 2). This process, the "kynurenine pathway" is irreversible. Tryptophan pyrrolase is primarily found in the liver and induced by tryptophan and cortisol. Thus an increased plasma tryptophan concentration results in enzyme induction and therefore a more rapid metabolism of the amino acid. The kynurenine pathway leads to the formation of nicotinamide adenine dinucleotide (NAD).

Figure 2. Metabolic pathway of tryptophan metabolism (kynurenine pathway)



There is a strong evidence that an increase in the rate of tryptophan catabolism by induction of tryptophan pyrrolase in the liver in some circumstances might alter tryptophan metabolism in the brain and influence 5-HT production. The decline in the free tryptophan pool owing to excess metabolism along the kynurenine pathway may be partly set off by compensatory protein catabolism.

Large amounts of tryptophan and other amino acids are held for longer periods of time in the free form in muscle.

Tryptophan is carried in the plasma largely ($\pm$ 85%) bound in a loose form at sites on serum albumine. These sites may be shared with free fatty acids (and certain drugs). The proportion of tryptophan in plasma that is in free solution depends not only on the albumin concentration but on that of the nonesterified fatty acids. The percentage of free tryptophan in human plasma is positively and significantly correlated with the concentration of the nonesterified fatty acids. A variety of pharmacological agents displace tryptophan from albumen; these agents include salicylates, probenecid, chlorpromazine, diphenylhydantoin, tolbutamine and benzodiazepines. There has been a great deal of controversy regarding the relative importance of "free" versus "total" tryptophan as a determinant of brain tryptophan concentration. It is now generally accepted that it is the nonalbumin-bound tryptophan, and not the total plasma tryptophan that controls the content of tryptophan in the brain.

Tryptophan shares a transport system with other large neutral amino acids, including leucine, isoleucine, valine, phenylalanine and tyrosine. These amino acids can inhibit tryptophan transport across the blood brain barrier and across the brain cell membrane. Accordingly, when rats ingest protein containing food, there is no change in the content of brain tryptophan, despite a large increase in the concentration of plasma tryptophan, for the protein containing food also elevates the concentration of the amino acids in plasma that compete with tryptophan for uptake into the brain. The level of brain tryptophan is correlated with the ratio of the concentration of plasma tryptophan to the sum of the concentrations of the other large neutral amino acids in plasma. (For review: see Young and Sourkes, 1977).

4.1.3 SYNTHESIS AND CATABOLISM OF SEROTONIN

In those cells that synthesize serotonin tryptophan is hydroxylated by tryptophan hydroxylase to yield 5-hydroxy tryptophan (5-HTP) (Figure 1). Tryptophan hydroxylase requires elementary oxygen and tetra hydrobiopterin as a cofactor. The hydroxylation of tryptophan is the rate-limiting step in the biosynthesis of 5-HT. In vivo tryptophan hydroxylase is not normally saturated with tryptophan. This means, in animal experiments, that an increase in tryptophan availability results in an increase of serotonin levels and vice versa. The way that the activity of the tryptophan hydroxylation in the brain is further modulated is not known.

5-Hydroxytryptophan is than decarboxylated into 5-hydroxytryptamine (serotonin) by 5-HTP decarboxylase which is present in cells in vast excess. 5-HTP decarboxylase requires pyridoxal phosphate as a cofactor.

The enzyme that is primarily responsible for catabolizing 5-HT is monoamine oxidase A (MAOA). This enzyme oxidizes the primary amine group of serotonin to form 5-hydroxyindoleacetaldehyde. This aldehyde is rapidly dehydrogenated to yield 5-hydroxyindoleacetic acid (5-HIAA), the major metabolite of serotonin.

In the cell MAO is probably located on the outer mitochondrial membrane. The enzyme has limited substrate specificity and oxidyzes tyramine, norepinephrine, epinephrine and dopamine as well.

4.1.4 SEROTONIN IN THE GUT

The enterochromaffin cells in the gastrointestinal mucosa contains a large proportion of the body's store of serotonin (Erspamer, 1958).

Little is known about the control of serotonin release from these cells. The enterochromaffin cells typically span the mucosa. In general, the apical pole of each cell borders the intestinal lumen and is striated with microvilli (Forsberg and Miller, 1983). The polimorphous secretory granules that contain the amine are most commonly found in the widened base of the cell but occasionally are found in the apical portion.

Presumably, dietary stimuli induce responses in the apical portion and

endocrine or nerve-derived factors induce responses in the basal portion. Muscarinic cholinergic receptors appear to mediate serotonin release from (rabbit) duodenal enterochromaffin cells in vitro (Forsberg and Miller, 1983). Neurones from the enteric nervous system (ENS) may act to stimulate the enterochromaffin cells to release 5-HT in response to acetylcholine. Conversely, the 5-HT released from enterochromaffin cells may act to stimulate sensory neurons to provide an important neural input into the ENS (Gershon and Erde, 1981). The cell bodies of the ENS lie within the myenteric and submucous plexuses. These neurones interconnect with each others to form an integrated network responsible for regulating gut function (motility, blood flow, transport, etc.). The ENS receives an extrinsic cholinergic input from the vagal nerve and an adrenergic input from the sympathetic nerve. The neuronal processing in the ENS is carried out by a great variety of neurotransmitters and neuropeptides like norepinephrine, acetylcholine, vasoactive intestinal peptide (VIP), 5-HT and others.

In vivo studies indicate that the enterochromaffin cells have the ability to secrete serotonin from their apical or basal sites, leading to increases in either luminal or blood concentrations of serotonin. Low luminal pH (Resnick and Gray, 1962), intraluminal sucrose solutions (Ohara et al., 1959), intraluminal pressure (Bulbring and Crema, 1959) and vagal nerve stimulation (Ahlman et al., 1981) were shown to increase serotonin levels within the intestinal lumen (Forsberg and Miller, 1983). On the other hand, vagal nerve stimulation (Pettersson et al., 1979), splanchnic nerve stimulation (Larsson et al., 1979), intra arterial nicotine, morphine, catecholamines and acetylcholine (Burks and Long, 1966a, 1967a,b), intraluminal glucose (Drapanas et al., 1962) and low luminal pH (Kellum and Jaffe, 1976) increased serotonin levels in the portal blood.

4.1.5 SEROTONIN IN THE BLOOD PLATELET

Almost all the serotonin is released from the enterochromaffin cells into the blood system and broken down by MAO in the lung and the liver or transported into the circulating platelets. Lacking nuclei, platelets

have no DNA and very little ability to synthesize proteins. Once the platelets are released from the marrow, they age slowly in the circulation and die in about 10 days, if they are not consumed before "in the line of duty" (Zucker, 1980). In the circulation, blood platelets appear as disc-shaped bodies with an outer membrane enclosing a cytoplasm differentiated into organells, such as granules, lysosomes, mitochondria, glycogen particles, microtubules and microfilaments.

Serotonin is accumulated from the blood by an active transport mechanism located in the cell membrane. In addition to this membrane transport system blood platelets possess an efficient intracellular storage mechanism which permits the accumulation and retention of high concentrations of 5-HT, thus protecting the amine from metabolism by cellular enzymes such as monoamineoxidase. Platelet MAO is localized in mitochondria of which there are about ten per human platelet. The mitochondria supply the energy required for the platelet functions.

Platelets behave as a multitransmitter storage site, in the sense that they have the ability to store several transmitter-like substances including, besides 5-HT, histamine, catecholamines, octopamine and the norepinephrine metabolite normetanephrine.

The 5-HT organelles in platelets are bounded by a typical unit membrane. Platelets have a high content of 5'-phosphonucleotides, mainly adenosine triphosphate (ATP), adenosine diphosphate (ADP), guanosine triphosphate (GTP) and uridine triphosphate (UTP). About 65% of the adenine nucleotides in human platelets is stored in the 5-HT organelles. High resolution nuclear magnetic resonance spectoscopy (NMR) investigations have revealed that the interaction between the intragranular components is very strong in human platelets. In rabbits ATPase of the 5-HT organelles is selectively activated by Mg^{2+} and partially inhibited by Na^{+} and Ca^{2+}. This ATPase probably generates the proton gradient across the 5-HT organelle membrane driving inwardly directed electrogenic proton pump. This ATPase dependant proton translocation is intimately linked to the 5-HT uptake. The phosphonucleotides in the 5-HT granules do not further take part in the metabolic activity of the platelet.

Uptake studies with isolated 5-HT organelles of rabbit platelets and chromaffin granules show that both organelles transport 5-HT and catecholamines quite efficiently. The concentration ratio between

platelet 5-HT and plasma 5-HT is probably more than 100.000. The concentration of 5-HT is about 200 times more concentrated in the 5-HT organelles than in whole platelets.

In a more general subcellular distribution actin has been found in platelets. In contrast, selective localisation of alpha actinin in membranes of electron microscopically pure fractions of 5-HT organelles has been demonstrated. Alpha-actinin could serve as an anchoring protein for microfilaments of the platelet contractile system attached to 5-HT organelle membranes, and such an alpha-actinin-actin microfilamentous system could be involved in the exocytosis and in the expulsion of the granular contents. A circumferential band of long microtubules just inside the external membrane serves as a skeletal frame that maintains the roughly discoid shape of the platelet. There are numerous open vesicles, or membranous chanels, some of which are connected with one another and with the surface of the platelet, much like channels of a sponge, to form what is called the surface connected system (Da Prada, 1981). The platelet serotonin stored in the nucleotide-containing granular vesicles can be released by drugs such as reserpine, tetrabenazine and amphetamine (Paasonen et al., 1965; Pletscher, 1978). Serotonin, secreted from the dense granules, is a weak aggregating agent and also a vasoconstrictor that narrows the blood vessel locally, whether they are cut or intact, and thereby helps to restrict bleeding (Zucker, 1980).

In normal circumstances the platelets are undersaturated with 5-HT and an excess storage capacity of platelets does exist.

It is not clear which aspect of 5-HT physiology is most important in setting blood levels in normal subject (Anderson et al., 1985d). Steady state levels of whole blood 5-HT are apparently determined by the rate of gut production and the balance between platelet uptake, storage and metabolism by monoamine oxidase. That these factors may influence blood 5-HT levels is evident. In carcinoid syndrome (Crawford et al., 1967) and celiac disease (Pimpakar et al., 1961) and after monoamine oxidase inhibition (Weissbach et al., 1961, Marshall et al., 1960) an increase of 5-HT levels in blood is seen.

After administration of imipramine (Marshall et al., 1960), chloripramine

(Ross et al., 1976), fenfluramine (Ritvo et al., 1983) or reserpine (Hanley et al., 1977) a decrease of 5-HT levels has been observed.

4.1.6 SEROTONIN IN THE BRAIN

In the brain serotonin serves as a neurotransmitter. Serotonin does not pass into the brain from the periphery, but it is synthesized in special nerve cells, stored in synaptic vesicles in the nerve endings and released by an exototic process.
The distribution of serotonin in the brain is uneven with relatively high concentrations in the hypothalamus, the midbrain and the brainstem.
The primary source of most of the serotonin in the brain are several clusters of neurons in the midline, the raphe of the pons, and mesencephalon. The more rostrally placed cellgroups project to the telencephalon and diencephalon. The more caudally placed cellgroups project to the medulla and the spinal cord. Serotonergic cells in the median raphe nucleus innervate preferentially structures of the limbic system. The cells in the dorsal raphe nucleus project primarily to the cerebral cortex, the neostriatum, the thalamus and the cerebellum.
Another important place serotonin is manufactured is the pineal body, where it is metabolized into melatonin.
Both serotonergic and nonserotonergic neurons have serotonin receptors. On serotonergic neurons, serotonin receptors ("autoreceptors") provides the mechanism for feedback inhibition of neuronal activity that is coupled to release of the transmitter. So, activation of the serotonergic autoreceptors reduces the neuron's rate of spontaneous firing.
The action of 5-HT receptors on nonserotonergic cells is predominantly inhibition of firing, although some excitatory serotonin receptors have been described (Brownstein, 1981).
Serotonin has been implicated in many central processes, including regulation of the anterior pituitary, sleep, appreciation of pain, thermoregulation, control of blood pressure, appetitive behavior, drinking, respiration, heart rate, rhythmic behavior and memory (Brownstein, 1981).

4.2 INFANTILE AUTISM AND SEROTONIN LEVELS IN BLOOD: HYPERSEROTONEMIA

4.2.1 INTRODUCTION

In 1961 Schain and Freedman were the first persons who reported an elevated serotonin level in the blood of some autistic children (Schain and Freedman, 1961). Of a group of 23 autistics the serotonin was elevated in 6 children. The mean level of the whole group was significantly higher than that of a contrast group consisting of mildly retarded children without autistic characteristics. Schain and Freedman also compared their group of autistics with a group of severely mentally retarded children. On an average these children had higher serotonin values than the slightly retarded children, but lower than the autistics. However, the latter difference was not significant.

After the publication by Schain and Freedman the finding of an elevated serotonin level in the blood of autistic children has been confirmed by many investigators. Thus, Ritvo et al. (1970), Yuwiler et al. (1971, 1975), Goldstein and Coleman (1976), Takahashi et al. (1976), Hanley et al. (1977), Hoshino et al. (1979b), Hoshino et al. (1984c), Anderson et al. (1985b, Appendix No. I) and Minderaa et al. (1985d, Appendix No. II) were among those who have found that the serotonin values in the blood were elevated. In total, more than 300 autistic children were involved in these investigations. There are not any results of investigations contradicting these findings. Only some investigators found the differences were not significant (Boullin et al., 1971a; Campbell et al., 1974a). Thus there is good agreement among investigators that mean whole blood 5-HT levels are elevated in autistic subjects. However, considerable overlap does exist for platelet 5-HT values among individuals in the autistic groups with those among normal control subjects.

It is difficult to compare the results of the different investigators. In the very first place there exists the problem of the diagnostic marking of the limits of the syndrome. The symptomatology differs from child to child and can change with time. Consequently, the view of the symptomatology has been evolved in the course of decades. This has led to a great variety of different diagnostic terminologies, which hamper comparison of the results of the investigations. In the literature the terms

childhood autism, primary autism, infantile autism, atypical development, childhood schizophrenia, symbiotic psychosis and early onset psychosis refer all to nearly the same symptomatology. It is possible that there exist diagnostic differences in the groups that were investigated.
Furthermore, it is important to note that in comparing the results of investigations on the serotonin levels various measurement techniques has been applied in the course of years. Schain and Freedman (1961) extracted the serotonin from homogenised blood by means of acetone. Serotonin was measured with a bioassay with heart of clams or rat uterus. Later various fluorometric methods were applied (Yuwiler et al., 1975; Goldstein and Coleman, 1976; Takahashi et al., 1976; Hanley et al., 1977; Hoshino et al., 1979b; Ritvo et al., 1970; Sankar et al., 1962). Recently Anderson (1985b) has employed high performance liquid chromatography (HPLC).
Another factor which plays a part is that by some the serotonin level was determined in serum (Ritvo et al., 1970; Hoshino et al., 1979b), by others in whole blood (Ritvo et al., 1970; Yuwiler et al., 1975; Goldstein and Coleman, 1976; Hanley et al., 1977), in platelet rich plasma (Hoshino et al., 1979b) or in platelet pellets (Takahashi et al., 1976).
As to the measurement in serum it is difficult to know which percentage of 5-HT stored in the platelets was released and was measured. In isolating platelet rich plasma or platelet pellets an important factor is that heterogeneous platelet populations can be acquired by centrifuging. Younger blood platelets are heavier and are characterized by a greater metabolic activity such as greater enzyme activity and more granules storing serotonin (dense granules) (Karpatkin, 1978; Murphy et al., 1978). Consequently, the isolation of platelets by centrifuging can lead to variable serotonin values (Young et al., 1982). Lastly the concentration of 5-HT has been expressed by some investigators per ml blood, and by others per number blood platelets or per mg protein.
In consequence, there are considerable differences between observed mean group values of autistic children and control groups (Anderson et al., 1985b, Appendix No. I Table I). The above mentioned problems concerning diagnostic procedures, measurement techniques and ways of expression can be causal aspects in this matter. With regard to control groups there are found blood 5-HT (ng/ml) elevations in groups of autistics of 137%

(Hanley et al., 1977), 117% (Schain and Freedman, 1961), 51% (Anderson et al., 1985b, Appendix No. I), 49% (Yuwiler et al., 1971), 41% (Minderaa et al., 1985d, Appendix No. II), 40% (Hoshino et al., 1984c), 33% (Yuwiler et al., 1975), 22% (Ritvo et al., 1970) and 18% (Goldstein and Coleman, 1976). Our findings of 51% and 41% (Anderson et al., 1985b, Appendix No. I) and 41% and 33% (Minderaa et al., 1985d, Appendix No. II) for ng/ml and ng/10^9 platelet respectively are approximately in the middle of this range.

Some investigators defined hyperserotonemia as a whole bood 5-HT level in the upper 5% of a normal control group. Using this criteria Hanley et al. (1977) found a percentage of 30% while our findings are 38% (Anderson et al., 1985b, Appendix No. I) and 29% (Minderaa et al., 1985d, Appendix No. II), percentages approximating each other.

4.2.2 STABILITY OF WHOLE BLOOD SEROTONIN OVER THE TIME

Only few studies dealt with the stability over the time of serotonin values in the blood. Yuwiler did not find any indications pointing to circadium rythmicity in normal nor in autistic children. In a normal control group Ritvo et al. (1971a) found constant 5-HT values in the blood over the period of a week. Yuwiler et al. (1971) also found a slight intra-individual variance in the 5-HT values obtained from male volunteers. However, the period between the blood drawings was not mentioned in this investigation. 5-HT values repeatedly measured over one year period of time, were also stable in 2 male control subjects. Hanley et al. (1977) reported several hyperserotonemic children were repeatedly confirmed to have high blood 5-HT levels over the one-year period of time.

In our own investigation (Minderaa et al., 1985d, Appendix No. II) 5-HT values appeared to be very stable over one year period with a very high significant intraclass correlation and a low mean percentage of change in 10 unmedicated autistics. In a series of articles Wirz-Justice described a variable pattern of 5-HT values over the day and a bimodal annual rythm in a group of normal volunteers (Wirz-Justice et al., 1977, 1978, 1979). However, the variation of 5-HT over time shown in these studies is very

modest. It might be concluded that blood 5-HT is stable over the time.

4.2.3 AGE

The relation between age and the serotonin level in the blood is unclear, both in control subjects and in autistics. After investigating a control group (N=164) in the age of 1-40 Ritvo et al. (1971a) found that the whole blood serotonin values (µg/ml) decreased from the first year of life up to puberty and remained constant afterwards. The number of blood platelets decreased in the same way with the climbing of age. In our study (Anderson et al., 1985b, Appendix No. I) no connection was found between whole blood serotonin levels and the age in a group of normal children (6 years and older) and young adults. Both Ritvo et al. (1970) and Takahashi et al. (1976) found differences between autistics and control groups to be the greatest in the younger age group up to the age of 6. This finding was confirmed by us, but only in young boys (Anderson et al., 1985b, Appendix No. I).

4.2.4 SEX

Ritvo et al. (1970) found no sex differences in their control group. In our investigation (Anderson et al., 1985b, Appendix No. I) significantly higher serotonin values were found in the blood of boys in the younger age group (1-6 years of age) of the control children in comparison with girls.

4.2.5 INTELLIGENCE

In his original study Schain and Freedman (1961) examined three groups: a group of mentally retarded children (IQ 60-80), a group of autistics functioning at a severely retarded level, and a group of seriously retarded non-autistic children with an IQ below 20. The serotonin levels of the first group were significantly lower than those of the second

group. However, the difference in serotonin values between the autistics and the most severely retarded children was not significant.
Hanley et al. (1977) employed a similar grouping. They defined hyperserotonemia as those values which were more than 1.65 times the standard deviation higher than the average serotonin values of the control group. According to this criteria 30% of the autistics had elevated serotonin levels, and 52% of the group seriously retarded children. Consequently, more than half of the seriously mentally retarded children without specific (manifest), metabolic or neurological disorders had hyperserotonemia. Surveying the studies which had been published up till then, they arrived at the impression that children with IQ values below 20 tend to have hyperserotonemia, whereas children with IQ values above 60 tend not to have it.
In a group of 11 seriously disturbed children (11 "schizophrenic" children, 2 autistics and 2 children with a "withdrawal reaction") Campbell et al. (1974a) tried to find a relation between serotonin values and intellectual functioning. She found that low intellectual functioning was the only parameter which seemed to correlate clearly with higher serotonin levels. In a later study Campbell et al. (1975) measured serotonin values in 23 children with "childhood schizophrenia" and 16 control children. The mean serotonin values in the autistics were elevated, although this was not significant. However, the serotonin values were significantly higher in a subgroup of children with floride psychoses, and with lower IQ's than of children in remission, partly remission or with higher IQ's.
The importance of these findings becomes more clear when one realises that in the literature 85% of autistic children are described as being mentally retarded. This leads to the question whether hyperserotonemia doesn't say more about mental retardation than about autism.

4.2.6 HYPERACTIVITY

Schain and Freedman (1961) examined the symptomatology of the 6 autistics with the highest serotonin values from their group of 23 and found no difference as to activity with the other autistics. In the group of 30

autistics of Takahashi et al. (1976) there were 21 with a high score for hyperactive behavior. However, the serotonin values of these children were only slightly higher than those of the whole group of autistics. In their contrast group of non-autistic psychiatrically disturbed children hyperactive children did have significantly higher serotonin levels than the rest of the children in this group. Neither Hoshino et al. (1979b) and Hanley et al. (1977) could find a connection between hyperactivity and elevated serotonin levels in autistic children.

4.2.7 NEUROLOGICAL EXAMINATION AND MEDICATION

Hanley et al. (1977) found no connection between results of neurological examination and the serotonin levels in the blood of their group of autistics. However, Schain and Freedman (1961) reported that none of the 6 hyperserotonemic autistic children in their group suffered from epilepsy, although this frequently occurred in the other autistics. Furthermore, it is reported that anti-epileptics did not seem to influence the serotonin values. Anderson et al. (1985b, Appendix No. I) recently found that autistics with anticonvulsive medication had significantly lower serotonin values than those without medication. In a follow-up study about this subject, the decrease seen was not statistically significant (Minderaa et al., 1985d, Appendix No. II). According to Schain and Freedman (1961) phenobarbital and chlorpromazine didn't have any influence on the serotonin level of the autistics either. Hoshino et al. (1979a) also reports that medication (methylphenidate and haloperidol) did not change the serotonin percentage in serum. However, Anderson et al. (1985b, Appendix No. I) also found significantly lowered serotonin values in autistics with neuroleptics in comparison with the unmedicated autistics. Minderaa et al. (1985d, Appendix No. II) found significantly decreased 5-HT values after the start of neuroleptic medication in 4 autistic individuals. In the same study in a group of 10 autistics with neuroleptic and anticonvulsive medication the 5-HT values were significantly higher for the second blood drawing compared to the first blood drawing when measured a year apart. But only a nonsignificant trend was found for a group time effect of the medicated group compared to the

unmedicated autistic individuals.
In some groups of mentally retarded children with a defined syndrome (e.g.: congenital rubella) elevated serotonin levels have also been found (Pare et al., 1960; Coleman, 1978), as well as lowered serotonin values as in Down's syndrome (Boullin et al., 1971b) and phenylketonuria (Pare et al., 1957, 1958). In the groups of mentally retarded children in which hyperserotonemia is found, autistic behavior characteristics have sometimes been described too. The percentage of hyperserotonemia did not differ when these subgroups were included or excluded from a group of autistics (Young et al., 1982c). Oikawa et al., (1978) found the degree of mental retardation in retarded adults correlated with the serotonin levels. Common symptoms in the individuals with increased serotonin levels included aggressiveness, seizures and hearing or visual defects. Finally, Minderaa et al. (1985f, Appendix No. VI) reported that a small group of unmedicated autistics with a visual rooting reflex showed significantly higher 5-HT values compared to those without a visual rooting reflex. This finding needs replication.

4.3 NUMBER OF BLOOD PLATELETS

The serotonin percentage circulating with the blood is almost completely situated in the blood platelets. Only a small part is situated in the plasma (1-5%). This gives rise to the question whether in hyperserotonemic autistics the number of blood platelets is elevated. This could be a possible explanation for elevated serotonin levels in blood. Thus, Boullin et al. (1971a) found that in 5 autistics the number of blood platelets per ml plasma was significantly elevated in comparison with control children.
Similarly, Ritvo et al. (1970) found that in 24 autistic children the number of blood platelets was elevated in comparison with a control group of same age. Although the serotonin level given per ml blood was significantly higher in the group of autistics, the serotonin values given per number of blood platelets did not differ with that of the control group. However, Yuwiler et al. (1975) found that in 12 autistics the serotonin values were elevated both when the concentration was given

per ml blood and per number of blood platelets.
Hanley et al. (1977) compared 4 autistics with high serotonin values with 4 mentally retarded children with normal serotonin values. The difference in number of blood platelets was small and could not account for the difference of serotonin values between the two groups. In our investigation (Anderson et al., 1985b, Appendix No. I, Minderaa et al., 1985d, Appendix No. II) there wasn't found any difference either in number of blood platelets between 21 unmedicated autistics and the normal children in the control groups (N= 87). In this study the serotonin level in autistics was elevated both when the concentration was given per ml blood and per number of blood platelets. So, the more recent findings point in the direction of an elevated serotonin level, both absolutely and per number of blood platelets. Consequently, it seems very unlikely that an elevated number of blood platelets should be the cause of elevated serotonin values in autistics.
Hanley et al. (1977) found a positive correlation between serotonin levels (ng/ml) in the blood and the number of blood platelets in autistics. In our first report (Anderson et al., 1985b, Appendix No. I) no correlation was found between the 5-HT (ng/ml) levels in the blood and the platelet count in unmedicated autistics. On the contrary, in the second report (Minderaa et al., 1985d, Appendix No. II) a correlation was found between whole blood 5-HT (ng/ml) levels and platelet count. Together with a lack of correlation between 5-HT ng/10^9 platelets and platelet count in unmedicated autistics this suggests that platelet count or function might play a causal role in hyperserotonemia.

4.4 FUNCTION OF BLOOD PLATELETS

Much work has been done on the in vitro capability of autistics' blood platelets to take up and store serotonin. The results are contradictory and controversial. In a series of articles Sankar (Sankar et al., 1962; Sankar, 1970; Sankar, 1971) demonstrated that the ability of blood platelets to take up serotonin is decreased in autistic and mentally retarded children. The lower the IQ, the greater the decrease seen. In normal children there was an increase of the serotonin-uptake of blood

platelets in vitro with climbing age. This was not found in autistic children. More recently this finding was replicated again and the author concluded that the defect is associated with the platelet and not with the plasma (Sankar, 1977).

On the other hand Boullin et al. (1971a) found a slight, not significant increase of the ability of blood platelets in vitro to take up serotonin. This was attended with an also slight increase in the adenosine triphosphate (ATP) level of blood platelets.

Lucas et al. (1971b) did not find any difference in the serotonin-uptake ability between 16 male "schizophrenic patients", with an average age of 12.9 and a control group with children with serious personality disturbances.

In 1975 Yuwiler et al. investigated 12 autistic children and 15 hospitalized otherwise psychiatrically disturbed children. The aim was in particular to measure the serotonin-uptake ability according to 2 different methods and to compare these methods with each other. In one method manupulation of the fragile trombocytes was prevented as much as possible. In the other method centrifuging and resuspensing was applied with an increased change of cell damage. With the first method the serotonin uptake of blood platelets in the autistics appeared to be slightly but not significantly decreased. In the second method the uptake was somewhat higher, but there were not any differences between the groups either. Finally, Rotman et al. (1980) investigated 2 subgroups of "autistics". They called the four children of the first group "affectively centripetal children". These children resisted contact and preferred to be left alone. According to the authors they were most similar to the original autistics described by Kanner. The 6 children of the second group were called "affectively centrifugal children". These children were directed on the outer world and were considered to be more aphasic or specific brain-organically disturbed. Rotman found the serotonin-uptake ability of blood platelets of the first group to be significantly higher than those of the second group. These findings are interesting in particular because an elevated uptake ability of 5-HT of blood platelets could be a factor in elevated serotonin values in the blood. However, in this investigation whole blood 5-HT values are not mentioned. Furthermore, 8 out of 10 children used psychoactive drugs.

Although the authors indicate that psychopharmacological medication had no significant effect on 5-HT uptake in vitro, the results should be viewed with caution.

The outcome of the investigation on the release of serotonin from blood platelets, is as contradictory as the results of the investigations mentioned above. In the investigation of 5 autistic children Boullin et al. (1970) found that the the ability of the blood platelets to store the radioactively labeled serotonin, was decreased. In a following study (Boullin et al., 1971) they were able to blindly predict, on the basis of experimental data concerning the serotonin efflux of blood platelets, which six out of ten psychotic children were autistic and which not, according to a specific scoring procedure. The criterium was that the efflux had to be three time the control value in order to predict the presence of autism according to the scores. In the vesicles in the blood platelets 5-HT forms a binding with adenosine-tri-phosphate. The ATP values in blood platelets were not abnormal and the ATP-5-HT ratio was normal in autistics. In consequence, a possible shortage of ATP could not be an explanation for the decreased ability of the blood platelets to store 5-HT. However, Yuwiler et al. (1975) could not confirm these spectacular findings. These investigators found no differences in serotonin efflux from blood platelets between autistics and control children. Later, the latter investigation was attacked in detail by Rimland (1976), one of the coworkers of the investigation of Boullin. The essence of the criticism is that Yuwiler used wider inclusive criteria for the experimental group. Boullin selected by means of score of +20 or higher on the Rimland E-2 Diagnostic Checklist. This list has specially been constructed to select the 10% of children with classical early infantile autism (Kanner's syndrome) from the larger group of children with autistic characteristics. Rimland argues that Yuwiler's investigation group can be compared with Boullin's control group. In a collaborative study Boullin, Yuwiler, Rimland and others (Boullin et al., 1982) have most recently reported that the serotonin efflux of blood platelets in autistic subjects is normal.

In our investigation (Anderson et al., 1984, Appendix No. IV) the imipramine binding of blood platelets was investigated. The imipramine binding sites are supposed to play a role in the regulation of the

serotonin uptake of blood platelets and recently low molecular weight substances in plasma have been detected that inhibit both high-affinity imipramine receptor binding and serotonin uptake in platelets (Brusov et al., 1985). The imipramine binding of blood platelets in 11 autistics without medication appeared not to differ from that of a control group. Therefore, this finding lends no support to alterations in the serotonin uptake of blood platelets in autistics.

Hanley et al. (1977) studied the effect of reserpine on the serotonin values of the blood in 4 hyperserotonemic autistics and 5 slightly retarded children with normal serotonin levels. Reserpine expels serotonin from the storage vesicles in the blood platelets so that the serotonin level in the blood is strongly reduced. In both groups the serotonin percentage was not to be measured any longer after some days. In both groups the recovery developed at the same rate. After 17 days the serotonin values were back to baseline levels. This finding is not in favour of a disturbance existing in the ability of blood platelets to store 5-HT.

Platelets have been reported to contain a neurone-specific enolase (Marangos et al., 1980; Campbell et al., 1980,1981). This enzyme was previously found only in neurones and in cells of the APUD-system (amine precursor uptake and decarboxilation system). Its presence in platelets suggests that platelets, like the argentaffine cells in the gut that produce serotonin, may belong to the Diffuse Neuroendocrine System (DNES). The DNES, the somatic and the autonomic nervous system are judged as the three devisions of the mammalian nervous sytem (Pearse, 1977). This might mean that ontogenetically the blood platelet and the argentaffine cell in the gut are related to each other. With Campbell (1983) we state that were this to be the case, conceptual problems concerning the relationship of various aspects of platelet function, argentaffine cell function and psychopathology might better be understood in the future.

4.5 PLASMA CYCLIC AMP

Cyclic AMP (adenosine 3'5'-monophosphate, cAMP) is supposed to be a

"second messenger" for neurotransmitters such as serotonin in the brain. These neurotransmitters elevate intracellular cAMP after interacting with the membrane receptors. Winsberg et al. (1980) reported that levels of cAMP in cerebrospinal fluid of autistic children were elevated after probenecid administration. However, no control group was used in this study. Hoshino et al. (1979b) measured the concentration of plasma cAMP in a group of 20 autistic children. They found the percentage of cAMP in plasma significantly higher than in the control children. At the same time, they, found a positive correlation of this value with the serotonin percentage in serum. There also existed a correlation of cyclic AMP concentration with the hyperactivity scores of the autistic children. The more active these children were, the higher the cAMP concentration in plasma.

Goldberg et al. (1984) reported plasma cyclic AMP significantly elevated by over 100% in 18 patients with childhood autism and in 7 patients with pervasive developmental disorders compared to 12 age- and sex-matched normal controls. Plasma guanine monophosphate (cGMP) was normal in these groups. The origin of plasma cAMP remains unclear. The compound has been assumed to be derived from peripheral organs, such as the liver, kidney, lung and adrenal glands, as well as the brain (Anderson et al., 1985b). So, the relation between plasma cAMP and whole blood serotonin is uncertain.

4.6 ATP AND ATPASE ACTIVITY

Boullin et al. (1971a) found the concentration of ATP in blood platelets in autistics not changed in comparison with controls. But the range appeared to be greater. However, Sankar (1971) found significant differences in ATPase activity before and after lysis of the blood. He concluded from this that a difference possibly existed in membrane function of the red cells between autistic and normal children.

4.7 MONOAMINE OXIDASE ACTIVITY IN BLOOD PLATELETS

Research on monoamine oxidase (MAO) in blood patelets is reported in various studies. MAO degrades serotonin (in addition to other amines) and decreased MAO-activity could be a cause of the elevated serotonin levels. However, the MAO activity in blood platelets of autistic children, measured under a variety of conditions, has appeared to be normal (Boullin et al., 1976; Campbell et al., 1976a; Cohen et al., 1977d; Lake et al., 1977; Roth et al., 1976). No correlation was found between MAO activity and the serotonin concentration in autistics (Cohen et al., 1977d).

4.8 TRYPTOPHAN

Hoshino et al. (1979b) measured free tryptophan in serum in 10 autistic children and found no differences with a control group of adults. Two children had a remarkably high free tryptophan level. These children were very hyperactive and showed aggressive behavior. However, in the whole group of autistics there was no connection between the free tryptophan level in serum and scores of degree of active behavior. The same research group recently compared 37 autistics with 76 control children (Hoshino et al., 1984c). The plasma free tryptophan level was significantly higher in autistic children than in normal control subjects. No differences were found for total TRP levels. There tended to be a significant positive correlation between the plasma free tryptophan level and scores of clinical symptoms and signs of autism at the Children's Psychiatric Rating Scale and at the Werry-Weiss-Peters Activity Scale and a negative correlation between plasma free tryptophan level and the Developmental Quotient. No correlation was found between 5-HT levels and behavior scores or between 5-HT levels and free tryptophan levels.

Yamamoto et al. (1982) treated autistic children during six months with haloperidol and showed that during this treatment plasma free levels of TRP normalised concomitant with clinical improvement.

In our first study (Anderson et al., 1985b, Appendix No. I) we found slight differences in total tryptophan levels in the blood of 40 autis-

tics in comparison with an age-matched control group. The tryptophan level in the autistics was lower. However, this difference was only significant for the medicated autistics. Takatsu et al. (1965) reported that the total TRP level was at the lower limit of normal or lower in atypical autism. Sylvester et al. (1970) reported that young psychotic children excreted significantly lower amounts of urinary TRP compared to normal controls.

In our second study (Minderaa et al., 1985d, Appendix No. II) no differences were found in total TRP between unmedicated autistics and control subjects. Hoshino et al. (1979b) found no differences either of total TRP levels between autistics and controls. It can be concluded from these several studies that the total TRP levels in the blood of autistics are normal.

Several investigators have experimented with a tryptophan loading test in autistic children. Schain and Freedman (1961) gave an oral tryptophan loading in 4 autistic children. This did not lead to consistent changes in the serotonin level of the blood. After an oral loading with 3 g of tryptophan Shaw et al. (1959) saw no difference in the increase of 5-hydroxyindoleacetic acid (5HIAA) excretion in the urine between 11 schizophrenic children and 10 non-schizophrenic children with different psychiatric problems. Sutton et al. (1958) described an 18 months old autistic girl with a decreased ability to convert tryptophan into 5-HIAA. After oral tryptophan loading (0.25 g/kg) there was found decreased concentration of indoleacetic acid, indolelactic acid and 5-hydroxy-indoleacetic acid in the urine. Without loading the excretion of these metabolites was normal. No deviations were found in the excretion of metabolites of the kynurenine pathway such as xanthurenic acid and kynurenic acid.

Finally Hanley et al. (1977) administered an oral dose of tryptophan to 4 hyperserotonemic autistics and 4 mildly mentally retarded children with normal serotonin levels during a period of 3 days. Both before and after the tryptophan loading the serotonin values in the blood and the urine excretion of serotonin and 5-HIAA were higher in the autistics. Tryptophan loading caused a slight fall in the serotonin values in the blood in both groups, while the urine 5-HIAA excretion doubled. After loading the serotonin excretion via the urine increased in the hyper-

serotonemic autistics, but decreased in the retarded children. The investigators concluded that in hyperserotonemic autistics 5-HT can be converted adequately into 5-HIAA via the MAO dehydrogenase enzymes. Furthermore they concluded that it is unlikely that there exists a serious blockade in the kynurenine pathway, as the 5-HIAA rise after TRP loading was proportionally equal in both groups. Finally, they concluded that the uptake of TRP by the APUD cells in the gut, and the different steps to convert TRP to 5-HTP and 5-HT were not greatly altered in autism.

Heeley and Roberts (1965) investigated the tryptophan metabolism in 16 psychotic children. They studied in particular the ratio between the excretion of 3-hydroxy kynurenine (HK) and 3-hydroxyanthranilic acid (HA) after tryptophan loading (0.1 g/kg). This HK-HA ratio was elevated in 9 out of 16 children, which points to a decreased kynureninase- activity. As kynureninase is very pyridoxine sensitive, this is interpreted as an indication for a dietary or functional pyridoxine deficiency. There were no other indications for diet deficiency of pyridoxine in these children. Four children with deviating values were treated with oral pyridoxine suppletion. After a week the HK-HA ratio had been normalised after tryptophan loading. Consequently, according to the authors, there are indications for a decreased availability of pyridoxine, for the kynureninase step. There are investigators who also claim a favourable result of administrating Vitamine B6 in regard to the behavior of some autistic children (Rimland et al., 1978; Martineau et al., 1981).

Hoshino et al. (1983, 1984b) administered L-5-hydroxytryptophan (5-HTP) to 6 autistic children and nine normal controls. Although the baseline values of blood 5-HT were significantly increased in the autistics, blood serotonin showed a suppressed increase after 5-HTP, compared with normal controls.

4.9 5-HYDROXYINDOLEACETIC ACID IN URINE

5-HIAA is the main metabolite of serotonin and can be measured in urine. The urine excretion of 5-HIAA seemed mainly derived from peripherely produced serotonin. Shaw et al. (1959) found no difference in absolute

values of 5-HIAA in urine between a group of 11 "schizophrenic children" and 10 non-schizophrenic psychiatrically disturbed children. Partington et al. (1973) also found a normal 5-HIAA excretion in mentally retarded children with elevated 5-HT values in blood.
Schain and Freedman (1961) did find significant higher 5-HIAA values in 24-hours urine in a group of autistics compared with a group of mildly mentally retarded children, if it was expressed per mg creatinine.
However, the creatinine concentration in the autistics was much lower than in the mentally retarded children, so that the results concerning the 5-HIAA excretion can not be considered to be deviating. However, Hanley et al. (1977) found significantly elevated 5-HIAA values (mg/day) in 4 autistics with hyperserotonemia compared with a control group of 4 slightly mentally retarded children with normal serotonin values. This elevated 5-HIAA excretion increased further after an oral TRP loading, at the same rate as in the slightly mentally retarded children.
We assessed urinary 5-HIAA excretion in a group of 16 unmedicated autistic individuals (Minderaa et al., 1985a, Appendix No. III). No significant differences were found between unmedicated autistics and normal controls. Furthermore, a lack of correlations between urinary 5-HIAA and whole blood 5-HT was observed for the autistics as well as for the control subjects. These findings suggested that elevation of whole blood 5-HT is not due to differences in 5-HT gut production in the intestine. However, urinary 5-HIAA excretion was significantly greater in hyperserotonemic autistic subjects compared to normal controls and tended to be higher compared to normoserotonemic autistics. These findings might suggest that some relationship between gut 5-HT production and increased blood 5-HT levels do exist in hyperserotonemic autistic individuals.

4.10 5-HYDROXYINDOLEACETIC ACID IN SPINAL FLUID

Up to the present only a few research groups have done spinal taps in autistic children in order to measure metabolites of monoamine metabolites. Cohen et al. (1977c) found in a group of 18 autistic children after administering probenecid the CSF concentration of 5-HIAA increased less than in a contrast group consisting of non-autistic psychotic

children. Within the group of autistic children there is searched for correlations between metabolite concentrations in the CSF and behavior scores. Autistic children who scored better as to social responsiveness and the ability to concentrate had higher 5-HIAA concentrations in the CSF. This also held true when a larger group of neuropsychiatrically disturbed children was examined. So, a greater turnover of serotonin, as measured via the metabolite concentrations after probenecid loading, was correlated with less disturbance in social functioning and attention functioning in autistic children and in a larger group of neuropsychiatrically disturbed children. Winsberg et al. (1980) found in a few autistic patients no increase in CSF 5-HIAA after probenecid administration. However, in this study, no control group was used. In a recent investigation Gillberg et al. (1983a) compared a group of 13 autistic children with a group of 22 children with other psychoses and with an age-matched control group. They also examined, among others, a group of 8 children with "simple" mental retardation. The CSF baseline 5-HIAA values of the autistics were not significantly higher than those of the control children. The same held true for the non-autistic psychotic children and the mentally retarded children. Of these 3 groups of children the 5-HIAA values were the highest in the last group. However, neither in the group with autistic children, nor in the group with other psychoses did there exist a significant correlation between IQ and metabolite values.
So, although the findings with respect to CSF 5-HIAA are inconclusive, there might be some suggestion of decreased values in autistic patients.

4.11 BUFOTENIN EXCRETION IN URINE

Perhaps prompted by the interest in the transmethylation hypothesis of schizophrenia, there also existed an interest in the bufotenine excretion (N, N-dimethylserotonin) with regard to psychotic children. Bufotenin is a dimethylated derivate of serotonin and was considered to have an hallucinogenic effect. Related compounds with hallucinogenic effects are N,N-dimethyltryptamine (DMT) and 5-methoxy-N,N-dimethyltryptamine (5-MeODMT).
Perry (1963) found that 2 out of 18 "juvenile psychotics" excreted bufotenin, while they were medicated with a MAO blocker. The bufotenin

concentrations did not differ from those of the control children, of whom 4 out of 6, with MAO blockers, excreted bufotenin. Himwich et al. (1972) compared 6 children suspected of autism with 6 normal children, under dietary circumstances. Five of the autistics and none of the control children sporadically excreted bufotenin. None of the patients excreted DMT of 5-MeODMT.
Finally, Narashimharchari and Himwich (1975) reported three out of six autistic patients excreted DMT compared to none of their 13 controls. Urinary bufotenin was detected in five out of six autistic individuals compared to one out of 13 of the control subjects.
As far as is known, methyldonors, as methionine, has never been administered to autistics.
Nicotinic acid or nicotinamide can serve as methylgroupacceptors. Their effect on autistic children are much disputed, as will be discussed in the following section.
So it might be suggested that unusual methylated metabolites of indoleamines are present more often in autistic individuals. The significance of these findings is unknown, at present they require replication.

4.12 MEDICATION WHICH HAS AN IMPACT ON THE SEROTONERGIC SYSTEM

Until recently medication which was primarily directed to the serotonergic system was applied only sporadically. Campbell et al. (1971) treated 10 autistic and schizophrenic children with imipramine. This resulted in a mixture of stimulating, tranquilizing and deteriorating effects. Three children functioned obviously better, five worse. In these 5 children there was an increase in the serotonin levels in blood. Sverd et al. (1978) treated 3 autistic children with the precursor of serotonin, L-5-hydroxytryptophan, in combination with Carbidopa, according to a double blind design. Changes in behavior were observed which, however, were not correlated with the treatment. Zarcone et al. (1973) administered L-5-HTP orally to 2 autistic boys, 7 and 8 years old, during a period of 8 days. Except the increase in REM-sleep no changes in behavior were observed.
Furthermore, in the 1960's several investigators tested the effect of LSD

on the symptomatology of autistics. Bender (1966) gave LSD (1-methyl-D-lysergine acid butonalamide bimaleate) to 26 schizophrenic children and a methylderivate of LSD, methysergide, to 28 schizophrenic children during the period of about a year. This resulted in an overall improvement in behavior, in particular with regard to the social responsiveness. However, in some children an increase of anxiety and chaotic behavior was observed.

Simmons et al. (1966) obtained variable or negative results with LSD.

Finally, Fish et al. (1969) administered methysergide to 11 mentally retarded children. Only 2 children showed some symptom-improvement.

Ritvo et al. (1971b) investigated, in 4 autistic children, whether the serotonin values in the blood would decrease with oral L-Dopa therapy. In the youngest three children a fall of the serotonin levels were observed. However, no symptom-improvement appeared.

In a double-blind crossover study Campbell et al. (1976b) gave L-Dopa to 12 psychotic children in the age varying from 3 to 6 , in daily doses of 900 to 2250 mg. Five out of 12 children improved, in particular by decreasing negativism, an increase in playing, energy and motor initiative. Four children showed progress in speaking, affective responsiveness and a decrease in irritability. These differences were not significant and there was no correlation with changes in the serotonin values. The four children with decreased serotonin values did not improve. In three children there were no changes in the serotonin levels, five of them showed a rise in serotonin levels.

A great number of American centers have lately started an investigation on the effect of fenfluramine. These activities started after publications by Ritvo et al. (1983) and are led by him: he treated 14 autistics with fenfluramine in a double-blind ABA crossover design. Fenfluramine depletes the blood platelets of serotonin and thus decreased the serotonin concentration in the blood. After one month of treatment the serotonin concentration did decrease to an average of 50 % of the starting value. It normalized again within a month after stopping the therapy. Both in autistics with normal and with elevated serotonin levels it was true that certain symptoms decreased with medication and returned on placebo. August et al. (1984) found after 4 months fenfluramine medication in autistic children an average reduction of 5-HT values of

60% which was coupled with significant decrease of certain symptoms such as distractability, motor activity and mood disturbances.
In the 1960's the therapeutic effect of a number of substances was investigated which are normally present in the organism, but which in some disorders may be functionally deficient in certain brain areas. Research within this "Orthomolecular Psychiatry" (Pauling, 1968) focussed its attention on schizophrenia in particular. Substances investigated were for example ascorbic acid, thiamine, pyridoxine, folic acid, glutamic acid and also nicotinic acid and nicotinamide. A number of these substances have also been administered to children and influence, among other things, the tryptophan and serotonin metabolism.
Hoffer (1970) found nicotinamide and ascorbic acid effective in psychotic children in comparison with a placebo.
However, Greenbaum (1970) could not confirm these results. He saw no difference in a double-blind investigation in which 17 children received nicotinamide and 24 a placebo. In a big survey with 190 children treated as outpatients Rimland (1973) investigated the effect of vitamines in high doses. Thirty seven of these 190 children were described as autistic. Nicotinamide, ascorbic acid, pyridoxine and pantothenic acid, were administered according to a fixed scheme. Changes in behavior were scored by parents and the medical attendant. The group of classical autistic children improved most. The strongest effect was by Vitamin B6 (150 mg/day), in particular in causing an increase in the intention to speak and the total verbal production. At the beginning of the therapy side effects such as an increased activity, irritatability and sleeplessness were observed in some children, both with nicotinamide (more than 1000 mg/day) and with Vitamin B6. These symptoms disappeared after some weeks. The overall result of the Vitamin regime was an improvement in 45% of the children, a possible improvement in 41%, no improvement in 10,5% and a worsening of the symptomatology in 3%.
In a follow-up study 16 autistic children who had reacted positively on Vitamin B6 therapy, were switched over double-blind on either Vitamin B6 or placebo. Behavior worsened significantly in the children who didn't receive Vitamin B6 (Rimland et al., 1978).

4.13 GENETIC FACTORS AND SEROTONIN

Recently, Jackson et al. (1984) studied 24 male fragile X patients, 13 of them (54%) being autistic. Of these 13 autistic fragile X patients, none demonstrated hyperserotonemia. They conclude that if blood serotonin levels differentiate subgroups of autistic patients, the subgroup with hyperserotonemia does not include fragile X autistic patients.
Finally, Kuperman et al. (1985) examined 30 autistic children and 84 of their first-degree relatives for serotonin blood levels and platelet counts. Significant correlations were found between sets of relatives (mother-child, father-child, sib-sib, either probands being included or not included) for platelet rich plasma (PRP) serotonin levels. The correlations between PRP serotonin levels of mothers and fathers was not significant. The authors conclude that there is a strong indication for familial resemblance of PRP serotonin levels and that the significant positive correlations of PRP serotonin levels between sets of blood relatives may be attributed to genetic similarity. So, it might be very interesting to trace further 5-HT values in blood in autistic and nonautistic families.

4.14 IMMUNOLOGICAL FACTORS AND SEROTONIN

Weizman et al. (1982) reported a cell-mediated autoimmune response to human myelin basic protein using a macrophage migration inhibition factor test in 13 (76%) of 17 autistic children examined. They conclude that their results indicate the existence of a cell-mediated immune response to brain tissue in autism. Westall et al. (1983) point to the fact that myelin basic protein contains a serotonin binding site in its tryptophan peptide region. This region is immunologically active and responds by activating lymphocytes. As one of possible explanations of Weizman's findings and reported increased whole blood values in autism they formulate that an autoimmune response to the serotonin binding site on myelin basic protein might result in an agonistic effect on the serotonin system concomitant with dumping of excess serotonin into the blood. A second hypothesis they offer is that serotonin binds to myelin basic

protein that is released during normal protein turnover, stabilizing it against proteolytic digestion so that it can be immunologically processed, which results in the activated lymphocytes.
Finally, in an intriguing report, Todd and Ciaranello (1985) described the discovery of autoantibodies against 5-HT binding proteins present in the blood and cerebrospinal fluid of an autistic child. These antibodies are of the IgG class, discriminate between 5-HT_{1A} and other 5-HT binding proteins and are specific for human 5-HT_{1A} sites. The investigators speculate that a subgroup of autistic patients may be suffering from an autoimmune disorder. In a second study they reported of 13 autistic children, 7 have circulating antibodies directed against brain 5-HT receptors. In contrast, these antibodies were not seen in 13 normal children.

4.15 CONCLUSION

Research on neurochemical aspects of infantile autism has been unsystematic and incomplete. Only a very few research groups have done consistent investigation on neurochemical variables and their relation to the psychopathology of autistic children during a considerable period of time. This results in a series of isolated or often contradictory findings. Accordingly, up to the present, few replicated neurochemical differences have been found between autistics and control subjects. The fact that many groups have confirmed the finding of elevated whole blood serotonin concentration in autistics is remarkable. Hyperserotonemia has been found in approximately 30% of the autistics. The serotonin concentration in the blood is stable, if measured over a year's time in autistic individuals. The serotonin concentration is elevated both when measured absolutely and expressed per blood platelet. A relation with specific behavioral aspects of autistics has not been found. The finding of an elevated serotonin level in the blood in various groups of non-autistic mentally retarded children is of importance. This gives rise to the question of the specificity of the finding.
The cause of hyperserotonemia in autistics is not known. The increase of urinary 5-HIAA excretion in hyperserotonemic autistic subjects seen by us

suggests that increased 5-HT gut production might play a role in the hyperserotonemia observed. Differences between autistics and controls in 5-HT uptake, storage or release ability of blood platelets have not emerged. However, platelet counts were observed to be increased and 5-HT levels (ng/ml) and platelet counts were significantly correlated in unmedicated autistics, so a role for the blood platelet in the hyperserotonemia of autism is certainly worth considering. No indications have been found for decreased degradation of 5-HT by MAO. Research on the tryptophan metabolism in autistics does not permit any conclusion. Accurate studies with precisely characterized children while use is being made of recent analysis techniques, will have to clarify this subject.

A number of aspects needs further investigation. More insight is required into those factors influencing the production of 5-HT and release of 5-HT from the gut into the blood.

Furthermore, uptake and release of 5-HT in platelets of autistics might be better characterized using recently developed, modified assay methods (Arora and Meltzer, 1981).

The relation between hyperserotonemia in autistics and the central serotonergic functioning in the brain is unclear. Parallel measuring of 5-HT and free tryptophan levels in the blood and concentration of the other amino acids, and 5-HIAA and tryptophan levels in the spinal fluid seems useful.

Another possible line of research is on the ontogenetic aspects of whole blood 5-HT. Measurements in baby's and young children may give more insight into the age factor which plays a role in the serotonin concentration of the blood. Also the sex factor in young autistics ought to be investigated further. More research could be done on the relation of the serotonin concentration in the blood of healthy family members, and between the concentrations of autistics and those of their relatives. Genetic aspects may have an influence. Furthermore, it is tempting to speculate about a possible relationship between the recently described auto-antibodies against 5-HT binding proteins present in the blood and the spinal fluid of some autistic children and blood 5-HT values. Further assessment of this finding is warranted.

Generally speaking better characterisation will have to be found of the various behavioral aspects of autistic children. It is more likely that

neurochemical variables, related to the functioning of the central neurotransmitter systems, will correlate with psychic and motor function disturbances, than with the whole of a syndrome.
Neuropsychological and neurophysiological research techniques will be of importance in this prospect. Animal models have to be used to assess specific target behaviors like stereotyped behavior, attachment, etc., to unravel their underlying pathophysiologic mechanisms. At the same time one has to search for the human analogue of behavior variables which seem to be important on the basis of manipulation of neurochemical systems in animal models, like e.g. contact comfort (Panksepp, 1980).
Modern chemical analytical techniques will have to be used, in which pharmacological manipulations both on the behavioral level and on the chemical level are monitored. In the future this will hopefully lead to more insight into the pathophysiology of the intriguing syndrome of infantile autism.

APPENDIX I

WHOLE BLOOD SEROTONIN IN AUTISTIC AND NORMAL SUBJECTS

George M. Anderson,[1]
Fred R. Volkmar,[2]
E. Lawrence Hoder,[3]
Peter McPhedran,[4]
Ruud B. Minderaa,[5]
J. Gerald Young,[6]
Carl R. Hansen,[7]
Donald J. Cohen,[8]

Departments of Laboratory Medicine[1,4] and Pediatrics[3,6,8] and the Child Study Center,[1,2,5-8] Yale University School of Medicine, New Haven, CT, USA.

SUMMARY

Whole blood serotonin (5-HT) and tryptophan (TRP) were measured in 87 normal children and young adults, and in 40 autistic subjects, (DSM-III criteria) having a similar age distribution. Whole blood 5-HT was significantly elevated in the drug-free (N=21) autistic group (205±16 ng/ml) compared to normal subjects (135±54 ng/ml). Serotonin values were Gaussianly distributed in both the normal and autistic groups. When the 95th percentile of the normal group was used to define hyperserotonemia, 38% of the autistic subjects could be termed hyperserotonemic. Autistic subjects on anticonvulsants and neuroleptic had significantly lower 5-HT levels than drug-free autistic subjects.
The elevated group mean for whole blood 5-HT seen in autism cannot be explained by group differences in age, sex, platelet counts or tryptophan levels.

INTRODUCTION

A number of investigators have reported, that group means of blood serotonin (5-HT) concentrations are elevated in autistic subjects compared to normals (Schain and Freedman, 1961; Ritvo et al., 1970; Yuwiler et al., 1971; Campbell et al., 1974; Yuwiler et al., 1975; Goldstein et al., 1976; Hanley et al., 1977; Takahashi et al., 1976; Hoshino et al., 1979a; Hoshino et al., 1984; Coleman et al., 1973; Young et al., 1982). Research on this "hyperserotonemia" of autism, which spans nearly 25 years, is reviewed in Table I.
Although the ten studies listed are consistent in their finding of increased 5-HT levels in autism, there is poor agreement as to the magnitude of the levels observed and the extent of the difference. While differences in the normal and autistic population samples selected for study may account for some of the discrepancies, it was felt that a number of basic aspects concerning the apparent hyperserotonemia of autism required further clarification.

Table I

Author	Year	Technique	Sample Type	Autistic Subjects	Controls	Notes
Schain and Freedman	1961	bioassay	serum	141±78 ng/ml, N=23	65±17 ng/ml, N=4 normals 72±33 ng/ml, N=12 (mildly retarded)	Mean autistic age 10.8.
Ritvo et al	1970	acid fluorescence	whole blood	263±63 ng/ml, N=23	216± ng/ml, N=36	3-8 year old subjects; levels declined with age; no group differences when expressed per platelet.
Yuwiler et al	1971	acid fluorescence	whole blood	272±53 ng/ml, N=7 760±112 ng/10^9 plat., N=7	183±28, N=4 494±42 ng/10^9 plat., N=4	8 A.M. samples; similar group differences seen at 12 noon. No circadian rythm seen.
Campbell et al	1974	acid fluorescence	platelet-rich plasma	280 ng/ml, N=11	170 ng/ml, N=6	mean age autistics 5; mean age normals 6.
Yuwiler et al	1975	acid fluorescence	whole blood	273±30*ng/ml, N=12 911±106*ng/10^9 plat., N=12	205±17*ng/ml, N=12 650±48*ng/10^9 plat., N=12	Platelet 5HT uptake, efflux similar in both groups. Mean autistic and normal ages 5 and 8 years respectively age-matched children.
Goldstein et al	1976	acid fluorescence	whole blood	86±36 ng/ml, N=72	73±23 ng/ml, N=71	
Hanley et al	1976	acid fluorescence	whole blood	135±57 ng/ml, N=27	57±49 ng/ml, N=6 (normals) 97±38 ng/ml, N=23 (mildly retarded)	7-22 year old subjects; no age effect seen; urinary 5HIAA also higher in autistics.
Takahashi et al	1976	ninhydrin fluorescence	platelet pellet	980±357 ng/mg, N=30	807±202 ng/ml, N=30	Expressed as ng/mg platelet protein; Mean age 5 years; no age effect from 2-12 years
Hoshino et al	1979	acid fluorescence	serum	218±27 ng/ml, N=42	175±60 ng/ml, N=20	Mean age autistic 5.7; mean age normals 9.3.
Anderson et al (this study)		HPLC-fluorometric	whole blood	205±73 ng/ml, N=21 776±348 ng/10^9 plat., N=16	136±50 ng/ml, N=87 522±213 ng/10^9 plat., N=67	Mean age autistics 14.5; mean age normals 14.6. drugs reduced 5HT levels.

A major issue involves the influence of age upon the 5-HT levels observed. In the few reports (Ritvo et al., 1970; Hanley et al., 1977; Takahashi et al., 1976; Hoshino et al., 1984) examining the age-related changes, little agreement exists concerning the presence or magnitude of the effect. The present study was designed with large groups so that the age-effect could be examined in both the normal and autistic populations. Important questions also existed concerning the group means observed for platelet counts and blood tryptophan (TRP) levels, and the relationships for these measures to 5-HT levels. The possibility remained that group differences in platelet (storage site of blood 5-HT) counts might contribute to, or cause, the reported differences in 5-HT levels. The measurement of TRP is important as it might indicate whether the metabolism of this amino acid precursor to 5-HT is altered in autism.

Two additional aspects which have been only very briefly examined are a possible sex-difference and the effect of medications on 5-HT levels. The influence of sex is of particular interest because of the increased prevalence of autism in males. The possibility of a drug-effect is of special concern due to the extensive use of potent neuroleptic and anticonvulsant medications in autistic subjects.

Questions regarding analytical methodology center on the type of blood sample used and the 5-HT assay employed in the various studies. For several reasons, the studies using serum (Hoshino et al., 1979a), platelet-rich-plasma (PRP) (Campbell et al., 1974), or isolated platelets (Takahashi et al., 1976) are difficult to compare to the majority of studies where whole blood 5-HT was measured (See Table I). The use of serum presents difficulties in estimating recovery of native (platelet-bound) 5-HT and in determining the effective sample volume, while the isolation of a platelet pellet or PRP for 5-HT analysis is complicated by the heterogeneity of the platelet population and the possibility of obtaining varying platelet yields in different subjects. The six studies (Ritvo et al., 1970; Yuwiler et al., 1971; Yuwiler et al., 1975; Goldstein et al., 1976; Hanley et al., 1977; Hoshino et al., 1984) of whole blood 5-HT avoid these problems; however, a discrepancy in the group means reported for whole blood 5-HT levels is evident. Here, serotonin was measured in whole blood, rather than in PRP or a platelet pellet, for the reasons mentioned above. A high performance liquid

chromatographic (HPLC) fluorometric assay for 5-HT (Anderson et al., 1981) was employed instead of the acid fluorescence assay which had been used in nearly all previous studies. While the acid fluorescence method has been carefully checked, it was thought that an HPLC method might be less prone to undetected interferences and would, at least, allow an alternative method to be employed.

METHODS

Subjects

All autistic subjects had deficits in social relatedness and communication sufficient to make a diagnosis of Autism (299.00) according to DSM-III criteria (DSM-III, 1980). Nearly all were enrolled in special programs specifically designed for autistic individuals. Care was taken to exclude aphasic subjects and non-autistic, retarded subjects. Unmedicated subjects had been drug-free for at least six months prior to blood drawing, while medicated individuals were on an constant drug regimen for at least two months. Young normal subjects were recruited from subscribers to the Yale University Health Service. All youngsters (ages 1-20) who were scheduled for routine "well child" visits were eligible for participation. Parents were asked to complete a brief form describing their child's general health and any current illnesses or medications. Normal, young adult subjects (ages 21-32) were recruited from healthy medical students and laboratory staff. Blood was drawn from young normals throughout the day (8:00 AM - 4:00 PM), from adult normals at 12:00 PM - 2:00 PM, from autistic subjects at 10:00 AM - 11:30 AM. Data specifying the age and sex distributions in the populations studied are presented in Table II.

Table II

Age and Sex Distributions in Normal and Autistic Groups

	Sex	Age (years)				
	M/F	Range	Median	Mean ± SD	(SEM)	N
Normals	45/42	2.0-32	14.2	14.6 ± 7.47	(0.80)	87
Autistics						
Total group	29/11	2.1-27.4	17.8	16.8 ± 6.04	(0.96)	40
Drug-free	15/6	2.1-27.4	15.0	14.5 ± 6.64	(1.44)	21
Medicated	14/5	7.5-26.7	18.6	19.3 ± 4.20	(.96)	19

ANALYSIS

Whole blood 5-HT and TRP were analyzed using an HPLC-fluorometric method (Anderson et al., 1981). Blood (approximately 1 ml) was obtained in EDTA-containing Vacutainer tubes, mixed by gentle inversion, and kept at room temperature for no longer than three hours before preparation. A 1-2 ml blood sample was obtained at the same time for automated (Clay-Adams Ultraflow 100) determination of platelet count. Samples (5-HT) were prepared for storage by placing 250 µl of mixed whole blood into a 1.5 ml polypropylene micro-centrifuge tube. Fifty microliters of 1.5 M ascorbic acid, 10 µl of 10 ng/ml 5-hydroxytryptophan (5-HTP), and 50 µl of 3.4 M perchloric acid were added in order. Immediately after the addition of the perchloric acid, the tube was capped and vortex-mixed for 10-15 sec, then placed on ice for 5-15 min. After centrifugation at ~13000 x G for 5-10 min, the supernate was poured into a micro centrifuge tube and stored at -80°C. Thawed samples were briefly centrifuged and 20 µl injected on a .39x30 cm u Bondapak C_{18} reversed-phase HPLC column. A mobile phase of 95% pH 4.25 .01 M sodium acetate - 5% methanol was delivered at a flow-rate of 2.0 ml/min. The compounds were detected using an Aminco Fluoromonitor with a low-pressure mercury lamp (254 nm) excitation source and a 360 nm peak transmittance emission filter. Recoveries of 5-HT, TRP, and 5-HT average 80% and concentrations are

calculated knowing the internal standard concentration (400 ng/ml) and the peak height ratios obtained for 5 ng standards. The quality control sample analyzed over a two-year period, a span of time within which all samples included in this study were run, had a day-to-day coefficient of variation (CV) of 7.2% (mean ± S.D.: 208±15 ng/ml, N=36).

RESULTS

Group Means:

Group means determined for whole blood 5-HT levels, TRP levels, and platelet counts in the normal and unmedicated autistic populations are given in Table III and the individual autistic data plotted in Figures 1a and 1b. Significantly ($p<.001$) higher levels of 5-HT were observed in the unmedicated autistic group when compared to normals. This was true whether the 5-HT concentration was expressed as ng/ml or as ng/10^9 platelet. When the former units were used, the autistic group mean was elevated 51%; within the latter units, a 48% elevation was present. Serotonin and TRP concentrations, and platelet counts, were Gaussianly distributed in the normal, drugfree autistic, and medicated autistic groups.

Table III
Group Means for Normal and Autistic Subjects

	mean ± SEM (N)			
	Serotonin (5-HT)		Platelet Count	Tryptophan
	(ng/ml)	(ng/10^9 platelets)	(per nl)	(ng/ml)
Normal Subjects	136±5.4(87)	522±26(67)	281±9.4(67)	6820±170(87)
Autistic Subjects (unmedicated)	205±15.7(21)	776±87(16)	279±16 (16)	6120±330(21)
p value from t-test	< .001	< .001	<.3	<.01

Figure 1A. Whole blood 5-HT levels (ng/ml) versus age in the drug-free (N = 21) and medicated (N = 19) autistic subjects. Medicated subjects were being treated with anticonvulsants (carbamazepine [4], valproic acid [2], and phenytoin [3]) and neuroleptics (thoridizine [5] and haloperidol [2]) in usual clinical doses

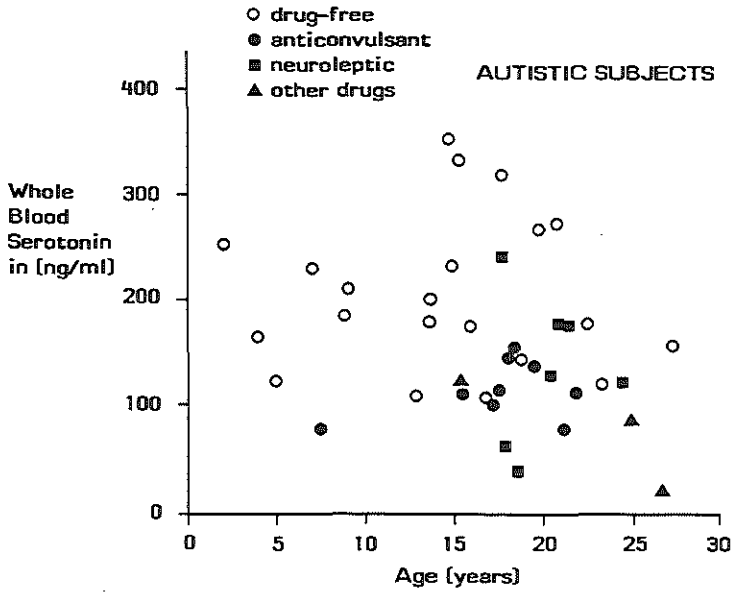
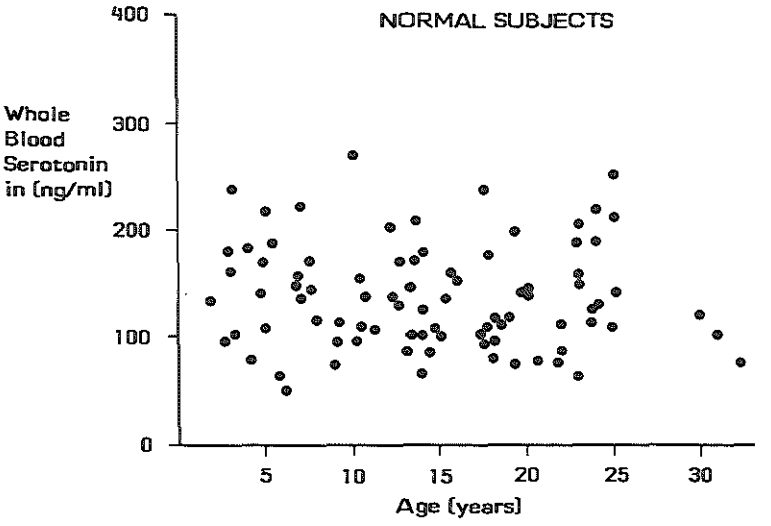


Figure 1B. Whole blood 5-HT levels (ng/ml) versus age in normal subjects (N = 87)



Influence of Age and Platelet Count:

Although platelet counts were similar in autistic and normal subjects and, therefore, presumably not a source of the differences seen between groups, several important relationships between platelet count, age, and 5-HT concentrations were examined in detail (see Table IV). A highly significant negative correlation of platelet count with age ($r=-.59$, $p<.001$) was observed. When the normal population (N = 67) was broken into age groups of 1-6, 7-12, 13-16, and over 17, means ($\pm$SEM [N]) platelet counts (per nl) observed in the respective groups were 346$\pm$21 (16), 302$\pm$19 (13), 292$\pm$23 (9), and 234$\pm$8.2 (30). An analysis of variance (ANOVA) for these groups was significant at the p=.001 level (DF 17, F 11,6).

Table IV

Correlations★ in Normal and Autistic Groups r (p)

Normal Subjects

	5-HT/10^9	TRP/ml	platelet count	age
5-HT/ml	.73(.001)	.17(.028)	.198(.11)	-.05(.66)
5-HT/10^9	----	.11(.40)	-.48(.001)	.315(.009)
TRP/ml	----	----	-.08 (.52)	.08(.47)
platelet count	----	----	----	-.59(.001)

Autistic Subjects (Drug-Free)

	5-HT/10^9	TRP/ml	platelet count	age
5-HT/ml	.73(.001)	.44(.46)	.27(.31)	-.016(.94)
5-HT/10^9	----	.134(.62)	-.425(.19)	.10 (.71)
TRP/ml	----	----	.224(.40)	.09 (.69)
platelet count	----	----	----	-.258(.34)

★ In the normal group, sample size (N) was 67 for correlations involving 5-HT/10^9 or platelet count, and 87 for remaining correlations. Sample sizes were 16 and 21 for the corresponding correlations in the drug-free autistic group.

Age was significantly positively correlated (r=.32, p=.009) with 5-HT level when the concentration was expressed in terms of ng/10^9 platelets. This positive relationship, and the negative correlation (r=-.48, p=-.001) seen between 5-HT expressed as ng/10^9 platelets and the platelet count, apparently result from including the platelet count in the denominator of the concentration units. When the partial correlation of age and 5-HT (ng/10^9 platelets) was calculated, with the platelet count partialed out, no age-effect was seen (r=.04). Similarly, no significant correlation was seen between age and 5-HT concentration expressed as ng/ml (r=.05, p=.66). The constancy of 5-HT levels (ng/ml) over a broad age range was confirmed by dividing the normal group (N = 87) into 1-12, 13-16, and over 17 age groups. Means ± SEM (N) observed in these three groups were 140±8.8 (37), 127±10 (15), and 136±8.6 (35), respectively. We further examined the relationship of age and 5-HT levels within the 1-12 age group. An age-related decline was seen in the 1-12 year-old group; however, because a sex difference was noted, this finding will be discussed in the following section.

Table V

Comparison of Sexes in the Normal Population (Mean ± SEM [N])

	All Ages (1-32 Years Old)			
	Serotonin (5-HT)		Platelet Count	Tryptophan
	(ng/ml)	(ng/10^9 platelets)	(per nl)	(ng/ml)
Males	144 ± 7.0(45)	596 ± 36(32)[a]	274 ± 15(32)	7100 ± 210(45)
Females	127 ± 8.1(42)	453 ± 35(35)[a]	287 ± 12(35)	6520 ± 260(42)
	Young (1-6 Year-Old) Children			
Males	181 ± 15(9)[b]	562 ± 63(8)[c]	340 ± 38(8)	7110 ± 540(9)
Females	113 ± 15(9)[b]	357 ± 54(8)[c]	352 ± 20(8)	7100 ± 950(9)

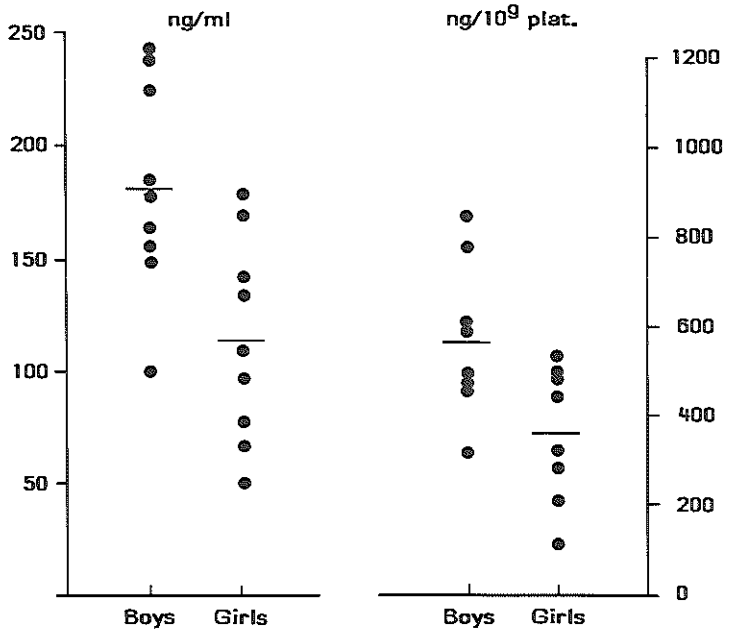
p values from two-tailed t-test: a .006, b .006, c .020

Average (± SD) ages in the groups listed: 1-32 year-old males, 15.2±8.0; 1-32 year-old females, 14.0±7.1; 1-6 year-old males, 4.9±1.8; 1-6 year-old females, 4.3±1.4.

Sex Differences:

In Table V, the normal group has been divided by sex and means presented (5-HT, TRP, and platelet count) for all subjects of alle ages and for the 1-6 year old group. Normal males (all ages) are seen to have significantly ($p=.006$) greater levels of 5-HT compared to normal females when ng/10^9 platelet concentration units were used. Serotonin only tended ($p=.1$) to be higher in males when expressed as ng/ml; the difference in mean platelet counts was also not significant. When the sexes were compared within age subgroups (1-6, 7-12, 13-16, and over 17), significant sex differences were seen only in the youngest group. Scatter diagrams for 5-HT concentrations (ng/ml and ng/10^9 platelets) in the 1-6 year-old group are presented in Figure 2.

Figure 2. Distribution of whole blood 5-HT measures in young (1-6 years old) male and female normal subjects. Additional data concerning tryptophan, platelet count, and age for the two sexes are given in Table V



Young normal males were observed to have significantly (p=.006) elevated levels of 5-HT compared to young females and compared to older males and/or females. However, in the 7-12 year-old male group, the mean 5-HT concentration was similar to adult (>17) levels: 129±11 ng/ml (N=10) vs 138±12 (N=17). Within the group of 1-12 year old males, 5-HT (ng/ml) levels were only weakly correlated (r=.37, p=.12) with age. Serotonin (ng/ml) levels in normal females were similar, and no significant correlations with age were observed, in alle age ranges examined. Finally, no difference in 5-HT levels was seen between males (204±16 ng/ml, N = 16) and females (208±37 ng/ml, N = 5) when the sexes were compared within the unmedicated autistic group (average ages: males 14.8 years, females 13.7 years).

Drug Effect

The effect of medication on blood measures in the autistic population was examined by calculating group means for unmedicated (drug-free) and medicated subjects. The medicated group was further divided into neuroleptic- and anticonvulsant-treated groups. As seen in Table VI, the mean 5-HT levels in the medicated autistic group were significantly lower than in the drug-free autistic group. This was true for the combined medicated group and for the neuroleptic and anticonvulsant-treated subgroups, except in the neuroleptic group when 5-HT concentration was expressed as ng/10^9 platelets. The individual data has been graphed in Figure 1a. It is apparent that most of the medicated autistic subjects are older than the mean age (14.5 years) of the drug-free group. (See also Table II). Although we observed no significant correlation with age in the autistic (or normal) group age-matched controls were used to ensure that the finding of lowered 5-HT levels in medicated autistics was not due to an age effect. When autistic subjects being treated with anticonvulsants were age-matched with unmedicated autistics, mean (± S.D.) ages of 17.4±4.2 (N = 9) and 17.9±4.8 (N = 9) years were obtained, respectively. Average (± SEM) levels of whole blood 5-HT in these age-matched anticonvulsant and unmedicated groups were 117±9.3 ng/ml and 219±9 ng/ml. The difference was highly significant (P=.006) using a one-tailed t-test. When a similar age-matching was performed with neuroleptic-treated and drug-free autistic subjects (N = 7), average ages

in the two groups were 20.2±2.4 and 19.8±2.5, while mean (± SEM) 5-HT levels were 136±26.5 and 201±31.1 ng/ml, respectively. The difference was significant only at the p=.068 level.

Table VI

Group Means ± SEM (N) for Drug-Free and Medicated Autistic Subjects

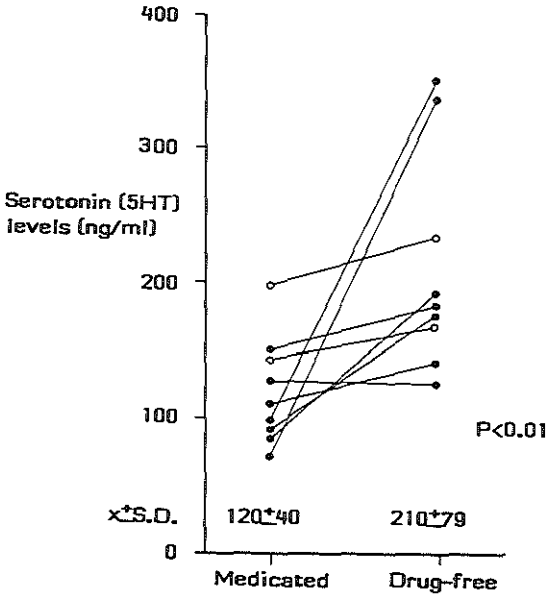
	Serotonin (5-HT)		Platelet Count	Tryptophan
	(ng/ml)	(ng/10^9 platelets)	(per nl)	(ng/ml)
Drug-Free Autistics	205±15.7(21)[abc]	776±87(16)[def]	279±16(16)	6120±330(21)
Medicated Autistics (all drugs)	117±11.9(19)[a]	473±53(19)[d]	253±10(19)	5680±340(19)
Anti-convulsant-treated Autistics	117± 9.3(9)[b]	463±66(9)[e]	262±17(9)	5590±400(9)
Neuroleptic-treated Autistics	136±26.4(7)[c]	571±111(7)[f]	239±14(7)	6110±660(7)

p values from two-tailed t-test: a:<.001; b:<.001; c:<.033;
d:<.004; d:<.018; f: <0.1

In order to further test the neuroleptic effect, an additional group of autistic subjects was examined during and after neuroleptic treatment. The group of nine subjects all had been drug-free or medicated (7 with haloperidol, 2 with thioridazine) for at least three months when the first blood sample was obtained. There was an average time-span of 7.6 months between an individual's samples, and all were drug-free for at least three months before the second sample was obtained. The individual data, on and off drug, is presented in Figure 3. The average (± SEM) drug-free level of 210±26.3 ng/ml was significantly (p=.009) higher than the 120±13.4 ng/ml level seen when the same subjects were treated with

neuroleptics. When the two subjects who showed the largest change were excluded from the comparison, 5-HT means (± SEM) of 173±11.3 and 130±13.1 ng/ml were obtained, and the t-test was still significant($p = .025$).

Figure 3.
Whole blood serotonin (ng/ml) of autistic subjects during treatment with neuroleptics (closed circles haloperidol, open circles thoridizine) and during a drug-free period



DISCUSSION

While the data confirm the general finding of hyperserotonemia in autism, they are at variance with the previous reports in some important ways. Most apparent is the poor agreement with a number of the previous observations in terms of the absolute levels of 5-HT seen in autistics and normals. Many of the whole blood 5-HT group means previously reported for normal populations are substantially lower (Schain and Freedman, 1961; Goldstein et al., 1976; Hanley et al., 1977) or higher (Ritvo et al., 1970; Yuwiler et al., 1975) than those observed in this study. Discrepancies are also apparent between the levels seen here in the autistic group and many of the previous reports. This issue is of importance in attempting to ascertain the magnitude of the 5-HT elevation seen in autism and in defining hyperserotonemia. Prior studies have

indicated that autistic populations have whole blood 5-HT (ng/ml) elevations of 137% (Hanley et al., 1977), 117% (Schain and Freedman, 1961), 49% (Yuwiler et al., 1971), 40% (Hoshino et al., 1984), 33% (Yuwiler et al., 1975), 22% (Ritvo et al., 1970), and 18% (Goldstein et al., 1976), compared to normals. Our finding of a 51% elevation in the autistic group falls in the middle of these estimations and agrees most closely with two studies of Yuwiler and co-workers (Yuwiler et al., 1971; Yuwiler et al., 1975) and Hoshino and colleagues (Hoshino et al., 1984). We have chosen to define hyperserotonemia as a whole blood 5-HT level in the upper 5% (mean plus 1.65 S.D.) of the large, Gaussianly distributed, normal group studied. An individual having a 5-HT value greater than 220 ng/ml would therefore be considered hyperserotonemic. Using this criteria, 8 of 21 (38%) of the unmedicated autistic subjects were hyperserotonemic. This percentage is slightly higher than the 30% incidence found in the one previous study where a similar definition (upper 5%) was employed (Hanley et al., 1977).

The Gaussian distribution of 5-HT values in the autistic group strongly suggests that the elevated group mean is not due to a subgroup of autistic subjects. Rather, it appears that all values in the autistic group have been shifted upward compared to a normal population. Although autistic subjects with higher levels might be more fruitfully studied for the cause(s) of the hyperserotonemia, it is misleading to consider those termed hyperserotonemic as forming one pole, or subgroup, of a bimodal distribution.

The observation of a remarkable similarity of platelet counts in the autistic and normal groups (Table III) is in disagreement with a previous study (Ritvo et al., 1970) which reported significantly higher platelet counts in young autistics compared to normals. This was an important aspect of the study (Ritvo et al., 1970) referred to because the elevation observed for whole blood 5-HT (ng/ml) was suggested to be due, possibly, to the elevated platelet counts seen in autistics. It should be pointed out that a subsequent report from several of the same investigators (Yuwiler er al., 1975) found no difference in platelet counts between groups while continuing to show increased 5-HT in autism.

The downward trend for platelet count with age has been reported before for autistic and normal subjects (Ritvo et al., 1970); however, it has been commonly thought that platelet counts do not vary systematically with age (Wintrobe, 1974).

The observation of an age-effect and platelet count effect on 5-HT levels when expressed as ng/10^9 platelets, and the relatively constant nature of the ng/ml value with respect to age and platelet count, indicate the latter units are probably to be preferred. Even so, both units should be employed when expressing whole blood 5-HT concentrations.

An age-related decline in 5-HT values in children has been previously reported (Ritvo et al., 1970; Hoshino et al., 1984) without a distinction being made between sexes. Here we see a decline with age only in young males. The cause of the high group mean observed in young (1-6 year-old) males and the reason for the maturational decline are unknown. It is also unclear whether these factors are related to those which cause the increase in autism; the possibility that hyperserotonemia reflects a maturational failure needs further study. Our findings, and those of other groups (Ritvo et al., 1970; Hoshino et al., 1984), do suggest that, at least in younger children, age- and sex-matched controls should be used when studying blood 5-HT. Although based on a small group of autistic females (N = 5), the similarity of group means in male and female autistics (see Results) suggests that the hyperserotonemia of autism occurs in both sexes.

The observation of significant reductions in whole blood 5-HT brought about by anticonvulsants and neuroleptics is surprising. In previous studies of drug-effects on blood 5-HT, neither anticonvulsants (Partington et al., 1973) nor neuroleptics (Hoshino et al., 1979b; Hoshino et al., 1982; Stahl et al., 1983; DeLisi et al., 1981) were observed to lower 5-HT in retarded (Partington et al., 1983), autistic (Hoshino et al., 1979b), or schizophrenic (Hoshino et al., 1982; Stahl et al., 1983; DeLisi et al., 1981) subjects. The study examining anticonvulsants (Partington et al., 1973) was mainly concerned with the effect of phenobarbitone, and two drugs common in the autistic group studied here, carbamazepine and valproic acid, were not studied. The reported (Hoshino et al., 1979) lack of an effect of Haldol on autistics' 5-HT

levels might have been due to the acute (2-week) administration and the relatively low doses (.3 - 1.5 mg/day) employed previously. It is fairly clear from our data that neuroleptics do lower whole blood 5-HT levels. Neuroleptics are known to occasionally cause thrombocytopenia (Stahl et al., 1983; Holt et al., 1984), and a trend ($p=.10$) to lower mean platelet counts (239 vs. 279 per nl -- see Table V) was observed in the neuroleptic-treated group. This, coupled with the possible 5-HT uptake-inhibiting effects of neuroleptics (Laubscher and Pletscher, 1979; Arora and Meltzer, 1983; Hussein et al., 1978; Oxenkrug, 1978), might explain the lowering observed. The situation is even less clear with respect to the anticonvulsants because it cannot be determined from the data whether the lower levels observed are a result of the drug treatment or a trait of the subgroup of autistic subjects with seizures.

We have made what we feel to be the most accurate estimation of autistic and normal group mean whole blood 5-HT levels. The normal ranges and means established are in relatively poor agreement with a number of prior reports; however, reasonable agreement with several previous studies (Yuwiler et al., 1971; Yuwiler et al., 1975; Hoshino et al., 1984; Swanson and Cook, 1977) is noted. The approximate 50% elevation seen here in autism, which is intermediate compared to previous estimations, is of unknown etiology.

The possible causes of the hyperserotonemia of autism have been considered in detail by several groups (Hanley et al., 1977; Takahashi et al., 1976; Hoshino et al., 1984; Young et al., 1982). Hypotheses most frequently center around the platelet, 5-HT synthesis, and monoamine oxidase (MAO) activity. These three possibilities have also been raised when attempting to explain the blood 5-HT elevation often seen in schizophrenia (Stahl et al., 1983; DeLisi et al., 1981; Freedman et al., 1981). Theorizing is difficult due to uncertainty regarding what factor(s) control blood levels of 5-HT in normal subjects. In certain situations, alterations in the platelet, in 5-HT synthesis, or in MAO activity all have been observed to affect 5-HT levels. For instance, pharmacologically inhibited (Marshall et al., 1960; Ross et al., 1976) or pathologically decreased (Tu and Partington, 1972) platelet 5-HT uptake results in lower 5-HT levels. On the other hand, the increased 5-HT synthesis which occurs in carcinoid syndrome (Crawford et al., 1967) and

coeliac disease (Pimpakar et al., 1961) or after TRP loading (Yuwiler et al., 1981) increases blood 5-HT, as does the inhibition of monoamine oxidase (Marshall et al., 1960; Weisbach et al., 1961). Unfortunately, these and other related studies do not point to which factor usually predominates in setting levels in normal or autistic subjects. The picture has been clarified somewhat, with respect to autism, since Hanley, Stahl and Freedman's complete survey of the field (Hanley et al., 1977).

It now seems less likely that an alteration in the platelet accounts for the hyperserotonemia. No group differences have been seen in the platelet count, in platelet uptake or efflux of 5-HT (Yuwiler et al., 1975; Boullin et al., 1971; Sankar et al., 1962; Boullin et al., 1982), or in the regulatory site for platelet 5-HT uptake (Anderson et al., 1984). Although several aspects of platelet structure and function, including size, shape, aggregation response, and intracellular compartmentalization of 5-HT remain poorly studied, the platelet studies performed to date are generally consistent in finding no differences between autistic and normal subjects.

The possibility of increased synthesis of 5-HT in autism is probably best approached by urinary measurements of the end-point metabolite, 5-hydroxyindoleacetic acid (5-HIAA). It is well established that most 5-HT is metabolized to, and excreted as, 5-HIAA (Davis et al., 1966; Udenfriend et al., 1959). Previously, it had appeared that autistic subjects excreted greater amounts of 5-HIAA (Hanley et al., 1977). However, we have recently observed no difference in urinary concentration or excretion rate of 5-HIAA between autistic and age-matched normal individuals (Minderaa, Anderson, Volkmar, Akkerhuis, Cohen, submitted, 1985). Data presented here (see Table III) suggest, in a simplistic way, that if 5-HT synthesis is increased in autism it is not a result of increased precursor availability as whole blood TRP levels tended, in fact, to be slightly lower in the autistic group. It should be noted that this finding is at variance with that of Hoshino et al. (1984), who found elevated plasma TRP levels in autistics. Because nearly all 5-HT is synthesized in the enterochromaffin (EC) cell of the intestine (Toh, 1954; Erspamer and Testini, 1959), further investigation might be aimed toward attempting to assess more directly turnover of gut 5-HT in

autistic subjects. It is not clear what measurements, short of those made in gut biopsies, would serve this purpose. Measurement of plasma levels of gut peptides might be a step in this direction. In particular, the determination of plasma levels of Substance P, which is co-localized with 5-HT in certain EC cells (Sandler et al., 1977), should allow a more definite statement to be made regarding the function of the EC cell in autism.

A decrease in the in-vivo activity of MAO is the least well examined of the possibilities mentioned. A number of groups have reported that platelet MAO is not lowered in autism (Roth et al., 1976; Boullin et al., 1976; Takahashi et al., 1977; Lake et al., 1977; Cohen et al., 1977). However, it has been recognized that, because platelet MAO is of the A-type while 5-HT is a better B-type substrate, the relevance of the measurements to the hyperserotonemia of autism is not clear. The in-vivo oxidation of 5-HT is thought to occur principally in the lung and liver (Thomas and Vane, 1967; Junod, 1972), and obvious difficulties have prevented an estimation of the MAO acitivity of these sites in human subjects.

This area might be studied further by determining plasma kinetics of other amines also catabolized by MAO. Studies of plasma levels and half-lives of selected amines and their metabolites should be informative. Most substrates of MAO, unlike 5-HT, would not be taken up by the platelet, allowing their kinetics to be studied without this confounding variable.

The search for the etiology of the 5-HT increase seen in autism should also take into account the fact that 5-HT is elevated in mental retardation (as well as in schizophrenia, coeliac disease, and other disorders). It is possible that the elevation seen in retarded and autistic groups is a reflection of behavioral similarities between the groups. Inverse correlations between 5-HT levels and IQ (Campbell et al., 1974; Hanley et al., 1977), and between 5-HT and activity levels (Campbell et al., 1974; Takahashi et al., 1976), have been observed previously in autistic and retarded populations. In recent studies of the relationships of IQ and specific behavior to blood 5-HT levels in unmedicated autistic individuals we have not observed a correlation of 5-HT with IQ. However, negative correlations have been observed with

several of the behaviors examined, including stereotypies, echolalia, and self-injurious behavior. A detailed report of the behavioral observations in unmedicated and medicated autistic subjects is in preparation (Volkmar, Minderaa, Anderson, Hoder, and Cohen, 1985).

In summary, although the hyperserotonemia of autism and some of the factors which might contribute to it have been well studied since Schain and Freedman's original observation (Schain and Freedman, 1961), the cause(s) remains elusive. It can be pointed out that the basic finding has been characterized better and, at least to some extent, the field of possible causes, narrowed.

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APPENDIX II

WHOLE BLOOD SEROTONIN AND TRYPTOPHAN IN AUTISM: TEMPORAL STABILITY AND THE EFFECTS OF MEDICATION

Ruud B. Minderaa
George M. Anderson
Fred R. Volkmar
Diane Harcherick
Grard W. Akkerhuis
Donald J. Cohen

SUMMARY

Whole blood serotonin (5-HT) levels, tryptophan (TRP) levels and platelet counts were determined in a large group of unmedicated autistic subjects (N=17) and compared to an age- and sex-matched control group (N=20). The effects of neuroleptic and anticonvulsant medication were examined by measuring the species in a group of medicated autistics (N=23). The temporal stability of the measures was examined by making repeated determinations in a smaller group of medicated (N=16) and drugfree (N=10) autistic subjects.

Whole blood 5-HT was significantly increased in the drugfree autistic group compared to the normal controls, however TRP values and platelet counts were similar in unmedicated autistics and normal subjects. No significant differences of 5-HT values were seen between medicated and unmedicated groups of autistic individuals. TRP concentrations were significantly lower in the medicated group compared to unmedicated autistics and a significant negative correlation was found between dose of neuroleptic medication and TRP values.

Highly significant intraclass correlation coefficients and low mean percent differences were found for the repeated measures of 5-HT and the platelet count in the unmedicated group. In contrast, autistic subjects on fixed doses of neuroleptics or anticonvulsants and autistics for whom drug treatment was initiated or increased during the study exhibited significantly greater variability in 5-HT levels and platelet counts. TRP values were highly variable over time in both the medicated and drugfree autistic groups.

INTRODUCTION

Schain and Freedman (1961) were the first to describe increased group values of whole blood serotonin (5-HT) in autism. Since then, this finding has been confirmed by many investigators (Ritvo et al., 1970; Yuwiler et al., 1975; Goldstein et al., 1976; Takahashi et al., 1976; Hanley et al., 1977; Hoshino et al., 1979; Hoshino et al., 1984; Anderson et al., 1985b). The cause and significance of the elevation is unclear.

Assessment of tryptophan levels in blood or tryptophan metabolism (Hoshino et al. 1979; Schain and Freedman, 1961; Shaw et al., 1959; Heeley et al., 1965; Anderson et al., 1985b; Hoshino et al., 1984), assessment of 5-HT serotonin uptake and release and other membrane properties of blood platelets (Sankar, 1970; Boullin et al., 1971; Lucas et al., 1971; Yuwiler et al., 1975; Anderson et al., 1984) and of catabolism of serotonin (Campbell et al., 1976; Cohen et al., 1977) did not result in clear insight into the causes of increased blood 5-HT values in autistics.
Furthermore it is not clear if the "hyperserotonemia" observed is related to possible dysfunctions of the central serotonergic system, although recently blood 5-HT level has been used as a target phenomenon for pharmacological treatment of autistics with fenfluramine (Ritvo et al., 1983; August et al., 1984,1985).

Besides these unanswered questions several other issues require further clarification. An important point considered in several reports is whether platelet count is increased in autistics and whether hyperserotonemia could be due to elevated platelet counts. Ritvo et al. (1970) found significantly increased platelet counts in 24 autistic children compared to an age-matched control group. Although the mean 5-HT values per ml blood were significantly higher in autistics compared to the controls, no differences were reported if 5-HT was expressed per number of platelets. However, the same group (Ritvo et al., 1977) found in a later study that 5-HT values in autistics were increased whether concentration was expressed as ng/platelets or as ng/ml. When Hanley (1977) compared 4 hyperserotonemic autistic subjects with 4 normoserotonemic retardeds, no substantial difference was found in platelet count. Anderson et al. (1985b) recently found similar platelet counts in autistic and normal groups.

A related issue involves the question whether serotonin (ng/ml) values are correlated with platelet count in autistic groups. A correlation between 5-HT ng/ml levels and platelet count might give support to the hypothesis that platelet number, and/or platelet function, might play a role in the regulation of 5-HT levels in blood. Hanley et al. (1977)

found a positive correlation between 5-HT values (ng/ml) and platelet counts in autistic individuals. Anderson et al. (1985b) found no such correlation in a group of unmedicated autistics, nor in the control group.

Furthermore in the literature there is some disagreement as to the effect of different kinds of antiepileptic or neuroleptic medication on mean values of whole blood 5-HT. Recently Anderson et al. (1985b) found significantly decreased serotonin levels in autistic patients on anti-psychotic or anticonvulsant medication, while several previous reports have indicated the drugs have little effect on 5-HT levels (Schain et al., 1961; Hoshino et al., 1979; Stahl et al., 1983; Jackman et al., 1983).

Only a few studies have dealt with the stability of serotonin values over time. In normal controls blood serotonin levels were relatively constant over a period of a week (Ritvo et al., 1970). Yuwiler et al. (1981) described small intraindividual variance in values obtained from six determinations in normal male volunteers. The same conclusion could be drawn from five and twentythree samples respectively of two males over the period of about a year. Wirz-Justice et al. (1976, 1977, 1979) found a pronounced diurnal pattern of 5-HT and a bimodal seasonal rythm in normal volunteers. Yuwiler et al. (1971) did not find a circadian rhythmicity in normal and autistic children. The stability of whole blood serotonin over a longer period of time and the effect of medication on this stability in autistic children has not been previously assessed.

SUBJECTS AND METHODS

The autistic group was comprised of 40 students enrolled in a school for autistic individuals. A diagnosis was made by several child psychiatrists and confirmed for this study by another child psychiatrist (RM) based on psychiatric evaluation, anamnestic information and observations by the school staff before the data were known. Thirty eight individuals received the diagnosis of autism, full syndrome present, according to DSM-III criteria (299.00). Two received the diagnosis of infantile autism, residual state (299.01). Blood was drawn from 10 AM - 11 AM.

Table 1A
Subjects Characteristics Total Sample

Subjects	N	Sex	Age (years)	
		M/F	Mean ± SD	Range
Normal controls	20	15/5	22.0 ± 7.5	9.2 - 36.1
Autistics unmedicated	17	11/6	19.4 ± 5.3	10.3 - 28.7
Autistics all medicated	23	17/6	19.4 ± 4.5	8.9 - 26.4
phenothiazines	7	6/1	22.0 ± 3.1	18.1 - 26.4
haloperidol	7	4/3	17.6 ± 4.0	10.5 - 22.8
anticonvulsants	9	7/2	18.7 ± 4.3	8.9 - 23.4

Table 1B
Subject Characteristics Temporal Stability Subsample

Subjects	N	Sex	Age (years)	
		M/F	Mean ± SD	Range
Unmedicated group	10	6/4	19.5 ± 6.0	10.3 - 28.7
Medicated group*	10	6/4	19.7 ± 4.9	8.9 - 26.4
Group with changed medication**	6	5/1	20.1 ± 2.8	16.7 - 23.3

* 5 used phenothiazines, 1 used haloperidol and 4 used antivonculsant medication (carbamazepine and valproic acid)

** 2 started haloperidol medication, 1 increased dose of haloperidol (2 → 6 mg per day), 1 increased dose of thioridazine (100 → 400 mg per day), 2 started with valproic acid, while medicated with thioridazine

Whole blood 5-HT and TRP were analysed using a HPLC-fluorometric method as described elsewhere (Anderson et al., 1981).

Seventeen of the autistic subjects were unmedicated for at least 6 months before the blood drawing. Seven of the 23 unmedicated autistics used phenothiazines, seven used haldol and nine used anticonvulsant medication. These medicated subjects used their medication in a constant manner for more than 3 months before the blood was drawn. A control group was comprised of 20 high school students, teachers and hospital employees, comparable in sex and age. All reported being in good physical health and using no medication. The group sex and age data for the different groups are given in Table IA.

In a subgroup (N=26) of the autistic subjects two blood samples were obtained approximately one year apart in order to test long term stability. These subjects were divided into further subgroups according to their medication history. Ten were unmedicated for at least 6 months before the first blood drawing and remain unmedicated throughout the study period. Another ten were medicated with a fixed dose for the period extending from at least 3 months before the first blood drawing through the time of the second drawing. A final subgroup was comprised of six autistic subjects who started or increased the dose of the medication between the first and the second blood drawing. Group, sex and age data for the subjects of the temporal stability study are given in Table IB. The mean time span ($\pm$ S.D.) between the two blood drawings for the unmedicated group, the medicated (fixed dose) group and the group with changed medication was 12.7 ($\pm$ 0.7) months, 11.4 ($\pm$ 4.9) months and 12.3 ($\pm$ 1.6) months respectively. Blood was drawn from all subjects at 10 AM - 11 AM.

RESULTS

Group means and correlations of blood 5-HT, TRP and platelet count

Group means of 5-HT ng/ml, 5-HT ng/10^9 platelets, platelet count and TRP are given in Table II. A one tailed t-test comparing unmedicated autistics with normal controls showed significantly higher values of 5-HT in the unmedicated autistic individuals if expressed as ng/ml ($p = .01$) as

well as expressed as ng/10^9 platelets ($p = .02$). When the control group is used to describe a normal range for 5-HT ng/ml an individual having a 5-HT value of greater than 174 ng/ml would be in the upper 5% (mean + 1.65 SD) and could be termed hyperserotonemic. By this criteria 29% of the unmedicated autistics (5 out of 17) and 30% of the total group of medicated autistics (1 out of 9 using anticonvulsive medication, 2 out of 7 using phenothiazines and 4 out of 7 using haloperidol) could be judged hyperserotonemic.

No significant differences were found for whole blood tryptophan levels or platelet count between unmedicated autistics and normal controls. However, hyperserotonemic unmedicated autistics showed significantly higher platelet counts (308±86.1 per nl) using a one-tailed t-test compared to normoserotonemic autistics (249±53.7 per nl, $p = .05$).

Table II

Whole blood serotonin and tryptophan in autistic and normal subjects (Mean ± SD)

Subjects	N	Serotonin (5-HT)		Platelet count	Tryptophan (TRP)
		ng/ml	ng/10^9 platelets	per nl	µg/ml
Normal controls	20	116 ± 35.0[a]	465 ± 162[b]	258 ± 47.7	5.73 ± 1.18
Autistics unmedicated	17	163 ± 81.7[ag]	620 ± 303[b]	267 ± 67.8[eg]	5.38 ± 1.13[cdf]
Autistics all medicated	23	146 ± 53.2	569 ± 192	265 ± 72.7	4.71 ± 1.14[d]
phenothiazines	7	128 ± 45.8[g]	561 ± 223	234 ± 42.8[g]	5.21 ± 1.25
haloperidol	7	176 ± 75.5	550 ± 197	324 ± 73.5[e]	4.52 ± 1.19[f]
anticonvulsants	9	136 ± 26.9	591 ± 163	242 ± 68.0	4.48 ± 1.02[c]

One tailed t-test unmedicated autistics versus normal controls:

a: $p = .01$ b: $p = .02$

One tailed t-test medicated groups versus unmedicated autistics:

c: $p = .03$ d: $p = .04$ e: $p = .05$ f: $p = .07$ g: $p = .09$

Table III

Pearson product-moment correlation coefficients in normal and unmedicated autistic groups

Normal controls (N = 20)	5-HT ng/10^9 platelets	platelet count	TRP	Age
5-HT ng/ml	0.86**	0.07	0.07	0.12
5-HT ng/10^9 platelets		-0.45*	-0.18	0.13
platelet count			0.48*	0.07
TRP				-0.19

Unmedicated autistics (N = 17)	5-HT ng/10^9 platelets	platelet count	TRP	Age
5-HT ng/ml	0.75**	0.49*	-0.33	-0.30
5-HT ng/10^9 platelets		-0.14	-0.32	-0.17
platelet count			0.10	-0.20
TRP				-0.21

All medicated autistics (N = 23)	5-HT ng/10^9 platelets	platelet count	TRP	Age
5-HT ng/ml	0.61**	0.45*	0.14	0.29
5-HT ng/10^9 platelets		-0.40	0.10	0.20
platelet count			-0.03	-0.52*
TRP				0.17

* $p < 0.05$

** $p < 0.005$

In Table III the Pearson Product-Moment correlation coefficients are shown for the values of 5-HT ng/ml, 5-HT ng/10^9 platelets, platelet count, TRP and age in the normal controls, the unmedicated autistics and the total medicated group. A highly significant correlation was found between 5-HT ng/ml and 5-HT ng/10^9 platelets in all groups. In the group of normal controls no correlation was found between 5-HT ng/ml and platelet count. Furthermore a significant negative correlation was found between 5-HT ng/10^9 platelets and platelet count ($r = -0.45$ $p = 0.04$). On the contrary, in the unmedicated autistics 5-HT ng/ml values were significantly correlated with platelet count ($r = 0.49$ $p = .04$). In this group no correlation was found between 5-HT ng/10^9 platelets and platelet

count. In the total medicated group a significant correlation has been observed between 5-HT ng/ml and platelet count ($r = 0.45$ $p = 0.03$). A trend for a negative correlation between 5-HT ng/10^9 platelets and platelet count did not reach the level of significance ($r = -0.40$ $p = 0.06$).

In the control group TRP was significantly correlated with platelet count, ($r=0.48$ $p=0.04$).

No correlation has been observed between age and any of the measures except for a negative correlation in the total medicated group between platelet count and age ($r = -0.52$ $p = 0.01$). A t-test did not show significant differences between males and females for any of the measures in the control group or the group of unmedicated autistics.

Effect of medication on group means

Group means for 5-HT levels, TRP levels and platelet counts in neuroleptic-treated, anticonvulsant treated and unmedicated autistics are presented in Table II. A one tailed t-test comparing medicated groups with unmedicated autistic individuals did not show significant differences for 5-HT values. Only a nonsignificant trend was seen for lower 5-HT values if expressed as ng/ml for the autistics on phenothiazines ($p=.09$).

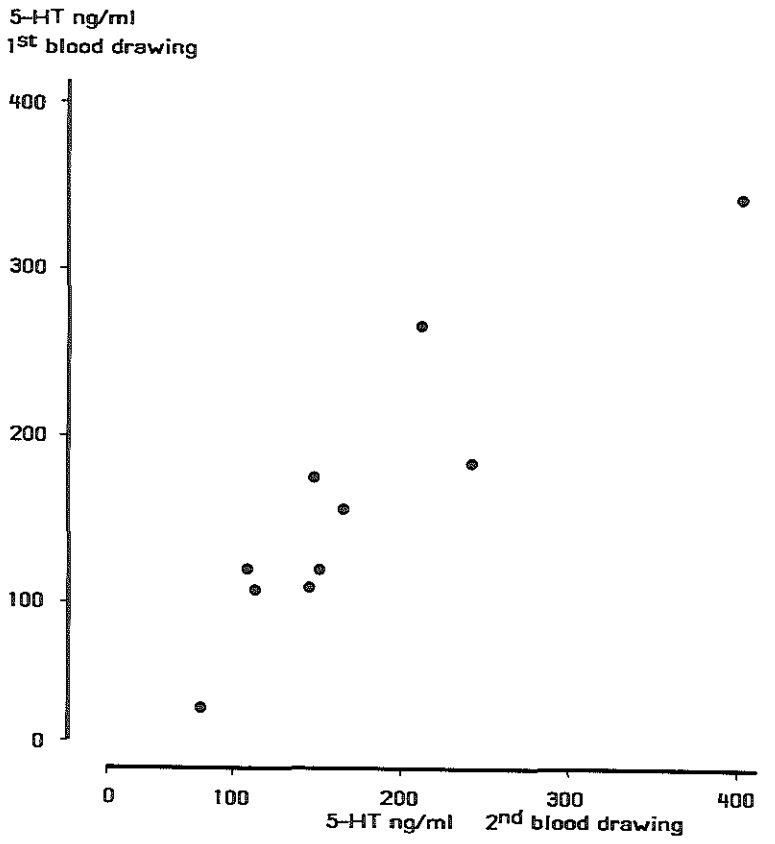
TRP concentrations were significantly lower in the total medicated group compared to the unmedicated autistics ($p=0.04$). In the medicated subgroups this medication effect was greatest in the group on anticonvulsants ($p=0.03$) and the group on haloperidol ($p=0.07$). To assess further this medication effect on TRP levels, we recalculated the neuroleptic dose for the groups on haloperidol and phenothiazine medication in equivalents of 100 mg chlorpromazine. For the total group on neuroleptic medication ($N=14$) a significant negative correlation was found between dose of medication and TRP values ($r=-0.57$ $p=0.003$).

In the total medicated group the platelet counts were similar to those of the unmedicated autistics, however the platelet counts of the medicated hyperserotonemic autistics (314 ± 73.7 per nl) were significantly higher ($t=2.38$ $p=.01$) compared to the medicated autistics with normal 5-HT levels (243 ± 62.7 per nl). The platelet count of the autistics on haloperidol was significantly higher ($p=.05$) compared to the unmedicated

autistics while a trend for lower values was seen in the group on phenothiazines (p=.09).

In the medicated group a significant correlation was found between 5-HT ng/ml and platelet count. Furthermore, an almost significant negative correlation was observed between 5-HT ng/10^9 platelets and platelet count ($r = -0.40$ $p = 0.06$). However, platelet count was significantly correlated with age ($r = -0.52$ $p = 0.01$). When the partial correlations of 5-HT (ng/ml and ng/10^9 platelets) and platelet count was calculated with age partialled out, no significant correlations were seen ($r = 0.37$ $p = 0.09$ and $r = -0.35$ $p = 0.11$ respectively).

Figure 1. Whole blood 5-HT values (ng/ml) first blood drawing versus second blood drawing in unmedicated autistics (r=.92 p<.01)



Long term stability

The mean values of 5-HT (ng/ml, and ng/10^9 platelets), the platelet count, and TRP µg/ml obtained 12 months apart are given in Table IVA. A within-group paired t-test showed no significant differences between the values of the first and the second blood drawing in the drugfree group of autistic individuals for any of the measures. In the medicated group values of 5-HT (ng/10^9 platelets) of the second blood drawing were significantly increased compared to the first drawing (p=.05). With respect to 5-HT values expressed as ng/ml this difference was close to the level of significance (p=.06). In the group with initiated or increased medication TRP values of the second blood drawing were significantly lower compared to the first blood drawing (p=0.01).

Table IVA

Serotonin and related measures between initial sampling and 12 months follow-up

		Drugfree group (N = 10) Mean ± SD	Medicated group, fixed dose (N = 10) Mean ± SD	Group with changed medication (N = 6) Mean ± SD
5-HT ng/ml	initial	167 ± 81.8	104 ± 36.9[a]	201 ± 86.3
	follow-up	177 ± 91.9	136 ± 30.8[a]	130 ± 50.2
5-HT ng/10^9 pl.	initial	542 ± 170	431 ± 178[b]	783 ± 337
	follow-up	581 ± 218	562 ± 188[b]	533 ± 242
Platelet count	initial	281 ± 65.9	248 ± 42.1	258 ± 74.1
	follow-up	289 ± 68.1	255 ± 61.9	261 ± 68.8
TRP µg/ml	initial	6.36 ± 1.55	5.61 ± 1.36	6.72 ± 1.01[c]
	follow-up	5.67 ± 1.24	5.02 ± 0.84	3.95 ± 1.56[c]

p values from paired t-test: a: .06
b: .05
c: .01

Table IVB

Intraclass correlations (R) and mean percentage (Δ%) of change for serotonin and related measures between initial sampling and 12 months follow-up

	Drugfree group R(Δ%)	Medicated group (fixed dose) R(Δ%)	Medicated group (changed medication) R(Δ%)
5-HT ng/ml	.92* (15%)	-.05 (33%)	-.51 (54%)
5-HT ng/10^9 platelets	.86* (14%)	.35 (42%)	-.61 (68%)
Platelet count	.93* (7%)	.43 (20%)	.07 (19%)
TRP	.04 (28%)	-.09 (25%)	-.43 (51%)

*$p < .01$

A one-tailed t-test did show significantly lower 5-HT values (ng/ml) in the medicated group (fixed dose) compared to the unmedicated group for the first blood drawing ($t = 2.22$ $p = 0.02$). For the second blood drawing only a trend was found for lower 5-HT values (ng/ml) in the medicated group compared to the unmedicated autistics ($t = 1.34$ $p = 0.10$). For this reason and because of the observed tendency for increased 5-HT levels (ng/ml) in the second blood drawing compared to the first blood drawing in the medicated group (fixed dose) we determined if a group-time effect was present using a MANOVA procedure. No significant effect was found ($F = 1.21$ $p \leqslant 0.12$).

Intraclass correlation coefficients (Bartko, 1976) and the mean percentages of change between the variables of the first and the second blood drawing of the three experimental groups are shown in Table IVB. Highly significant intraclass correlations were found between the values of the first and the second blood drawing for 5-HT ng/ml ($r=.92$ $p<.01$) (Figure 1), 5-HT ng/10^9 platelets ($r=.86$ $p<.01$), and the platelet count ($r=.93$ $p<.01$) in the unmedicated group. In the unmedicated group there

was no significant correlation between the values of the first and the second blood drawing for TRP. No significant correlation for any of the measures were found in the medicated group, or in the group with changed medication.

DISCUSSION

Group differences and correlation in blood 5-HT, TRP and platelet count

As expected we confirmed the finding of elevated mean 5-HT values in unmedicated autistic subjects compared to normal controls as reported in many previous studies (Schain and Freedman, 1961; Ritvo et al., 1970; Yuwiler et al., 1971; Campbell et al., 1974; Yuwiler et al., 1975; Goldstein et al., 1976; Hanley et al., 1977; Takahashi et al., 1976; Hoshino et al., 1979; Anderson et al., 1985b; Hoshino et al., 1984). This 5-HT elevation of 41% (ng/ml) and 33% (ng/10^9 platelets) is in agreement with our previous findings (Anderson et al., 1985b) and those of others (Yuwiler et al., 1971; Yuwiler et al., 1975). Other groups have found smaller (Goldstein et al., 1976; Ritvo et al., 1970) or larger (Schain and Freedman, 1961; Hanley et al., 1977) elevations of whole blood 5-HT, compared to normals.

Using a criteria of hyperserotonemia as a whole blood 5-HT level in the upper 5% of the control group, 29 percent of the unmedicated autistic subjects were judged as hyperserotonemic. This percentage is similar to that found in one previous study (Hanley et al., 1977) and slightly lower than our previous findings (Anderson et al., 1985b). However, the small size of the control group might limit the accuracy of the estimation of hyperserotonemia.

Platelet count in the normal controls and the unmedicated autistics are very similar. This finding is in agreement with that of two previous reports (Anderson et al., 1985b; Hanley et al., 1977) while one study reported significantly higher platelet counts in autistic subjects compared to normals (Ritvo et al., 1970). The finding of normal platelet counts and the significantly increased 5-HT values expressed as ng/10^9 platelets strongly suggest that the elevation observed for whole blood 5-HT (ng/ml) is not due to elevated platelet counts.

To approach further the question of a possible role of platelet count or function in hyperserotonemia we looked at the difference between the hyperserotonemic (5-HT level greater than 174 ng/ml) and the normoserotonemic autistics in terms of platelet count and at correlations between 5-HT values and platelet counts. Platelet counts in unmedicated hyperserotonemic autistics were significantly higher compared to the normoserotonemic autistics. This is in contrast to the findings of Hanley et al. (1977), who reported that platelet counts were similar in 4 hyperserotonemic autistic individuals compared to 4 retarded subjects with normal 5-HT values. Furthermore, in the group of normal controls we found a lack of correlation between platelet count and 5-HT ng/ml and a significant negative correlation between platelet count and 5-HT ng/10^9 platelets. These two findings are similar to those of a previous study (Anderson et al., 1985b). In contrast, in the unmedicated autistic subjects a significant correlation is seen between 5-HT ng/ml and platelet count and no correlation of platelet count and 5-HT expressed as ng/10^9 platelets is found. These findings, the increased platelet count in hyperserotonemic unmedicated autistics, the significant correlation between 5-HT ng/ml and platelet count, and the lack of correlation between 5-HT ng/10^9 platelets and platelet count, would suggest that in these unmedicated autistics platelet number or function might play a role in the 5-HT elevation observed.

Blood total TRP values were similar in unmedicated autistics and in normal controls. This is in general agreement with the findings of Hoshino et al. (1979, 1984) and Anderson et al. (1985b) where no differences were found between groups of unmedicated autistic subjects and normal controls. On the basis of these studies and the data presented here, it might be concluded that total TRP blood levels in autistics are normal. However, Hoshino et al. (1984) did find free (non protein bound) plasma TRP was significantly higher in autistic children than in normal control subjects. There tended to be a significant correlation between the free plasma TRP level and several clinical rating scales in autistic children, but no correlation between blood 5-HT and free TRP levels.

The question of whether increased precursor availability might contribute to increased 5-HT levels in autism remains open, further study of free TRP levels appears warranted.

Effect of medication

No significant differences of 5-HT values were found between medicated and unmedicated groups of autistic individuals. A tendency toward lower 5-HT values was observed in the total medicated group and in two of the three medicated subgroups. However, the differences were not as marked or as consistent as seen in the previous study of Anderson et al. (1985b). Several other studies have not observed an effect of neuroleptic or anticonvulsant medication on blood 5-HT levels in retarded (Partington et al., 1983), autistic (Schain and Freedman, 1961; Hoshino et al., 1979;) or schizophrenic (Stahl et al., 1983; Jackman et al., 1983) subjects.

A problem in interpreting these data is the fact that the original 5-HT values before the start of the medication are unknown. Medicated subjects might be comprised of a group with 5-HT values different from the mean values of unmedicated autistics.

Increased platelet counts were observed in medicated hyperserotonemic autistic subjects compared to medicated normoserotonemic subjects. This increase was also observed in unmedicated hyperserotonemic autistics and, as discussed, might play a role in the increased blood 5-HT levels observed.

The finding of significantly lower TRP levels in medicated autistic subjects is consistent with those of a previous report (Anderson et al., 1985b), where a nonsignificant trend for lower TRP values in neuroleptic and anticonvulsant medicated autistics have been observed. These findings, together with the observed negative correlation between dose of neuroleptics and TRP values, strongly suggest that neuroleptics and anticonvulsant medication lowers blood total TRP levels in autistics.

Long term stability

The highly significant intraclass correlation coefficients for the two observations of 5-HT (ng/ml and ng/10^9 platelets) and platelet count, and the relatively low mean percentage of change of these values (Table IVB) show that these variables are stable in unmedicated autistics over a time span of more than a year. For these variables one can say that if they are low, they stay low and if they are high, they stay high over a years time. For the 5-HT values this is more true if expressed as ng/ml

compared to ng/10^9 platelets, the intraclass correlation coefficients for 5-HT being higher than for 5-HT ng/10^9 platelets. With respect to stability over time it therefore seems preferable to express 5-HT levels as ng/ml rather than as ng/10^9 platelets.

In the medicated group and the group with changed medication no significant correlations were found between any of the variables for the first and second blood drawing. Furthermore rather high mean percentages of change of 5-HT values (ng/ml and ng/10^9 platelets) and platelet counts were observed in the medicated groups (Table IVB). It appears that anticonvulsant, phenothiazine- and haloperidol medication have a large negative influence on the temporal stability of 5-HT levels and the platelet count. These findings are in agreement with those of Jackman et al. (1983). They found significant shifts, either upwards or downwards, of platelet 5-HT levels in schizophrenic patients determined after 2 to 8 weeks of treatment with haloperidol or chlorpromazine.

One possibility to look at influences of medication on 5-HT ng/ml levels is given by the group that changed medication. Neuroleptic medication caused in the group that started medication or increased the dose, a decrease of 5-HT values in all cases (N = 4). For this subgroup using a paired t-test the values of 5-HT (ng/ml) were almost significantly ($p = 0.06$) and those of 5-HT ng/10^9 platelets significantly lower ($p = 0.02$) for the second drawing compared to the first drawing. Although no firm conclusions can be made on the basis of these data, they might indicate that the initiation of neuroleptic medication or a robust increase in dose of neuroleptics result in a drop of 5-HT values. This is in agreement with the lowering of 5-HT values in neuroleptic medicated groups previously reported (Anderson et al., 1985b).

An increase in dose of depakine did not show a decrease of 5-HT levels. However, the baseline values might well have been suppressed by the previous neuroleptic medication. So for the total group that started medication or increased the dose, the difference in 5-HT levels did not reach the level of significance.

However, as discussed above, most studies of medication effects have not observed significant decreases in whole blood 5-HT levels after neuroleptic treatment. In most cases the acute effects of medication have been studied; in this sense the studygroups are probably more comparable to

the group reported here with initiated or increased medication. It is not clear why the data are not more consistent, however the increased variability in 5-HT values which occurs after administration of neuroleptic (or anticonvulsant) medication has probably hindered studies in this area.

Because of the observed tendency for increased 5-HT (ng/ml) levels for the second blooddrawing compared to the first blooddrawing in the medicated group (fixed dose), we considered the possibility that, after an extended period of medication, compensating mechanisms might be triggered which could counter the lowering effect of medication on the 5-HT values. However, using a MANOVA procedure only a nonsignificant trend for a group-time effect was found.

No significant intraclass correlations were found between the TRP values of the first and second blood drawing for any of the three groups. Furthermore, relatively high mean percentages of change were seen in all groups. This means that TRP values are not very stable over a years period of time in both unmedicated and medicated autistic subjects. A paired t-test showed a significant intra-individual difference ($p=.01$) between the two TRP values for the group with changed medication, with the values of the second blood drawing being lower than the values of the first blood drawing. This gives further support to the conclusion made above that neuroleptic (and anticonvulsant) medication lowers blood 5-HT values in autistic subjects.

This study on the temporal stability and the effects of medication on whole blood 5-HT, TRP and platelet counts has indicated, to some extent, what precautions and directions might be taken in research on the hyperserotonemia of autism.

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APPENDIX III

URINARY 5-HYDROXYINDOLACETIC ACID AND WHOLE BLOOD SEROTONIN AND TRYPTOPHAN IN AUTISTIC AND NORMAL SUBJECTS

Ruud B. Minderaa
George M. Anderson
Fred R. Volkmar
Grard W. Akkerhuis
Donald J. Cohen

SUMMARY

Urinary 5-hydroxyindoleacetic acid (5-HIAA) excretion in two consecutive collection periods (5 PM - 11 PM and 11 PM - 8 AM) and whole blood serotonin (5-HT) and tryptophan (TRP) were measured in groups of unmedicated autistics (N = 16), medicated autistics (N = 20) and normal controls (N = 27). Whole blood 5-HT values were significantly higher in unmedicated autistics compared to normal controls. No significant differences were found in 5-HIAA excretion (µg/mg creatinine, mean ± SD) between unmedicated autistics (4.07 ± 1.52) and normal controls (3.50 ± 1.07), nor beteen medicated (5.35 ± 2.93) and drugfree autistic individuals.

No correlations were found between 5-HT values and urinary 5-HIAA excretion. Urinary 5-HIAA (µg/mg creatinine, mean ± SD) was significantly greater in hyperserotonemic autistic subjects (4.88 ± 0.87) compared to normal controls (3.50 ± 1.07, total collection period, $p = 0.002$). A slight trend ($p = 0.10$) to higher overnight (11 PM - 8 AM) 5-HIAA excretion (µg/mg creatinine, mean ± SD) was observed for unmedicated hyperserotonemic autistic subjects (5.12 ± 1.06) when compared to unmedicated autistics with normal whole blood 5-HT levels (3.86 ± 1.78). In the medicated autistic group significantly higher overnight and total excretion of 5-HIAA was observed in subjects with elevated whole blood levels. The relevance of these findings to the possibility that increased gut production of 5-HT might cause the elevated whole blood 5-HT levels seen in autism is discussed.

INTRODUCTION

In 1961 Schain and Freedman reported that the mean value of whole blood serotonin (5-HT) in a group of 23 autistic children was increased compared to a contrast group of mildly retarded children without autistic features. Since then the finding of elevated serotonin levels in autistics has been replicated by a number of researchers (Ritvo et al., 1970; Yuwiler et al., 1975; Goldstein et al., 1976; Takahashi et al., 1976; Hanley et al., 1977; Hoshino et al., 1979; Hoshino et al., 1984;

Anderson et al., 1985b; Minderaa et al., 1985). Group mean elevations ranging from 18% (Goldstein et al., 1976) to 137% (Hanley et al., 1977) above group means of normal controls have been observed. The cause of this hyperserotonemia in autistic children is unknown.

A number of factors have been investigated as possible cause of the 5-HT elevation including precursor availability, platelet uptake and release of 5-HT, and catabolism by monoamine oxidase (MAO). Hoshino et al. (1977) found normal levels of serum tryptophan, the precursor of serotonin, in 10 autistic children, while Anderson et al. (1985b) found slightly lower tryptophan levels in a group of 40 autistic individuals. By some investigators (Sankar et al., 1962; Sankar, 1970, 1971, 1977) the in vitro uptake of serotonin by blood platelets has been reported to be decreased, by others to be increased (Rotman et al., 1980) or unchanged (Lucas et al., 1971; Boullin et al., 1972; Yuwiler et al., 1975). The same can be said about the in vitro release of serotonin by blood platelets. Initial reports indicated the capacity of autistics' platelets to retain serotonin in vitro was decreased (Boullin et al., 1970; Boullin et al., 1971). However, it now appears this aspect of platelet functioning is normal in autism (Yuwiler et al., 1975; Boullin et al., 1982). Finally, Anderson et al. (1984) found the imipramine binding of blood platelets of autistic children was normal. Thus, as yet, no definite deficiencies in platelet membrane functions in respect to serotonin uptake, storage or release have been detected. Furthermore some investigators assessed the catabolism of serotonin by monoamine oxidase (MAO). The MAO activity in autistics appeared to be normal (Boullin et al., 1976; Campbell et al., 1976; Cohen et al., 1977; Roth et al., 1976) and no correlation between MAO activity and serotonin levels was found (Cohen et al., 1977).

Of all 5-HT in the human body, about 90% is found in the gut, about 8% in the bloodplatelets and 1 or 2% in the brain. The 5-HT found in the blood is produced in the enterochromaffin cells in the mucosa of the gut, released into the plasma, from where it is actively taken up by the blood platelets. As a possible cause of the elevated group means seen in autism, several workers (Hanley et al., 1977; Anderson et al., 1985b) have suggested that the 5-HT production in these enterochromaffin cells in the gut might be increased. 5-Hydroxyindoleacetic acid (5-HIAA) is the

major metabolite of serotonin and urinary 5-HIAA excretion is seen as a measure of total body 5-HT turnover (Udenfriend et al., 1959). With almost all 5-HT being produced in the gut, 5HIAA excretion can be considered a measure of gut 5-HT production, assuming the catabolism of 5-HT is normal. If the production of 5-HT in the intestine of autistic subjects is increased, it seems reasonable to expect that the urinary 5-HIAA would also be increased.

In order to test this hypothesis and to study the relationship between urine 5-HIAA and blood 5-HT, we have measured these species in 36 autistic and 27 normal subjects.

SUBJECTS AND METHODS

The experimental group was comprised of 36 students enrolled in a school for autistic individuals. A diagnosis was made by a child psychiatrist (RM) based on psychiatric evaluation, anamnestic information and observations made by the school staff, before the data were known. Thirtyfour individuals received the diagnosis of infantile autism, full syndrome present, according to DSM-III criteria (DSM-III, 1980, 299.00). Two subjects received the diagnosis of infantile autism, residual state (299.01). Urine was collected for two consecutive collection periods, namely from 5 PM - 11 PM and from 11 PM up to 8 AM. During collection the urine was kept at 3 ^{0}C. Urinary 5-HIAA was analysed using a high performance liquid chromatographic method (HPLC) (Anderson et al., 1985a). Sixteen of the autistics were unmedicated for at least six months before the urine collection. Out a total of 20 medicated autistic subjects, 6 used phenothiazines, 6 used haloperidol and 8 used anticonvulsant medication. These subjects were medicated in a constant manner for more than 3 months before the urine collection. A control group was comprised of 28 high school students, teachers, and hospital employees, comparable in age and sex. All reported being in good physical health and using no medication.

The group sex and age data for the different groups are given in Table I. Blood was drawn between 10 - 11 AM from all but two of the unmedicated autistics and all but one of the medicated autistic individuals.

Table I
Sex and Age Data of Study Subjects

Subjects	N	Sex	Age (years)	
		M/F	Mean ± SD	Range
Normal controls	27	19/8	20.3 ± 6.9	9.1 - 36.1
Autistics unmedicated	16	11/6	20.6 ± 4.6	14.3 - 28.7
Autistics all medicated	20	16/4	19.4 ± 4.1	8.9 - 26.4
phenothiazines	6	6/0	22.5 ± 3.2	18.3 - 26.4
haloperidol	6	4/2	17.8 ± 4.9	10.5 - 23.7
anticonvulsants	8	6/2	18.1 ± 4.2	8.9 - 23.4

Whole blood 5-HT and TRP were analysed using a HPLC-fluorometric method described previously (Anderson et al., 1981). A control group was comprised of 20 subjects of similar age and sex. Both blood and urine was obtained from seventeen of the normal control subjects.
For the autistics, the mean time span between blood drawing and urine collection was 22 days (range 1-252 days). Within this time span there was no change in medication for any of the autistics. For the control group the blood drawing was done the morning following the urine collection procedure.

STATISTICAL ANALYSIS AND RESULTS

Urinary 5-HIAA excretion

Group means of urinary 5-HIAA for the separate and combined collection periods in the control group, the unmedicated autistic group and the medicated autistic groups are given in Table II.
The individual data for the excretion of 5HIAA (µg/hr and µg/mg creatinine) are also presented in Figure 1A and 1B.

Table II

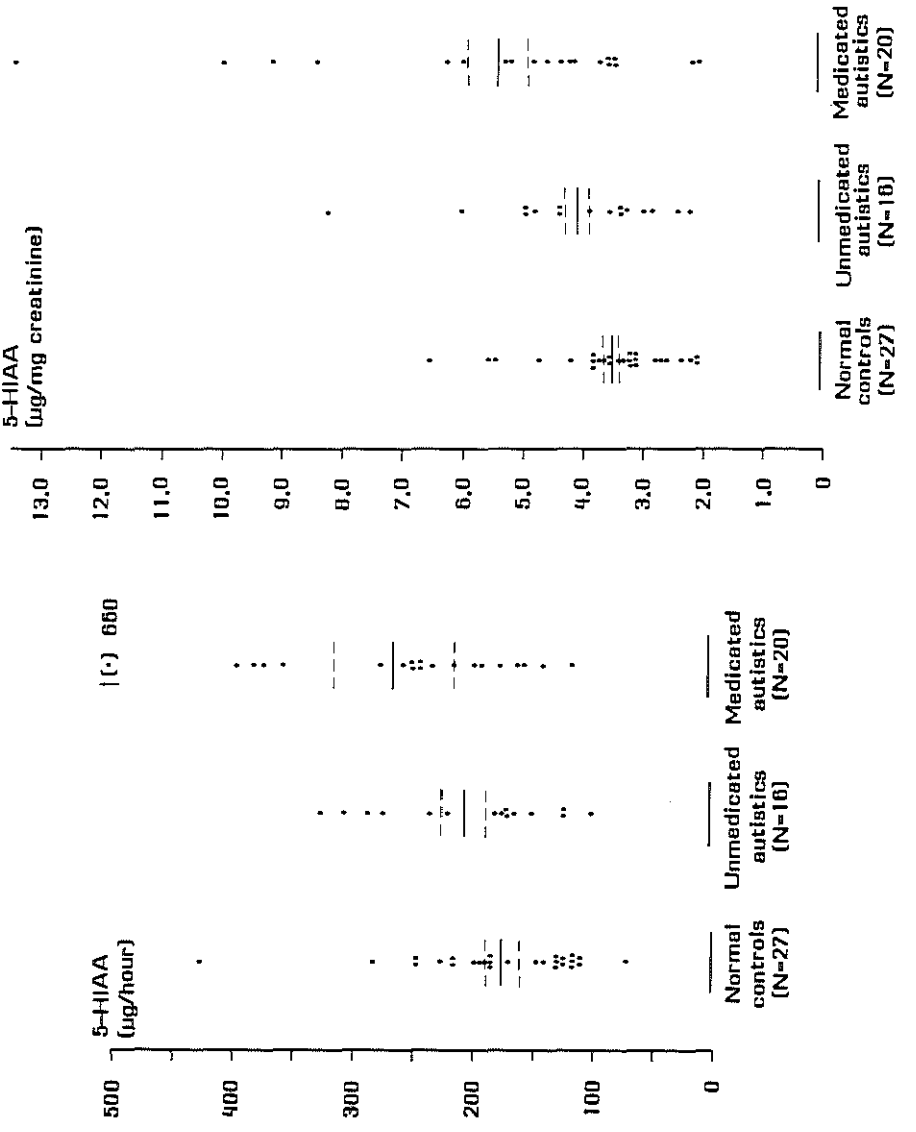
Urinary 5-HIAA excretion during evening (5 PM - 11 PM) and overnight (11 PM - 8 AM) collection periods in normal and autistic subjects

Subjects	N	5 PM - 11 PM	11 PM - 8 AM	5 PM - 8 AM
Normal controls	27			
5-HIAA µg/hr		174 ± 117	175 ± 66.5	174 ± 72.5
5-HIAA µg/mg creatinine		3.53 ± 1.62	3.44 ± 1.06[a]	3.50 ± 1.07[b]
Autistics unmedicated	16			
5-HIAA µg/hr		203 ± 75.3[c]	208 ± 89.9	205 ± 69.8[e]
5-HIAA µg/mg creatinine		4.00 ± 1.58	4.16 ± 1.70[ad]	4.07 ± 1.52[bf]
Autistics all medicated	20			
5-HIAA µg/hr		333 ± 243[c]	225 ± 94.0	263 ± 125[e]
5-HIAA µg/mg creatinine		5.77 ± 4.10	5.05 ± 2.58	5.35 ± 2.93[f]
Phenothiazine-treated	6			
5-HIAA µg/hr		374 ± 224	238 ± 87.7	280 ± 87.0
5-HIAA µg/mg creatinine		4.76 ± 1.98	4.92 ± 3.26	4.82 ± 2.39
Haloperidol-treated	6			
5-HIAA µg/hr		370 ± 369	249 ± 76.3	294 ± 179
5-HIAA µg/mg creatinine		7.91 ± 6.48	6.02 ± 2.25[d]	7.04 ± 3.85
Anticonvulsant-treated	8			
5-HIAA µg/hr		246 ± 120	201 ± 117	222 ± 103
5-HIAA µg/mg creatinine		4.73 ± 2.74	4.19 ± 2.29	4.44 ± 2.10

Unmedicated autistics versus normal controls:
one-tailed t-test: a: $p = 0.07$ b: $p = 0.1$

Medicated groups versus unmedicated autistics:
two-tailed t-test: c: $p = 0.07$ d: $p = 0.08$
e: $p = 0.09$ f: $p = 0.1$

Figure 1. Urinary 5-HIAA excretion (Fig. 1A: µg/hr; Fig. 1B: µg/mg creatinine) during combined collection periods (5 PM - 8 AM) in normal controls (N = 27) and in unmedicated (N = 16) and medicated (N = 20) autistic groups



Using a one-tailed t-test, 5-HIAA excretion rates in drugfree autistics and in normal controls were not significantly different. A nonsignificant trend ($p = .07$) was observed for slightly higher overnight 5-HIAA excretion (µg/mg creatinine) in unmedicated autistics when compared to normal subjects.

Two-tailed t-tests comparing 5-HIAA excretion in medicated autistics with unmedicated autistics did not show significant differences for any of the collection periods. Nonsignificant trends were observed for increased 5-HIAA excretion in several of the medicated subgroups examined (see Table II).

Several methodological issues of urinary 5-HIAA excretion were examined in detail. Highly significant correlations were found between urinary 5-HIAA excretion expressed as µg/hr and urinary creatinine excretion expressed as mg/hr for the evening ($r = 0.61$, $p = 0.001$), the overnight ($r = 0.74$, $p < 0.0005$) and the combined ($r = 0.61$, $P < 0.0005$) collection periods of the control group and for the overnight collection period ($r = 0.50$, $p = 0.04$) of the unmedicated autistics.

No significant correlation was found between urinary 5-HIAA excretion (µg/hr) and body surface area or the collection volume.

No significant differences were observed in urine volumes or creatinine excretion between unmedicated autistics and normal controls and between medicated and unmedicated autistic individuals.

No correlations have been found between urinary 5-HIAA excretion (µg/mg creatinine or µg/hr) and age in the normal controls, the unmedicated group or the medicated group of autistics over the range examined. A t-test did not show any significant differences in urinary 5-HIAA excretion between males and females in the normal controls or the unmedicated autistics.

A paired t-test comparing 5-HIAA values of the first and the second collection period did not show significant differences for any of the groups being expressed either as µg/hr or as µg/mg creatinine. However, in the total group of medicated autistics a tendency ($p = 0.06$) for lower 5-HIAA values (µg/hr) was found for the overnight collection period. So, urinary 5-HIAA excretion seems to be stable over the periods examined in unmedicated autistic subjects and normal controls.

Pearson product-moment correlation coefficients computed for the 5-HIAA

values (µg/mg creatinine) of the first and second urine collection showed a significant correlation in the normal controls ($r = 0.38$, $p = 0.05$), the unmedicated autistics ($r = 0.66$, $p = 0.005$), the medicated autistics ($r = 0.66$, $p = 0.005$) and the total group ($r = 0.59$, $P < 0.0005$). However, if the urinary 5-HIAA excretion was expressed as µg/hr no significant correlations were observed between the two collections in the normal controls or any of the autistic subgroups, although the relationship approaches significance ($r = 0.36$, $p = 0.06$) in the normal control group and was highly significant ($r = .34$, $P = 0.006$) in the total group.

5-HT and TRP values in blood

Group means of whole blood 5-HT, platelet count and TRP in the control group, the unmedicated autistic group and the medicated autistic groups are given in Table III.

Table III

Whole blood serotonin and tryptophan in autistic and normal subjects (Mean ± SD)

Subjects		Serotonin (5-HT)		Platelet count	Tryptophan (TRP)
		ng/ml	ng/10^9 platelets	(per nl)	(µg/ml)
Normal controls	17	113 ± 24.6[a]	443 ± 112[b]	261 ± 50.4	5.85 ± 1.24
Autistics unmedicated	14	163 ± 86.3[a]	630 ± 333[b]	262 ± 58.7[c]	5.35 ± 1.23
Autistics all medicated	19	147 ± 56.0	586 ± 191	261 ± 77.2	4.70 ± 1.15
phenothiazines	6	132 ± 48.7	599 ± 217	222 ± 29.3	5.03 ± 1.27
haloperidol	6	185 ± 85.7	569 ± 227	332 ± 86.7[c]	4.41 ± 1.42
anticonvulsants	8	136 ± 28.7	586 ± 173	245 ± 72.1	4.63 ± 0.99

one tailed t-test: a: $p = .01$ b: $p = .03$

two tailed t-test: c: $p = .03$

A one-tailed t-test was used to assess differences of 5-HT values between the group of unmedicated autistics and the control group. The values of 5-HT ng/ml and 5-HT ng/10^9 platelets for the unmedicated autistics were significantly higher than those for the controls (t value = 2.39, p = 0.01 and t value = 2.02, p = 0.03 respectively).

Using a two-tailed t-test no significant differences were found for the platelet count and TRP values between the unmedicated autistics and the normal controls.

Two-tailed t-tests between medicated groups and unmedicated autistics showed no differences for 5-HT values, platelet counts and TRP values except for significantly higher platelet counts in the group of autistics on haldol compared to the unmedicated group (p = 0.03).

Relationship of urinary 5-HIAA and whole blood 5-HT

A scatter diagram of urinary 5-HIAA excretion (µg/mg creatinine and whole blood 5-HT (ng/ml) levels is presented in Figure 2.

No significant correlations between urinary 5-HIAA and blood 5-HT were found for any of the groups (normal controls: r = -0.38, p = 0.13, unmedicated autistics: r = 0.17, p = 0.57 if urinary 5-HIAA expressed as µg/hr; normal controls: r = -0.25, p = 0.21, unmedicated autistics: r = 0.28, p = 0.29 if urinary 5-HIAA expressed as µg/mg creatinine).

There were also no significant correlations found between blood TRP values and urinary 5-HIAA excretion measures in any of the groups studied.

When the control group (N = 20) is used to describe a normal range of 5-HT ng/ml, an individual having a 5-HT value of greater than 174 ng/ml (mean + 1.65 SD) would be in the upper 5% and could be termed hyperserotonemic. By this criteria 25% of the unmedicated autistics (4 out of 16) and 25% of the total group of medicated autistics (1 out of 8 using anticonvulsive medication, 2 out of 6 using haldol and 2 out of 6 using phenothiazines) could be judged hyperserotonemic. The urinary 5-HIAA values of the hyperserotonemic autistics were compared with those of the autistic subjects with normal whole blood 5-HT ng/ml values and with those of the normal controls.

Figure 2. Urinary 5-HIAA excretion (µg/mg creatinine) during combined collection periods (5 PM - 8 AM) versus whole blood 5-HT (ng/ml) in normal controls (N = 17) and in drugfree (N = 14) and medicated (N = 19) autistic subjects

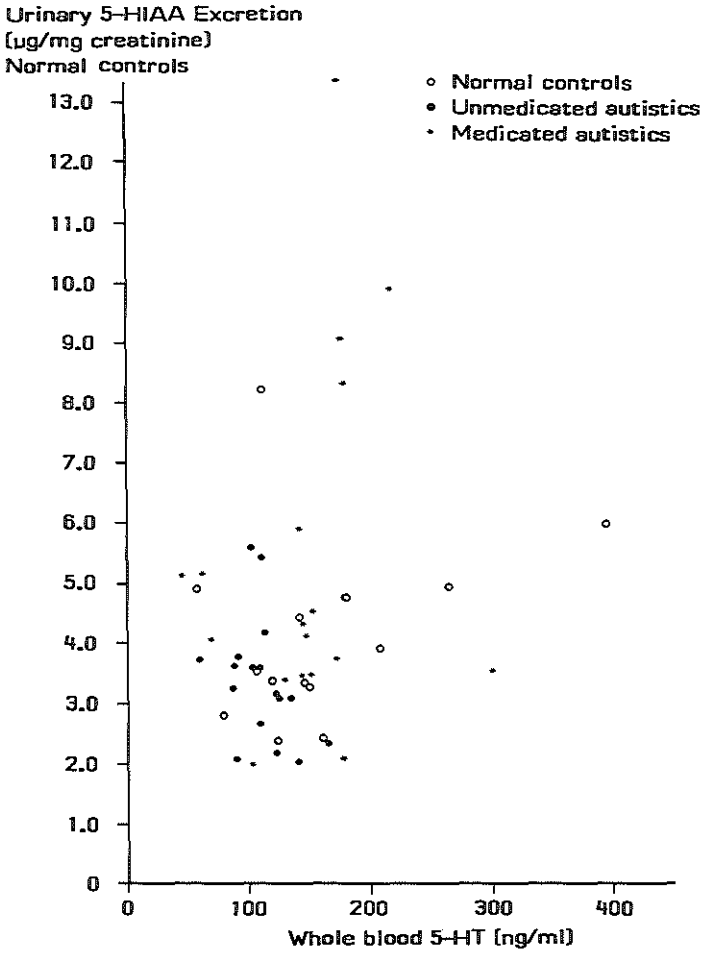


Table IV

Urinary 5-HIAA excretion in hyperserotonemic autistic individuals

A

Unmedicated autistics

	N	5-HIAA µg/hr	5-HIAA µg/mg creatinine
5 PM - 11 PM		Mean ± SD	Mean ± SD
hyper 5-HT	4	208 ± 75	4.57 ± 0.68[a]
normo 5-HT	12	206 ± 82	3.80 ± 1.77
normal controls	27	174 ± 117	3.53 ± 1.62[a]
11 PM - 8 AM			
hyper 5-HT	4	229 ± 70[d]	5.12 ± 1.06[bf]
normo 5-HT	12	206 ± 102	3.86 ± 1.78[b]
normal controls	27	175 ± 66.5[d]	3.44 ± 1.06[f]
5 PM - 8 AM			
hyper 5-HT	4	221 ± 71	4.88 ± 0.87[ce]
normo 5-HT	12	200 ± 74	3.89 ± 1.62[c]
normal controls	27	174 ± 72.5	3.50 ± 1.07[e]

a,b,c : $p = 0.1$

d : $p = 0.08$

e : $p = 0.02$

f : $p = 0.006$

B

Medicated autistics

	N	5-HIAA µg/hr	5-HIAA µg/mg creatinine
5 PM - 11 PM		Mean ± SD	Mean ± SD
hyper 5-HT	5	437 ± 416[e]	8.40 ± 6.57[ad]
normo 5-HT	13	270 ± 103	4.38 ± 1.34[a]
normal controls	27	174 ± 117[e]	3.53 ± 1.62[d]
11 PM - 8 AM			
hyper 5-HT	5	222 ± 95.0[b]	7.48 ± 3.33[gh]
normo 5-HT	13	225 ± 101	3.87 ± 1.23[g]
normal controls	27	175 ± 66.5[b]	3.44 ± 1.06[h]
5 PM - 8 AM			
hyper 5-HT	5	291 ± 197[c]	7.73 ± 4.22[fi]
normo 5-HT	13	249 ± 85.8	4.09 ± 1.04[f]
normal controls	27	174 ± 72.5[c]	3.50 ± 1.07[i]

a : p = 0.1
b : p = 0.09
c,d : p = 0.07
e,f : p = 0.05
g : p = 0.02
h : p = 0.01
i : p = 0.003

The unmedicated hyperserotonemic autistics showed significantly greater 5-HIAA excretion (µg/mg creatinine) compared to the normal controls ($p = 0.02$) (see Table IV). In the unmedicated autistics a nonsignificant trend was found for higher urinary 5-HIAA excretion (µg/mg creatinine) in the hyperserotonemic autistics compared to the normoserotonemic autistics in the overnight collection period ($p = 0.10$) and in the combined collection period ($p = 0.11$). When 5-HIAA excretion was expressed as µg/hr no differences were seen between the hyperserotonemic autistics and the normoserotonemic autistics or the normal controls. In the group of unmedicated hyperserotonemic autistics a high correlation was observed ($r = 0.86$) between urinary 5-HIAA excretion (µg/mg creatinine) and blood 5-HT (ng/ml) that tended to be significant ($p = 0.1$).
In the medicated autistic subjects significantly greater 5-HIAA excretion (µg/mg creatinine) was observed in the hyperserotonemic subgroup during the overnight collection period ($p = 0.02$) and the total collection period ($p = 0.05$) compared to the normoserotonemic autistic subgroup.
The medicated autistic subgroup also excreted significantly more 5-HIAA (µg/mg creatinine) compared to normal controls ($p = 0.003$).

DISCUSSION

The mean urinary 5-HIAA values in the control group are in agreement with those of others (Wiesel et al., 1982; Markianos et al., 1982; Challacombe et al., 1981; Anderson et al., 1985a).
Although the normal control and unmedicated autistic groups were well matched with respect to sex and age, and the expected hyperserotonemia was clearly observed in the group of unmedicated autistics, no significant differences were found in urinary 5-HIAA excretion (expressed as µg/hr of µg/mg creatinine). This is in agreement with the findings of several previous reports. Shaw et al. (1959) did not observe any difference in 5-HIAA excretion between a group of 11 schizophrenic children and a group of 10 psychiatrically disturbed children. Schain and Freedman (1961) did not find increased urinary 5-HIAA excretion in autistic subjects compared to mentally retarded children.

We did observe greater urinary 5-HIAA excretion (µg/mg creatinine) in a subgroup of autistic individuals with increased blood 5-HT values compared to normoserotonemic autistic subjects or normal controls. The difference between hyperserotonemic autistics and normoserotonemic autistics was not significant, however differences between hyperserotonemic autistics and normal controls were hightly significant.

These differences were more marked in medicated autistics and medicated autistics excreted greater amounts of 5-HIAA than unmedicated autistics. These findings are in agreement with those of Hanley et al. (1977) who reported increased urinary 5-HIAA excretion in 4 autistic subjects with increased whole blood serotonin values compared to 4 normoserotonemic mentally retarded children before and after oral tryptophan loading.

Some discussion of the large positive correlations observed between the hourly excretion of 5-HIAA and creatinine is called for. No significant correlations were observed between hourly 5-HIAA excretion and body surface area or urine collection volumes. The correlations between hourly rates of 5-HIAA and creatinine excretion remained significant after partialling out body surface area. These facts suggest the correlation was not simply due to larger subjects excreting greater amounts of both compounds, or to incomplete collections. The puzzling correlations between 5-HIAA and creatinine excretion remain unexplained and warrant further investigation, particularly since similar correlations were seen between other catecholamine metabolites and creatinine (Minderaa, unpublished). In the mean time it would appear advisable to express metabolite excretion using both units (per hour and per mg of creatinine).

No significant correlation was found between the urinary 5-HIAA excretion and whole blood 5-HT values in the normal groups, nor in any of the autistic groups. However, in unmedicated hyperserotonemic subjects a high correlation was found between these two measures that tended to be significant. These correlation data obtained in the autistic groups should be judged with caution because of the time span between the blood drawing and the urine collection. However, whole blood 5-HT values have been found to be very stable measures over a years time in unmedicated autistics (Minderaa et al., 1985) and in normal subjects (Yuwiler et al., 1971).

The lack of correlation between 5-HIAA excretion and whole blood levels of 5-HT in the autistic and normal groups is difficult to explain.
As Anderson et al. (1985c) pointed out, it is not clear which aspect of 5-HT physiology is most important in setting blood 5-HT levels in normal subjects. The rates of gut production, platelet uptake and storage, and catabolism by monoamine oxidase (MAO) might all play a role. In some pathological circumstances like carcinoid syndrome (Crawford et al., 1967) excessively increased 5-HT production does lead to increased blood 5-HT values and increased urinary 5-HIAA excretion. However, it appears that under most circumstances, gut production of 5-HT, to the extent which it can be assessed by measuring urinary 5-HIAA, does not play a predominant role in setting blood 5-HT levels in either normal, or autistic subjects.
Although the bulk of the data presented indicate similar gut production of 5-HT in autistic and normal individuals, the increase of urinary 5-HIAA excretion and the high correlation between urinary 5-HIAA excretion and blood 5-HT seen in hyperserotonemic autistic subjects suggests that, in fact, some relationship between gut production and blood levels might exist in these subjects.
While the increase seen in urinary 5-HIAA excretion is relatively small (ca. 35%) compared to the more than 2x fold elevation in whole blood 5-HT, its potential importance should not be overlooked. It has been estimated that only a small proportion of the total gut 5-HT production is ever taken up by the platelets. Because the platelet, or whole blood, pool of 5-HT is small compared to total gut production, one could speculate that a small increase in gut synthesis might have a relatively large effect on blood levels of 5-HT.

SUMMARY

One can say fairly confidently that the increased whole blood 5-HT values seen in autism are not due to a large increase in gut production of 5-HT. However, the possibility remains that the significant, but relatively small, examined increase in gut production seen in the hyperserotonemic autistic subjects might contribute to elevated whole blood 5-HT levels.

Further assessment of urinary 5-HIAA excretion in larger groups of hyperserotonemic autistic subjects might throw more light on this issue.

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APPENDIX IV

PLATELET IMIPRAMINE BINDING IN AUTISTIC SUBJECTS

George M. Anderson
Ruud B. Minderaa
Peter-Paul G. van Benthem
Fred R. Volkmar
Donald J. Cohen

George M Anderson, Ph.D., is Assistant Professor of Laboratory Medicine; Fred R. Volkmar, M.D., is Assistant Professor of Psychiatry and Pediatrics; and Donald J. Cohen, M.D., is Professor of Pediatrics, Psychiatry and Psychology, and Director, Child Study Center, Yale University, New Haven, CT. Ruud B. Minderaa, M.D., and Peter-Paul van Benthem, are at Erasmus University, Rotterdam, The Netherlands, where Dr. Minderaa is Associate Professor of Child Psychiatry. (Reprints requests to Dr. G.M. Anderson, Yale Child Study Center, P.O. Box 3333, New Haven, CT 06510, USA).

SUMMARY

Previous reports of elevated platelet serotonin (5-HT) concentrations in autistic subjects suggest that platelet 5-HT uptake might be altered in autism. Parameters of ^{3}H-imipramine (IMI) binding were measured in 11 drug-free autistic subjects and 10 normal volunteers. Similar means (± SD) for B_{max} (autistics, 1350 ± 171 fmole/mg protein; normals 1590 ± 206 fmole/mg protein and K_d (autistics, 0.98 ± 0.10 nM; normals 0.94 ± 0.13 nM) were found in the two groups. The normal number (B_{max}) and affinitity (K_d) of the IMI binding site in autistic subjects suggest that the regulation of 5-HT uptake is not different in autism.

INTRODUCTION

Binding sites for the tricyclic antidepressant, imipramine (IMI), have been observed in brain tissue and platelets of humans and rats (Briley et al., 1979; Raisman et al., 1979; Wennogle et al., 1981; Langer et al., 1981). In brain, the site is distributed similarly to serotonin (5-HT) neurons (Langer et al., 1981; Palkovits et al., 1981; Sette et al., 1981), and in both brain and platelets, the site is involved with the regulation of 5-HT uptake (Langer et al., 1980; Paul et al., 1981a; Rehavi et al., 1981; Raisman et al., 1982; Sette et al., 1983). The IMI binding site is apparently distinct from, though in close proximity to, the 5-HT uptake site (Talvenheimo et al., 1979; Ahtee et al., 1981; Barbaccia et al., 1983). The importance of the site and the existence of an endogenous ligand remain to be established. Clinical relevance is suggested by studies showing a positive correlation between mean clinical doses of tricyclic antidepressants and their dissociation constants (K_d) at the IMI site (Wennogle et al., 1981; Paul et al., 1981b).

Platelet IMI binding has been studied extensively in human depression. A number of groups have observed lower numbers (B_{max}) of platelet IMI binding sites in depressed patients compared to normals (Asarch et al., 1980; Briley et al., 1980; Paul et al., 1981b; Raisman et al., 1981, 1982; Wood et al., 1983). A lower B_{max} for IMI binding has also been observed in post-mortem brain (frontal cortex) tissue of suicides

(Stanley et al., 1982). However, several recent studies of IMI binding in platelets have not observed lower group means for B_{max} values in depression compared to normal controls (Berrettini et al., 1982; Mellerup et al., 1982; Whitaker et al., 1983). The discrepancy between studies might be a result of an apparently large seasonal variation in the B_{max} measure (Whitaker et al., 1983).

The consistent finding of elevated platelet 5-HT concentration in autism (Young et al., 1982) suggests an alteration in 5-HT uptake might be present in autistic subjects. Studies of 5-HT uptake in platelets of autistic subjects have not demonstrated a difference in rates or affinities of uptake (Boullin et al., 1982). We have measured IMI binding in drug-free autistic subjects and in normals, in order to ascertain whether this aspect of the platelet 5-HT system is altered in autism.

METHODS

Subjects

The control group consisted of 10 normal, healthy children and young adults with an age range (8-32 years) and distribution (mean $\pm$ SD = 14.0 $\pm$ 2.5) similar to that found in the autistic group (10-24; 17.3 $\pm$ 4.4). Subjects were determined to be in good mental health and to be unmedicated. The autistic population was composed of 11 individuals diagnosed as having infantile autism (299.0) as defined by DSM-III criteria (American Psychiatric Association, 1980). All were enrolled in special programs designed for autistic individuals. Care was taken to exclude aphasic and nonautistic retarded subjects. All autistic subjects had been drug-free for at least 6 months before blood drawing. Blood was obtained from normal and autistic subjects over a 4-month period (December-April); blood drawing was performed between 10 and 11 A.M.

Platelet Preparation

Thirty-five ml of blood were collected in six tubes containing 0.5 ml of 0.15 M disodium ethylenediaminotetraacetate (EDTA). The blood was pooled and spun in polypropylene tubes at 250g for 10 minutes (20°C) using a swinging-bucket rotor. The platelet-rich plasma (PRP) was carefully

measured and transferred to two additional plastic tubes. A 100 µl aliquot was removed from each tube for automated platelet counting (Clay Adams, Ultra-Flo 100) and the PRP was then centrifuged at 10,000g for 15 minutes using a fixed angle rotor. The plasma was decanted off the resulting platelet pellet, and each pellet washed with 3 ml of pH 7.0 (20°C), 0.05 M Tris buffer containing o.11 M sodium chloride and 0.02 M disodium EDTA (solution 1). The pellets were washed and resuspended by filling and emptying a plastic disposable pipette for 2 to 3 minutes. The pipette was finally rinsed with an additional 2 ml of solution 1, the 5 ml platelet resuspensions centrifuged at 10,000g for 15 minutes, and the wash procedured repeated. The twice-washed pellets were then resuspended, lysed, and homogenized by sonicating on ice in 2 ml of 0.005 M Tris containing 0.005 M disodium EDTA (pH 6.3). Pellets were sonicated for two 10-second periods using a Branson Polytron Sonicator (Bridgeport, CT) at a setting of 3.5; care was taken to avoid frothing of the sample. The sonicates were pooled and 100 µl removed for protein assay (Lowry et al., 1951). The membrane protein was pelleted at 39,000g (15 minutes, 4°C), the supernate decanted, and the membrane pellet stored at -80°C.

Binding Assay

The platelet membrane pellet was resuspended in 5 ml of pH 7.5, 0.05 M Tris buffer (containing 0.12 M NaCl, 0.005 M Kcl) by brief sonication (setting 3.5, 5 seconds on ice). Tubes, prepared in duplicate, contained 0.5, 1.0, 2.0, 4.0, 8.0, and 12.0 nM ^{3}H-IMI (55 Ci/nmole, New England Nuclear, Boston MA), with or without 10 µM desimipramine (DMI) in a total incubation volume of 300 µl. Both IMI and DMI were dissolved or diluted in assay buffer. Following the addition of 100 µl of platelet membrane resuspension (approximately 40-80 µg protein), the tubes were incubated for 45 minutes with gentle shaking at 0°C. Samples were then individually filtered through Whatman GF/C glass fiber filters after the addition of 5 ml of ice-cold assay buffer. Filters were washed with three 5 ml portions of cold buffer first added to the sample tube. The filtration procedure was typically performed in 17 minutes for 28 samples. After a 2-hour digestion in 1 ml Protosol, 9 ml of Econofluor (New England Nuclear, Boston MA) was added and the radioactivity determined by liquid scintillation counting. Specific binding was determined by subtracting

counts (cpm) observed in samples containing 10 μM DMI (blocked) from those observed in the corresponding unblocked tubes. Duplicated vials typically differed by less than 10%; values were discarded if agreement was not within 15%. The apparent specific activity (cpm/pmole ^{3}H-IMI) was determined for each experiment by counting 10 μl aliquots of the ^{3}H-IMI solution in similarly prepared vials. Values for receptor number (B_{max}) and affinity (K_d) were calculated from Scatchard analysis of the specific binding data.

RESULTS AND DISCUSSION

Methodological Findings

Saturable, specific binding was observed in the platelet membranes examined, whether isolated from whole blood or obtained from blood bank supplies of platelet concentrate. Nonspecific binding was linearly related to the concentration of added ^{3}H-IMI and was approximately 20% of the total binding at a concentration of 2nM. As has been reported (Mellerup et al., 1982), over 90% of the nonspecific binding observed at all concentrations of ^{3}H-IMI was due to binding to the filter. Attempts to eliminate this binding by silanizing the filters were unsuccessful due to large decreases in filter porosity. To test the effect of incubate protein concentration on the binding parameters (B_{max} and K_d), assays were performed using protein concentrations of 0.13-0.33 mg/ml. No systematic effect on either B_{max} of K_d was observed. The effect of endogenous and exogenous serotonin was also studied. Native serotonin concentrations determined (Anderson et al., 1981) in sonicated membrane suspensions were found to decline after washing of the sonicated membranes (Table I). No difference in K_d was seen for platelets analyzed after 0, 1, or 2 washes; however, the B_{max} for the unwashed membranes was lower. This difference is attributed to a purification of binding site protein (as will be discussed). When 5-HT was added to twice-washed membranes, a decrease in B_{max} and an increase in K_d was apparent (see Table I). Complex inhibition of ^{3}H-IMI binding by serotonin has been reported previously (Sette et al., 1983) and remains to be clarified. We are confident that after the one wash of sonicated membranes used in our

standard procedure the effects of 5-HT are negligible.

Table I

Effect of 5-HT concentration on platelet ^{3}H-IMI binding

5-HT levels and IMI binding after washing of sonicated membranes

No. of washes	Endogenous 5-HT concentration (μM)[1]	B_{max} (fmole/mg)	K_d (nM)
0	2.04	690	1.1
1	0.21	1450	0.69
2	0.03	1300	1.0

Effect of added 5-HT on B_{max} and K_d

Concentration added 5-HT (μM)[2]	B_{max} (fmole/mg)	K_d (nM)
0	1930	1.52
2	1455	4.35
7.5	1600	11.2
23	1210	12.0

1. 5-HT concentration measured in 5 ml total resuspension volume
2. Concentration of 5-HT in incubated assay tube A2μM concentration is equivalent to 6μM 5-HT in the resuspension

Measurements of B_{max} made in platelets isolated from whole blood or from platelet concentrate were consistently elevated compared to previous reports. A mean of approximately 1500 fmole/mg protein was observed compared to typically reported means of 500-600 fmole/mg. The difference is probably due in part to the use of sonication rather than of shearing homogenization for platelet membrane preparation. Sonication apparently results in the preferential isolation of binding site-containing membrane protein relative to other cellular proteins. Approximately one half of the platelet protein is eliminated after the sonication procedure described. When the B_{max} is expressed in terms of the protein present after the initial sonication (total unpurified platelet protein), values

calculated are in better agreement with previous results. This increase in B_{max}, observed in sonicated rat cerebral cortex (Arora et al., 1983), recently has been reported for platelet membraned prepared by freeze-thawing (friedl et al., 1983). Because of the low (<10%) recovery of freeze-thaw or sonicated membrane protein, and the lower number of binding sites observed per platelet in freeze-thaw membranes, it was suggested that ^{3}H-IMI binding is measured best in intact platelets (Friedl et al., 1983). However, we have observed good recovery of platelet protein (~60%), and B_{max} values (sites/platelet) are similar to those obtained for intact platelets. The disadvantages of using intact platelets remain; possible aggregation and/or shape change, possible 5-HT release, and a requirement for immediate assay. We do suggest that B_{max} values be expressed as fmole/mg total platelet protein, fmole/mg isolated protein (if applicable), and sites/platelet. The reporting of protein per platelet (mg/10^9), protein yields, and whole blood and PRP platelet count would further facilitate comparisons between studies.

The reproducibility of the method was assessed by each day running a stored aliquot of platelet membranes prepared from platelet concentrate. The total nonspecific and specific binding was determined for a ^{3}H-IMI concentration of 1.0 nM (in duplicate, blocked and unblocked). A day-to-day coefficient of variation of 20% (n = 17) was observed in this four-tube quality assessment sample. Full saturation curves of the stored aliquots were run every few weeks, and B_{max} and K_d values were calculated. Coefficients of variation for these measures were 20% and 18%, respectively, when determined over a period of 2.5 months (n = 4).

Clinical study

Mean values of B_{max} and K_d in normal control and autistic subjects are shown in Table II, along with related measures. The individual data for B_{max} and K_d in the two groups are shown in Figure 1. The B_{max} values tended to be lower in the autistic group, but the difference was significant ($p = 0.048$) only when B_{max} was expressed as fmole/mg of total initial platelet protein. Smaller and insignificant differences were observed when B_{max} was expressed in the more meaningful units (sites/platelet and fmole/mg isolated protein). As mentioned, the B_{max} means are elevated relative to most previous reports.

Table II

Platelet ^{3}H-IMI binding and related measures in autistic and normal subjects (mean ± SD)

Measures	Autistic subjects (n = 11)	Normal controls (n = 10)
B_{max} (fmole/mg isolated platelet protein)	1350 ± 568	1590 ± 650
B_{max} (fmole/mg total platelet protein)	616 ± 350[1]	973 ± 383
B_{max} (fmole/10^9 platelets	568 ± 275	783 ± 368
B_{max} (sites/platelet)	342 ± 166	471 ± 222
K_d (nM)	0.98 ± 0.33	0.94 ± 0.42
Platelet count (10^9/ml) in platelet-rich plasma (PRP)	0.466 ± 0.170	0.589 ± 0.109
Whole blood platelet count (10^9/ml)	0.268 ± 0.085	0.260 ± 0.053
Total TRP platelet protein (mg/10^9 platelets)	0.99 ± 0.39	0.80 ± 0.16
Total PRP platelet protein (mg)	5.83 ± 2.43	5.34 ± 1.40
Isolated platelet protein (mg)	2.97 ± 1.34	3.37 ± 0.96
Serotonin (ng/ml)	176 ± 97.1	123 ± 43.5
Serotonin (ng/10^9)	681 ± 380	488

1. $p = 0.048$, by two-tailed t-test

While the membrane preparation method probably accounts for much of the elevation, a recently reported seasonal variation may also contribute to the increase (Whitaker et al., 1983). The values for K_d are similar in the two groups and are somewhat lower than most prior determinations. No significant differences were seen between the groups for the whole blood or PRP platelet counts, or for the various protein measures. The expected trend to higher 5-HT levels was observed in the autistic group; however, the difference was not significant ($p = 0.12$).

Table III

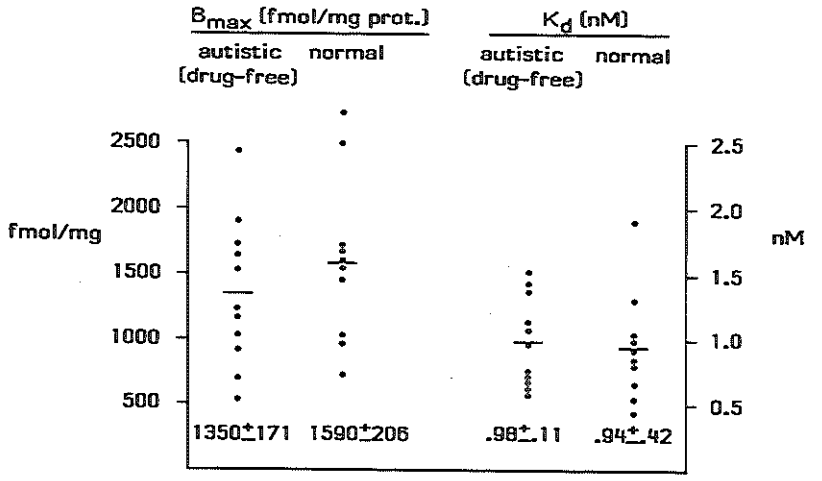
Correlation cross-table for combined population[1]

Variable	Variable[2] 1	2	3	4	5	6	7	8	9	10
1. B_{max}(fmole/mg isolated protein)	-	0.85 (0.001)*	0.78 (0.001)*	0.76 (0.001)*	0.11 (0.63)	0.27 (0.24)	0.80 (0.74)	0.34 (0.13)	0.001 (0.99)	0.004 (0.98)
2. B_{max}(fmole/mg total PRP platelet protein)	-	-	0.89 (0.001)*	0.78 (0.001)*	0.39 (0.88)	0.22 (0.27)	0.09 (0.71)	0.44 (0.06)	0.05 (0.85)	0.40 (0.09)
3. B_{max}(fmole/10^9 platelets	-	-	-	0.78 (0.001)*	0.11 (0.67)	0.32 (0.19)	0.17 (0.50)	0.19 (0.43)	0.25 (0.31)	0.42 (0.07)
4. K_d (nM)	-	-	-	-	0.07 (0.76)	0.28 (0.23)	0.17 (0.48)	0.18 (0.43)	0.30 (0.22)	0.15 (0.90)
5. 5-HT (ng/ml)	-	-	-	-	-	0.79 (0.001)*	0.38 (0.10)	0.17 (0.47)	0.30 (0.22)	0.03 (0.90)
6. 5-HT (ng/10^9 platelets)	-	-	-	-	-	-	0.19 (0.41)	0.18 (0.45)	0.03 (0.90)	0.11 (0.63)
7. Platelet count (whole blood)	-	-	-	-	-	-	-	0.62 (0.003)*	0.64 (0.005)*	0.47 (0.04)*
8. Platelet count (PRP)	-	-	-	-	-	-	-	-	0.07 (0.78)	0.26 (0.25)
9. Total PRP platelet	-	-	-	-	-	-	-	-	-	0.73 (0.001)*
10. Isolated platelet protein	-	-	-	-	-	-	-	-	-	-

1. The r values are listed along with p value in parentheses; significant correlations ($p < 0.05$) are indicated by an asterisk.

2. See variable column on left for variable number

Figure 1. Data presented are mean ± SEM. Autistic subjects, n = 11; normal subjects, n = 10. B_{max} is expressed as fmole/mg of isolated platelet protein. See Table II for additional measures of platelet binding and composition in the two groups



Shown in Table III is the correlation cross-table for the measures of interest in the combined population. The expressions of B_{max} are all highly correlated with one another and with K_d. The significant positive correlation of B_{max} with K_d has been observed previously (Asarch et al., 1980) and might reflect a compensating increase in affinity when fewer binding sites are present. Alternatively, it simply may be an artifact of Scatchard analyses. The B_{max} of K_d values were not correlated with platelet counts, platelet protein, or platelet 5-HT. The lack of a correlation of B_{max} and K_d with platelet 5-HT suggests that the state of the IMI binding site does not normally control blood 5-HT levels. Means (± SD) for B_{max} (1312 ± 375 fmole/mg protein) and K_d (0.80 ± 0.27 nM) in a subgroup (n = 3) of autistic subjects with elevated whole blood 5-HT (241, 267, and 401 ng/ml) were similar to control values. The absence of group differences between normal controls and autistic subjects, the lack of a relationship between IMI binding parameters and whole blood 5-HT

levels, and the normality of IMI binding in the few hyperserotonemic autistic subjects examined all indicate that the site is not altered in autism. Hence, the regulation of platelet 5-HT uptake, in addition to measures of 5-HT efflux and uptake rates and affinities, appears normal in autism. It remains to be determined if some other aspect of platelet functioning is responsible for the elevation of whole blood 5-HT in autism.

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APPENDIX V

SNOUT AND VISUAL ROOTING REFLEXES IN INFANTILE AUTISM

Ruud B. Minderaa,[1,2]
Fred R. Volkmar,[1]
Carl R. Hansen,[1]
Diane F. Harcherick,[1]
Grard W. Akkerhuis,[2]
Donald J. Cohen,[1]

[1] Yale University School of Medicine and the Child Study Center, New Haven, CT, U.S.A.
[2] Department of Child Psychiatry, Sophia Children's Hospital, Erasmus University, Rotterdam, The Netherlands.

ABSTRACT

A group of 42 autistic individuals and a normal control group matched for sex and age were assessed for the presence of a series of primitive reflexes: the snout reflex, the sucking reflex, the tactile rooting reflex, the visual rooting reflex, the grasp reflex, the palmomental reflex, the glabellar tap reflex, and the nuchocephalic reflex. The snout reflex and the visual rooting reflex were significantly more common in the autistic group. The visual rooting reflex was significantly less common in older autistic individuals.

INTRODUCTION

Physical and neurological assessments of autistic children have yielded an array of neurological findings, including hyperactive tendon reflexes, hypotonia, and spasticity (Gubbay, Lobascher, and Kingerlee, 1970). So-called minor physical anomalies have also been reported, such as low-set ears, partial syndactyly, hypertelorism, high arched palate, curving of the fifth finger, and increased distance between the first and second toes (Walker, 1977; Campbell, Geller, Small, Petti, and Ferris, 1978). Individually, none of these symptoms are found in a majority of autistic individuals. We have recently conducted extensive neurologic evaluations of 42 autistic individuals and were surprised to discover a consistently positive snout reflex in most of these patients. We were also struck by the difficulty of assessing the snout reflex as the subjects often made mouthing of pouting movements just before the reflex hammer touched the face. Sometimes a subject bent his/her head toward the reflex hammer and touched it with his/her mouth, nose, or forehead, a phenomenon suggestive of visual rooting. Based on these findings, we reassessed the group for a series of "primitive" reflexes, elicited, organized motor patterns which often are seen as normal phenomena in newborn infants and disappear in the first months of life but which reappear in several pathological conditions and then are interpreted as signs of diffuse cortical brain damage (Tweedy et al., 1982).

SUBJECTS

The parents of 42 of 52 students of a special university-affiliated school for autistic individuals gave written consent for the neurologic evaluations. Forty of these students satisfied DSM-III criteria for the diagnosis of infantile autism, full syndrome present (DSM-III, 1980). In two cases, the diagnosis was atypical pervasive developmental disorder. In five cases, an associated medical condition was present: e.g., meningitis in the second year of life, congenital rubella, kernicterus, Wallenberg's syndrome, and tuberous sclerosis. Fifteen of the students lived at home; 27 were enrolled in a residential program. The control group was composed of 42 normal individuals, matched for sex and age to the autistic population. Data about sex, age, and medication for both groups are given in Table I.

Table I
Number, Sex, Age, and Medication of Autistic Subjects and Normal Controls

	N of Subjects	Sex M/F	Age X	Age SD	Age Range	Medication no	Medication yes
Autistic	42	31/11	19.5	4.5	8.9-28.9	15	27
Control	42	30/12	19.6	6.2	7.4-29.9	42	0

METHODS

As a part of a thorough neurological assessment, each subject was systematically assessed for the presence of a range of so-called "primitive" reflexes. These reflexes included the following: the snout (elicited by tapping with a reflex hammer and by firm pressure), sucking, tactile rooting, visual rooting, grasp, palmomental, glabellar, and nuchocephalic reflexes.

Details of the elicitation of these reflexes are presented in the

Appendix.
All reflexes in both groups were elicited and rated by the same examiner (RBM).

RESULTS

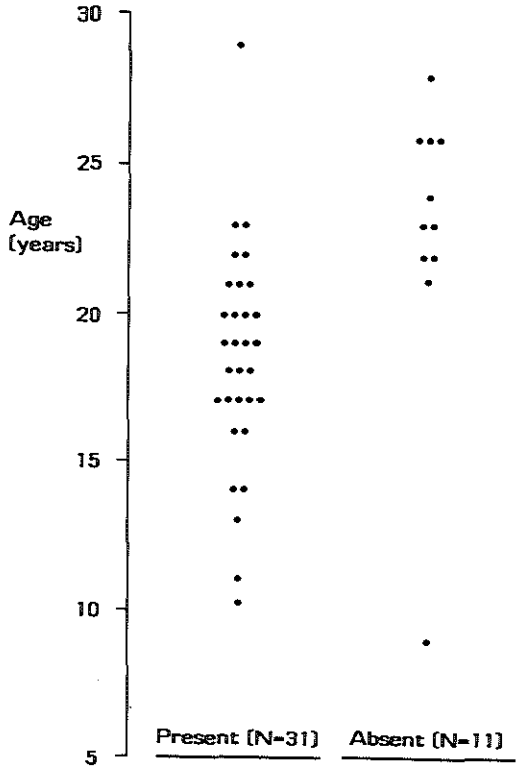
Comparative numbers and percentages of the reflexes elicited in the autistic subjects and the normal controls are given in Table II.

Table II
Number and Percentage of "Primitive" Reflexes in Autistic Subjects and Normal Controls

	Autistics (N=42)		Controls (N=42)		
	N	percentage	N	percentage	
Snout reflex to tapping	32	76.2	5	11.9	($p<0.0001$)
Snout reflex to manual pressure	10	23.8	2	4.8	($p<0.01$)
Overall snout reflex	34	81	6	14.3	($p<0.001$)
Visual rooting reflex to waving hammer	28	66.7	0	0	($p<0.0001$)
Visual rooting reflex to waving hand	31	73.8	0	0	($p<0.0001$)
Overall visual rooting reflex	33	78.6	0	0	($p<0.0001$)
Sucking reflex	1	2.4	0	0	(ns)
Tactile rooting reflex	2	4.8	0	0	(ns)
Palmomental reflex	2	4.8	0	0	(ns)
Grasp reflex	0	0	0	0	(ns)
Nuchocephalic reflex	5	11.9	5	11.9	(ns)

Differences between the autistic and the control group were tested with the X^2 statistic. Significant differences ($p<.01$ or less) were found for the snout reflex to tapping, the snout reflex to manual pressure and the overall snout reflex, as well as the visual rooting reflex to the hammer, the visual rooting reflex to the hand and the overall visual rooting reflex. No significant differences were found for the sucking reflex, the tactile rooting reflex, the palmomental reflex, the grasp reflex or the nuchocephalic reflexes.

Figure 1. Visual rooting reflex to the hand and age (years).



For the autistic group, t-tests were performed by grouping subjects based on the presence or absence of the snout reflex to tapping, the snout reflex to manual pressure and the visual rooting reflexes using chronological age and IQ as dependent measures. Only the age comparison for the visual rooting to hand was significant ($T = 2.99$, $df = 40$, $p<.005$). The age dependency of the visual rooting reflexes is presented in a scattergram in Figure I. Within the autistic group, no significant differences were found between males and females, or between medicated and non-medicated individuals for the snout reflex to tapping or the visual rooting reflex to the hand.

DISCUSSION

The increased incidence of positive snout reflexes and positive visual rooting reflexes in this group of autistic individuals is noteworthy. Some difficulty in eliciting these reflexes should be noted. The snout reflex, if present, is most obvious when the subject is relaxed, complicating attempts to elicite it in autistic individuals. Problems in evaluating this reflex may arise when there is a strong positive rooting reflex. Occasionally the patient begins pouting the lips before the reflex hammer even touches the face, and sometimes there are such prominent kissing-like movements of the lips that it is difficult to tap above the upper lip. In these cases, the attention of the subject must be diverted, or, as was once the case in this study, the subject must be blind-folded. Of the 32 positive reactions to tapping, 12 were associated with pouting or protrusion of the mouth. On five occasions there was a pursing or puckering movement. And on an additional five occasions there was a movement that was considered to be a combination of these two types of reactions. In eight cases, there was a short phasic contraction of the upper lip. Several times this brief reaction could be observed in combination with the longer, more tonic "pout" reaction.

The snout reflex is known to be a developmental phenomenon. Lip protrusion is described as a reflex in the normal human fetus after stimulation of the lip as early as at 14-17 weeks of gestational age (Paulson and Gottlieb, 1968). Based on his observations in a group of

normal infants, Touwen (1976) described the snout reflex, which he called lip-tap reflex, as a phenomenon with a very rapid developmental course that displayed little inter-individual differences. In a group of 51 normal newborn infants, all showed a tonic protrusion of the lip elicited by a brisk tap on the upper or lower lip. In 95% of the infants, this protrusion was rated as evident; in 5%, it was evanescent and equivocal. The number of subjects evidencing protrusion decreased to 10% when the infants were approximately 12 weeks of age. After the age of six months, this reaction was not seen except in two cases is which a phasic reaction persisted until the age of nine months.

The incidence of a positive snout reflex in normal youngsters and adults is not known. This phenomenon can be observed by electromyography in high percentages of normal individuals (Ekbom, Jernelius and Kugelberg, 1952). As a clinical sign and using pressure on the upper lip as a stimulus, Jacobs and Gossman (1980) found that the incidence of the reflex was zero in the third and fourth decade of life, 12% in the fifth decade and up to 30% in the ninth decade of life.

An increased incidence of snout reflexes is described in several clinical populations, e.g., demented geriatric patients (Tweedy, Bouchard, Cote, and Jus, 1974), patients with severe senile or presenile dementia (Paulson and Gottlieb, 1968), and older individuals with neuropsychological impairment (Jenkyn, Walsh, Culver and Reeves, 1977). Tweedy et al. (1982) found a significant relationship between the occurence of snout reflex and impaired performance on cognitive tests in a group of 103 patients referred to a clinic for patients with dementia.

The visual rooting reflex is clinically very impressive. In our study it revealed itself only when the eliciting object attracted the visual attention of the autistic individual, provided he was not otherwise distracted; on such occasion waving of the hammer or hand from a distance of 50 cm was enough to elicit the reflex in 5 of our 42 autistic subjects. Of the 28 positive rooting reflexes to the waving hammer, six subjects exhibited a pouting or pursing of the mouth. In 14 cases, the head moved forward as if to touch the object, and in eight cases, a combination of these two types of reaction occurred. In contrast to the tactile rooting reflex, the visual rooting reflex is not described as a developmental phenomenon in infants.

Tweedy et al. (1982) described the visual rooting reflex as a sucking reflex, elicited by visual stimulation in groups of geriatric patients with different types of pathology. If the reflex was positive, the patients reacted by opening the mouth in order to seize the object, to suck it, or to lick it. Sucking or licking, as part of the reaction, was seldom seen in our group of autistic individuals. Jenkyn et al. (1977) described the visual rooting reflex as any anticipatory opening of the mouth, with or without turning toward the object. In our patients, this orienting toward the object became obvious when they were approached from different directions within their visual field. Paulson and Gottlieb (1968), in their retrospective review of 85 patients with dementia, described "patients occasionally pursing the lips or moving forward to approach the blade", especially in very deteriorated patients when sucking movements also were present. Unfortunately, no systematic assessment of this reflex was done. The age-dependent nature of the visual rooting reflex found in our autistic group suggests that this reflex is a developmental phenomenon.

An intriguing question is whether these phenomena are specifically characteristic for autistically retarded individuals, in contrast to non-autistic retarded individuals or those with less severe forms of atypical development. The two subjects in our group with atypical pervasive developmental disorders did not show any of these "primitive" reflexes. Correlations between these phenomena and aspects of the symptom profiles, e.g., language development, IQ subscores, or behavioral phenomena would be of interest.

The significance of our findings on "primitive" reflexes remains to be clarified. The neurophysiological basis of these reflexes is not known. As developmental symptoms, they are seen as brain stem phenomena that disappear with further development of the dendritic arbor and subcortical myelinization in the first months of life (Ornitz, 1983). The "primitive" reflexes may be related to the frequent clinical observation that autistic children frequently mouth foreign objects, sometimes leading to pica; also, the ease of elicitation of primitive reflexes may be associated with the autistic child's continued use or preference for proximal sensory receptors (taste, touch) to distal receptors (sight, hearing) (Kootz and Cohen, 1981). What may appear as simply mouthing or

use of the mouth for sensory input may be a reflection of the engagement of reflexes, such as visual rooting. As pathological reflexes, when they fail to disappear, or when they reappear, they are taken as signs of a lack of inhibition or disinhibition of brain stem reflexes by "higher" cortical and subcortical centers. The reflexes, themselves, are all integrated in the brain stem and are influenced by cerebral activity, without actually requiring such activity. In this respect, it is remarkable that these "oral" reflexes occurred in autistic individuals suffering from severe dysfunctions involving speech and language. Further assessment of these primitive reflexes in autistic individuals may provide additional insight into the pathophysiology or biological correlates of this disorder.

APPENDIX

Method for elicitation of "primitive" reflexes.

1. The snout reflex was elicited by a light tap or touch with the reflex hammer beneath the nose on the upper lip or by exerting firm pressure with the index finger beneath the nose on the upperlip. Any puckering, pursing, protrusion, or pouting of the lips due to contraction of upper and lower portions of the orbicularis oris was noted, as were contractions of the muscles at the base of the nose.

2. The sucking reflex was elicited by striking the medial area of the upper and lower lip with the index finger. This reflex was judged positive if the lips closed around the finger or if sucking of licking movements were observed.

3. The tactile rooting reflex was elicited by striking the corners of the mouth and by striking the right and left cheek. Any turning of the head and mouth toward the stimulus with or without sucking movements was noted.

4. The visual rooting reflex was elicited by slowly approaching the subject's face with a waving reflex hammer. Any forward movement of the head in the direction of the object, as if to touch it, and protrusion or opening of the mouth was noted.

5. The visual rooting reflex was tested by slowly approaching the face with a waving, clenched hand. The same reactions as for the waving reflex hammer were noted.

6. The grasp reflex was elicited by stroking the palm of the subject's hands with the blade of the reflex hammer. Any flexion of the fingers was noted.

7. The palmomental reflex was examined by firmly striking the thenar eminence of the right and the left hand. This reflex was judged positive with any contraction of the ipsilateral mentalis muscle for five consecutive times.

8. The glabellar tap reflex was tested by tapping the glabellar region rapidly ten times. The examiner's index finger approached the subject from above the forehead outside of the visual field. The normal response was inhibition of reflex closure of the eyelids after two to three taps with the lids remaining open. The reflex was judged positive if continuous reflex blinking of either uper or lower lid or both occurred.

9. The nuchocephalic reflex was elicited by briskly turning the shoulder of the standing subject to the left and to the right. This reflex was present if the head did not actively turn in the direction of the shoulder movement after an interval of approximately half a second, but remained in the original position.

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APPENDIX VI

Brief Report

PRIMITIVE REFLEXES AND WHOLE BLOOD SEROTONIN VALUES IN AUTISTIC SUBJECTS

Ruud B. Minderaa
George M. Anderson
Fred R. Volkmar
Grard W. Akkerhuis
Donald J. Cohen

SUMMARY

The relationship between whole blood serotonin (5-HT) and the newly observed visual rooting reflex and snout reflex was examined in a group of 39 autistic individuals. In the unmedicated group the autistics with a visual rooting reflex showed significantly ($p = 0.05$) higher 5-HT values (184 ± 81 µg/ml, $N = 13$) compared to those without a visual rooting reflex (87.4 ± 32.1 ng/ml, $N = 3$). No difference was found for 5-HT values between autistics with a snout reflex ($n = 12$) and those without a snout reflex ($N = 4$). Further examination of the relationship between these phenomena in a greater number of autistic and mentally retarded subjects seems to be warranted.

INTRODUCTION

Recently the snout reflex and the visual rooting reflex have been found to be present in a majority of autistic individuals (Minderaa et al., 1985a; Minderaa et al., 1985b). These reflexes can be reliably elicited and are not correlated with each other in autistic and retarded individuals (Minderaa et al., 1985b). The visual rooting reflex is correlated with aspects of autistic behavior, especially with peculiarities of motoric behavior and objects use and is less common in older autistic individuals (Minderaa et al., 1985b).

These "primitive" reflexes occur transitory in the course of normal development. The snout reflex is described in normal infants up to the age of about 6 months after which the reflex disappeared (Touwen et al., 1976). The visual rooting reflex is seen in normal children appearing at the age of about 6-8 weeks and disappearing at the age of about 12-18 months (Minderaa, 1985d; Babkin, 1956). The presence of these reflexes in autistic and retarded individuals fits into a global hypothesis of a developmental delay of neuronal brain structures in these subjects.

Increased values of whole blood serotonin are consistantly found in about 30% of autistic individuals. Although in normal controls the relationship between age and whole blood 5-HT is not totally clear, several groups (Ritvo et al., 1971, Anderson et al., 1985a) have reported that whole

blood 5-HT levels in children decrease with age. Increased levels of whole blood 5-HT might represent a developmental delay in the ontogenesis of serotonergic systems. In order to examine the relationship between the newly observed primitive reflexes and whole blood 5-HT we have determined these behavioral and neurochemical measures in a group of 39 autistic individuals.

METHODS AND RESULTS

The experimental group was comprised of 39 students (28 males, 11 females) of a special school for autistic individuals. A diagnosis was made by several child psychiatrists and confirmed for this study by another child psychiatrist (RM). Thirty-eight of the students satisfied DSM-III criteria for the diagnosis of infantile autism, full syndrome present (DSM-III, 1980). In one case the diagnosis was autism, residual state. The mean age of the group was 19.2 $\pm$ 5.1 years (range 8.9-28.7). Sixteen of the students were drugfree. Twenty-three of the autistic subjects used neuroleptic or anticonvulsive medication.

The snout reflex was elicited by a light tap with the reflex hammer beneath the nose on the upperlip. Any puckering, pursing, protrusion or pouting of the lips due to contractions of upper and lower portions of the orbicularis oris was noted (Minderaa et al., 1985a).
The visual rooting reflex was elicited by slowly approachting the face with a waving, clenched hand or with a reflex hammer. Any forward movement of the head in the direction of the object, as if to touch it, and protrusion or opening of the mouth was noted (Minderaa et al., 1985a).
Blood was drawn between 10 AM and 11 AM. Whole blood 5-HT was analysed using a HPLC-fluorometric method as described elsewhere (Anderson et al., 1981, 1985b). Whole blood 5-HT was also measured in a control group comprised of 20 highschool students, teachers and hospital employees, comparable in sex and age (15 males, 5 females; mean age 22.0 years; age range 9.2 - 36.1 years).
In the unmedicated group 12 out of 16 autistic individuals showed a

positive snout reflex and 13 out of 16 showed a positive visual rooting reflex. In the medicated group these figures were 18 out of 23 and 16 out of 23 respectively.

In the unmedicated autistic group (N = 16) group means of whole blood 5-HT values (mean ± S.D.) were 166 (± 83.6) ng/ml and 640 (± 271) ng/10^9 platelets.

In the medicated autistic group (N = 23) mean whole blood 5-HT values were 145 (± 51.0) ng/ml and 569 (± 178) ng/10^9 platelets.

The mean whole blood 5-HT values of the control group were 116 ± 35.0 ng/ml. The 5-HT values of the unmedicated autistics were significantly higher compared to those of the normal controls, as previously reported (Minderaa et al., 1985c). No relationship between blood 5-HT values and age was observed in the group studied.

Table I

Primitive reflexes and mean 5-HT values in unmedicated autistic subjects

Subjects		N	Age Mean ± SD	5-HT ng/ml	5-HT ng/10^9 platelets
Autistics					
Visual rooting reflex					
	present	13	17.6 ± 4.53	184 ± 81.0	699 ± 303
	absent	3	25.6 ± 2.90**	87.4 ± 32.1*	379 ± 95.1*
Snoutreflex					
	present	12	18.6 ± 4.22	168 ± 92.1	662 ± 347
	absent	4	20.8 ± 8.36	161 ± 58.5	572 ± 86.3
Normal controls		20	22.0 ± 7.5	116 ± 35.0	465 ± 162

* $p < 0.05$

** $p < 0.01$

Serotonin values in the groups with and without reflexes were compared using a t-test (Table I). In the unmedicated group the autistics with a visual rooting reflex showed significantly higher 5-HT values being expressed as ng/ml or as ng/10^9 platelets ($p= 0.03$ and $p= 0.05$ respectively). The mean age (years $\pm$ SD) of the unmedicated autistics without a visual rooting reflex (25.6 $\pm$ 2.90) was significantly higher ($p = 0.01$) compared to those with a visual rooting reflex (17.6 $\pm$ 4.53). In the unmedicated autistics no difference was found for 5-HT values between autistics with snout reflex and autistics without snout reflex. In the medicated group no differences were found for any of the 5-HT measures between autistics with or without either of the reflexes.

DISCUSSION

In the unmedicated autistics the subjects who showed a visual rooting reflex did have significantly higher whole blood 5-HT levels than those without such a reflex. This does suggest that these phenomena, the occurance of a visual rooting reflex and hyperserotonemia in autistics might be related. However, this relationship was not seen in medicated subjects. This might have been caused by increased variability of blood 5-HT values in medicated autistics (Minderaa et al., 1985c).

No relationship was found between the occurence of a snout reflex and whole blood 5-HT levels. This is in agreement with the observed dissociation between the occurence of a snout reflex and the visual rooting reflex in autistic and non-autistic retarded individuals (Minderaa et al., 1985b).

Because of the small numbers in this study, no definite conclusion can be drawn. However, it seems interesting to assess further the relationship between these phenomena in a greater number of autistic and mentally retarded subjects.

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SUMMARY

Infantile autism is a syndrome defined in terms of behavior, the typical symptoms of which exist before the age of thirty months. The most significant symptoms are a disturbance in speech- and language development and the non-verbal communicative skills, a disorder in the relationship with people, objects and events and a disturbance in the reaction on sensory stimuli. In general the motor-, socially adaptive and cognitive development are seriously retarded, hampered or regressed and the mutual balance in the development has been disturbed. There is a serious disturbance of the ability of expression, the estimation and appreciation of emotions and in the regulation of anxiety and excitement.

This thesis deals with neurochemical aspects of infantile autism.
In **chapter I** the aims of the study are described. The experimental work is centered around the finding that group mean whole blood serotonin values are increased in autistic populations.
The study addresses to a number of basic questions in respect of characterization of this 5-HT elevation, and the elucidation of its causes.
In **chapter II** the behavioral and clinical aspects of the syndrome of infantile autism are reviewed and genetic and somatic factors that are related to the syndrome are outlined.
In **chapter III** studies on autistic individuals dealing with the catecholamine systems (norepinephrine and dopamine), neuroendocrine systems (cortisol, prolactin, growth hormone, luteinizing hormone, follicle-stimulating hormone and the thyroid hormones, and several hormone-releasing hormones) and neuropeptide systems are reviewed.
It is concluded that catecholamine systems in the brain might be involved in the syndrome. Indications are the therapeutic effects of medications influencing these neurotransmitter systems, increased baseline homovanillic acid values in spinal fluid after probenecid loading, and raised norepinephrine levels in plasma. Possibly a subgroup with high spinal fluid homovanillic acid concentrations is characterized by a more serious degree of stereotypy, repetitive behavior and locomotor activity, and in general a more severely disturbed behavior.
Furthermore, abnormalities have been found in neuroendocrine functioning

and in the peptide excretion. The most robust finding of which is an increased release of thyroid stimulating hormone after stimulation with thyrotropin-releasing hormone.

In **chapter IV** studies dealing with the finding of elevated blood serotonin levels in autistic subjects are extensively reviewed. "Hyperserotonemia" has been found in approximately 30% of the autistics. The serotonin concentration in the blood is stable, if measured over a years time in autistic individuals. The serotonin concentration is elevated both when measured absolutely and expressed per blood platelet. There exists a negative relation between serotonin level and intelligence. The cause of hyperserotonemia in autistics is not known. The increase of urinary 5-HIAA excretion seen in hyperserotonemic autistic subjects suggest that increased 5-HT gut production might play a role in the hyperserotonemia observed. One single interpretation of the deviation in the serotonin uptake, storage or release ability of blood platelets has not emerged. However, a role of the blood platelet function in the explanation of hyperserotonemia is certainly worth considering. So far, no indications have been found for decreased degradation of serotonin by monoamine oxidase.

The appendix is comprised of papers based on original experimental work related to hyperserotonemia in infantile autism.

In **appendix I** whole blood serotonin (5-HT) and tryptophan (TRP) were measured in 87 normal children and young adults, and in 40 autistic subjects, (DSM-III criteria) having a similar age distribution. Whole blood 5-HT was significantly elevated in the drug-free (N=21) autistic group (205$\pm$16 ng/ml) compared to normal subjects (135$\pm$54 ng/ml). Serotonin values were Gaussianly distributed in both the normal and autistic groups. When the 95th percentile of the normal group was used to define hyperserotonemia, 38% of the autistic subjects could be termed hyperserotonemic. Autistic subjects on anticonvulsants and neuroleptics had significantly lower 5-HT levels than drug-free autistic subjects.

The elevated group mean for whole blood 5-HT seen in autism cannot be explained by group differences in age, sex, platelet counts or tryptophan levels.

In **appendix II** whole blood serotonin levels, tryptophan levels and platelet counts were determined in a large group of unmedicated autistic subjects (N=17) and compared to an age- and sex-matched control group (N=20). The effects of neuroleptic and anticonvulsant medication were examined by measuring the species in a group of medicated autistics (N=23). The temporal stability of the measures was examined by making repeated determinations in a smaller group of medicated (N=16) and drugfree (N=10) autistic subjects.

Whole blood 5-HT was significantly increased in the drugfree autistic group compared to the normal controls, however TRP values and platelet counts were similar in unmedicated autistics and normal subjects. No significant differences of 5-HT values were seen between medicated and unmedicated groups of autistic individuals. TRP concentrations were significantly lower in the medicated group compared to unmedicated autistics and a significant negative correlation was found between dose of neuroleptic medication and TRP values.

Highly significant intraclass correlation coefficients and low mean percent differences were found for the repeated measures of 5-HT and the platelet count in the unmedicated group. In contrast, autistic subjects on fixed doses of neuroleptics or anticonvulsants and autistics for whom drug treatment was initiated or increased during the study exhibited significantly greater variability in 5-HT levels and platelet counts. TRP values were highly variable over time in both the medicated and drugfree autistic groups.

In **appendix III** urinary 5-hydroxyindoleacetic acid (5-HIAA) excretion in two consecutive collection periods (5 PM - 11 PM and 11 PM - 8 AM) and whole blood serotonin (5-HT) and tryptophan (TRP) were measured in groups of unmedicated autistics (N = 16), medicated autistics (N = 20) and normal controls (N = 27). Whole blood 5-HT values were significantly higher in unmedicated autistics compared to normal controls. No significant differences were found in 5-HIAA excretion (µg/mg creatinine, mean $\pm$ SD) between unmedicated autistics (4.07 $\pm$ 1.52) and normal controls (3.50 $\pm$ 1.07), nor between medicated (5.35 $\pm$ 2.93) and drugfree autistic individuals.

No correlations were found between 5-HT values and urinary 5-HIAA excretion. Urinary 5-HIAA (µg/mg creatinine, mean $\pm$ SD) was significantly

greater in hyperserotonemic autistic subjects (4.88 ± 0.87) compared to normal controls (3.50 ± 1.07, total collection period, $p = 0.002$). A slight trend ($p = 0.10$) to higher overnight (11 PM - 8 AM) 5-HIAA excretion (µg/mg creatinine, mean ± SD) was observed for unmedicated hyperserotonemic autistic subjects (5.12 ± 1.06) when compared to unmedicated autistics with normal whole blood 5-HT levels (3.86 ± 1.78). In the medicated autistic group significantly higher overnight and total excretion of 5-HIAA was observed in subjects with elevated whole blood levels. The relevance of these findings to the possibility that increased gut production of 5-HT might cause the elevated whole blood 5-HT levels seen in autism is discussed.

In **appendix IV** parameters of ^{3}H-imipramine (IMI) binding were measured in 11 drug-free autistic subjects and 10 normal volunteers.

Similar means (± SD) for B_{max} (autistics, 1350 ± 171 fmole/mg protein; normals 1590 ± 206 fmole/mg protein) and K_d (autistics, 0.98 ± 0.10 nM; normals 0.94 ± 0.13 nM) were found in the two groups. The normal number (B_{max}) and affinitity (K_d) of the IMI binding site in autistic subjects suggest that the regulation of 5-HT uptake is not different in autism.

In **appendix V** a group of 42 autistic individuals and a normal control group matched for sex and age were assessed for the presence of a series of primitive reflexes: the snout reflex, the sucking reflex, the tactile rooting reflex, the visual rooting reflex, the grasp reflex, the palmomental reflex, the glabellar tap reflex, and the nuchocephalic reflex. The snout reflex and the visual rooting reflex were significantly more common in the autistic group. The visual rooting reflex was significantly less common in older autistic individuals.

In **appendix VI** the relationship between whole blood serotonin (5-HT) and the newly observed visual rooting reflex and snout reflex was examined in a group of 39 autistic individuals. In the unmedicated group the autistics with a visual rooting reflex (N = 13) showed significantly higher 5-HT values ($p \leq 0.5$) compared to those without a visual rooting reflex (N = 3). No difference was found for 5-HT values between autistics with a snout reflex (N = 12) and those without a snout reflex (N = 4). Further examination of the relationship between these phenomena in a greater number of autistic and mentally retarded subjects seems to be warranted.

SAMENVATTING

Kinderlijk autisme is een syndroom dat wordt beschreven in termen van gedrag. De kenmerkende symptomen zijn aanwezig vóór de leeftijd van dertig maanden. De symptomen zijn een stoornis in de ontwikkeling van spraak en taal en van de nonverbale communicatieve vaardigheden, een stoornis in de relatie met mensen, dingen en gebeurtenissen en een stoornis in de reactie op sensore prikkels. In het algemeen zijn de motore- sociaal- adaptieve en cognitieve ontwikkeling ernstig vertraagd, gestagneerd of geregredieerd en de onderlinge balans in de ontwikkeling ervan verstoord. Er is een ernstige stoornis in het vermogen zich uit te drukken, in de taxering en waardering van emoties en in de regulatie van angst en opwinding.

Dit proefschrift handelt over neurochemische aspecten van kinderlijk autisme.
In **hoofdstuk** I worden de doelstellingen van het onderzoek beschreven. Het experimentele onderzoek richt zich op de bevinding dat het gemiddelde serotonine gehalte in het bloed van autisten is verhoogd. Het onderzoek levert een bijdrage aan de beantwoording van een aantal basale vragen betreffende kenmerken van deze serotonineverhoging in het bloed en betreffende de opheldering van de oorzaken ervan.
In **hoofdstuk** II worden klinische en gedragsaspecten van het syndroom kinderlijk autisme beschreven en een overzicht gegeven van genetische en somatische factoren die er mee in verband staan.
In **hoofdstuk** III wordt een overzicht gegeven van het onderzoek gericht op neurochemische aspecten van het syndroom en wel die betreffende de catecholamine systemen (noradrenaline en dopamine), neuroëndocriene systemen (cortisol, prolactine, groeihormoon, luteinizerend hormoon, follikelstimulerend hormoon en de schildklierhormonen, en verscheidene hormoon-releasing hormonen) en neuropeptide systemen.
Geconcludeerd kan worden dat dysfuncties in catecholamine systemen in de hersenen wellicht bij het syndroom zijn betrokken. Aanwijzingen hiervoor zijn de therapeutische effecten van medicaties die deze neurotransmitter systemen beïnvloeden, verhoogde basale homovanillinezuurwaarden in de liquor na belasting met probenecid, en verhoogde noradrenalinewaarden in

plasma. Mogelijk bestaat er een subgroup van autisten waarbij verhoogde homovanillinezuurwaarden in de liquor gepaard gaan met versterkt stereotiep en repetitief gedrag en verhoogde motore activiteit. Deze groep is in het algemeen ernstiger gedragsmatig gestoord.
Voorts zijn afwijkingen gevonden in functioneren van neuroëndocriene systemen en in de uitscheiding van neuropeptiden. De meest duidelijke bevinding is een verhoogde toename van schildklierstimulerend hormoon na stimulatie met thyrotropine-releasing hormoon.
In **hoofdstuk IV** wordt een overzicht gegeven van de studies die gericht zijn op de bevinding van verhoogde serotoninewaarden in het bloed van autisten. "Hyperserotonemie" wordt gevonden bij ongeveer 30% van de autisten. Het serotoninegehalte in het bloed van autisten is stabiel gemeten over de periode van een jaar. Het serotoninegehalte is verhoogd als het wordt uitgedrukt in absolute maat, maar ook indien berekend per bloedplaatje. Er is een negatief verband tussen serotoninegehalte in het bloed en intelligentie. De oorzaak van hyperserotonemie bij autisten is onbekend. De verhoogde uitscheiding van 5-hydroxyindolazijnzuur (5-HIAZ) in de urine in de hyperserotoneme autisten, doet vermoeden dat een verhoogde produktie van serotonine in de darm wellicht een rol speelt als oorzaak van hyperserotonemie. Duidelijk te interpreteren afwijkingen van de bloedplaatjes in het vermogen tot opnemen, opslaan en vrijmaken van serotonine zijn niet gevonden. Echter het is zeker zinvol de rol van het functioneren van bloedplaatjes voor een verklaring van de hyperserotonemie verder te onderzoeken. Er zijn geen aanwijzingen gevonden dat de afbraak van serotonine door monoamine oxidase afwijkend is.

De **appendix** bestaat uit artikelen gebaseerd op experimenteel werk. De artikelen zijn gericht op het probleem van de hyperserotonemie van autisten.
In **appendix I** werd het gehalte van serotonine (5-HT) en tryptofaan (TRP) gemeten bij 87 kinderen en jongvolwassenen, en bij 40 autisten (DSM-III criteria) met een zelfde leeftijdsverdeling. Het serotoninegehalte in het bloed was verhoogd in de ongemediceerde (N = 21) autisten (205 $\pm$ 16 ng/ml) vergeleken met de controlegroep (135 $\pm$ 54 ng/ml).
De serotoninewaarden waren zowel in de controle- als in de autistengroep normaal verdeeld (Gausse kromme). Indien de 95e percentiel van de

controlegroep werd gebruikt om "hyperserotonemie" te definiëren, dan bleek bij 38% van de autisten het serotoninegehalte verhoogd.
Autisten met anti-epileptica en neuroleptica als medicatie hadden significant lagere serotoninewaarden dan de ongemediceerde autisten. De verhoging van het gemiddelde serotoninegehalte van de groep autisten kan niet verklaard worden door verschillen in leeftijd, sexe en aantal bloedplaatjes in het bloed of tryptofaangehalte in het bloed.
In **appendix II** werden serotonine- en tryptofaangehalte, en hoeveelheid bloedplaatjes bepaald in het bloed van een grote groep ongemediceerde autisten (N = 17) en vergeleken met een in leeftijd en sexe overeenkomende controlegroep (N = 20). De effecten van neuroleptica en anti-epileptica werden onderzocht door de genoemde variabelen te meten in een groep gemediceerde autisten (N = 23). De stabiliteit over de tijd van deze variabelen werd onderzocht door herhaalde metingen in een groep van gemediceerde (N = 16) en ongemediceerde (N = 10) autisten.
Het serotoninegehalte in het bloed van de ongemediceerde autisten was verhoogd vergeleken met dat van de controlegroep. Echter het tryptofaangehalte en het aantal bloedplaatjes in het bloed van ongemediceerde autisten was niet verschillend van dat van de controlegroep. Er werden geen significante verschillen gevonden in serotoninegehalte tussen gemediceerde en ongemediceerde autisten. Het tryptofaangehalte in het bloed was significant lager in de gemediceerde autisten vergeleken met de ongemediceerde autisten en er werd een significante negatieve correlatie gevonden tussen tryptofaangehalte en de hoogte van de dosis van de neuroleptica.
Voor de herhaalde metingen van 5-HT en het aantal bloedplaatjes werden significant hoge intraclass correlaties en een laag gemiddeld verschilpercentage in de ongemediceerde groep gevonden. De autisten met een vaste dosis medicatie met neuroleptica en anti-epileptica en die autisten die met deze medicatie startten of bij wie deze werd verhoogd gedurende het onderzoek, vertoonden een grote mate van variabiliteit in het 5-HT gehalte en het aantal bloedplaatjes in het bloed. Tryptofaanwaarden in het bloed waren zeer variabel over de tijd, zowel in de ongemediceerde als in de gemediceerde groep autisten.
In **appendix III** werden de 5-hydroxyindolazijnzuur (5-HIAZ) uitscheiding in de urine in twee aaneensluitende verzamelperiodes (17 uur - 23 uur en

van 23 uur tot 8 uur) en het serotonine- en het tryptofaangehalte in het bloed gemeten in groepen ongemediceerde (N = 16) en gemediceerde autisten (N = 20), en in een controlegroep (N = 27). De serotoninewaarden in het bloed waren significant hoger in de ongemediceerde groep autisten vergeleken met de controlegroep. Er werd geen significant verschil gevonden in 5-HIAZ uitscheiding (µg/mg creatinine, gemiddelde ± SD) tussen ongemediceerde autisten (4.07 ± 1.52) en controles (3.50 ± 1.07), noch tussen gemediceerde (5.35 ± 2.93) en ongemediceerde autisten.
Er bestond geen correlatie tussen 5-HT waarden in het bloed en de 5-HIAZ uitscheiding in de urine. De uitscheiding van 5-HIAZ (µg/mg creatinine, gemiddelde ± SD) was significant hoger bij de hyperserotoneme autisten (4.88 ± 0.87) dan bij de controles (3.50 ± 1.07, gecombineerde verzamelperiode, p = 0.002). Een tendens (p = 0.10) tot hogere 5-HIAZ uitscheiding (µg/mg creatinine, gemiddelde ± SD) gedurende de nacht werd gevonden bij de ongemediceerde hyperserotoneme autisten (5.12 ± 1.06) indien vergeleken met de ongemediceerde autisten met normale 5-HT waarden in het bloed (3.86 ± 1.78). In de groep van gemediceerde autisten werd een significant hogere 5-HIAZ uitscheiding waargenomen gedurende de nacht en de totale verzamelperiode bij de autisten met de hoge 5-HT waarden in het bloed. Het belang van deze bevindingen ten aanzien van de mogelijkheid dat een verhoogde produktie van serotonine in de darm de oorzaak is van de verhoogde serotoninewaarde in het bloed bij autisten wordt besproken.
In **appendix IV** werden parameters gemeten van ^{3}H-imipramine (IMI) binding in 11 ongemediceerde autisten en 10 controle-vrijwilligers.
De gemiddelde (± SD) waarden voor de B_{max} (autisten, 1350 ± 171 fmole/mg eiwit; controles 1590 ± 206 fmole/mg eiwit) en de K_d (autisten, 0.98 ± 1.10 nM; controles 0.94 ± 0.13 nM) waren niet verschillend in de twee onderzochte groepen. Deze bevindingen duiden erop dat de regulatie van de opname van serotonine door bloedplaatjes niet afwijkend is bij autisten.
In **appendix V** werd een groep van 42 autisten en een in sexe en leeftijd overeenkomende controlegroep onderzocht op de aanwezigheid van een serie "primitieve reflexen": de snoutreflex, de zuigreflex, de tactiele rooting reflex, de visuele rooting reflex, de grijpreflex, de palmomentaal reflex, de glabella tap reflex en de nuchocephaal reflex. De snoutreflex en de visuele rooting reflex kwamen significant vaker voor bij de autisten dan bij de controles. De visuele rooting reflex kwam minder vaak

voor bij de oudere dan bij de jongere autisten.
In **appendix VI** werd onderzoek gedaan naar de relatie tussen de serotonine waarden in het bloed en de aanwezigheid van de visuele rooting reflex en de snoutreflex. In de ongemediceerde groep hadden de autisten met een visuele rooting reflex (N = 13) significant hogere serotoninewaarden ($p \leqslant 0.5$) dan die zonder visuele rooting reflex (N = 3). Er werd geen verschil in serotoninegehalte gevonden tussen de autisten met (N = 12) en die zonder snoutreflex (N = 4). Verder onderzoek naar de relatie tussen deze fenomenen bij een groter aantal autisten en mentaal geretardeerde kinderen lijkt zinvol.

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CURRICULUM VITAE

1947, 17 mei:	geboren te Zeist
1966:	eindexamen gymnasium beta, 1e Christelijk Lyceum te Zeist
1966-1972:	studie medicijnen aan de Medische Faculteit te Rotterdam
1972-1977:	opleiding tot psychiater
	. 1972-1974: afdeling Psychiatrie AZR Dijkzigt (opleider: Prof. Dr. G.A. Ladee)
	. november 1974-maart 1976: stage kinder- en jeugdpsychiatrie, Afdeling Kinder- en Jeugdpsychiatrie Sophia Kinderziekenhuis te Rotterdam (opleider: Prof. Dr. J.A.R. Sanders-Woudstra)
	. 1976-1977: stage neurologie, Afdeling Neurologie AZR Dijkzigt (opleider: Prof. Dr. A. Staal)
1977:	inschrijving in het specialistenregister als psychiater
1977 tot heden:	staflid afdeling Kinder- en Jeugdpsychiatrie Sophia Kinderziekenhuis (hoofd: Prof. Dr. J.A.R. Sanders-Woudstra); aanvankelijk in dienst van het Sophia Kinderziekenhuis, sedert 1978 als Wetenschappelijk Hoofdmedewerker, Erasmus Universiteit Rotterdam
	. 1978-1981: chef de policlinique
	. 1981-1983: research medewerker Child Study Center, Yale University, New Haven CT, U.S.A. (hoofd: A.L. Solnit, M.D.; sedert 1983 D.J. Cohen, M.D.)
	. 1983 tot heden: research coordinator
1977-1980:	IMP-opleiding analytische kindertherapie (IMP Rotterdam, directeur Prof. Dr. J.H. Thiel)
1980:	lid Nederlandse Vereniging voor Psychotherapie
1980 tot heden:	lid bestuur van de Sectie Kinder- en Jeugdpsychiatrie van de Nederlandse Vereniging voor Psychiatrie

ABBREVIATIONS

ACTH	Adreno corticotropic hormone
ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
cAMP	cyclic Adenosine monophosphate
COMT	Catechol-O-methyltransferase
CRF	Corticotropin-releasing factor
CSF	Cerebro Spinal Fluid
DBH	Dopamine beta-hydroxylase
DNA	Desoxyribonucleinic acid
ENS	Enteric nervous system
FSH	Follicle stimulating hormone
GRF	Growth hormone-releasing factor
GTP	Guanosine triphosphate
5-HIAA	5-Hydroxyindoleacetic acid
HPLC	High performance liquid chromatography
5-HT	5-Hydroxy tryptamine
5-HTP	5-Hydroxy tryptophan
HVA	Homovanillic acid
L-5-HTP	L-5-hydroxytryptophan
LC	Locus coeruleus
LH	Luteinizing hormone
LH-RH	Luteinizing hormone-releasing hormone
MAO	Monoamine oxidase
MHPG	3-Methoxy-4-hydroxy phenylglycol
αMSH	Alpha melatonin stimulating hormone
NAD	Nicotinamide adenine dinucleotide
NE	Norepinephrine
NMR	high resolution nuclear magnetic resonance spectoscopy
PIF	Prolactin inhibiting factor
PRF	Prolactin releasing factor
PRL	Prolactin
PRP	Platelet rich plasma
SRIF	Somatotropin releasing factor
T_3	3,5,3'-Triiodothyronine
T_4	Thyroxine
TH	Thyroid hormone
TRH	Thyrotropin-releasing hormone
TRP	Tryptophan
TSH	Thyroid stimulating hormone
UTP	Uridine triphosphate
VIP	Vasoactive intestinal peptide
VMA	Vanillyl mandelic acid