The Role of Tryptophan in Autism and Related Disorders

Paul Shattock & Paul Whiteley: Autism Research Unit, School of Health, Natural and Social Sciences, University of Sunderland, SUNDERLAND. SR1 3SD.

Introduction

Tryptophan is an essential amino acid found in many foods. It is abundant in dairy products but meats such as turkey and chicken are a particularly rich source. By the late 1970s it was commonly believed that the primary tryptophan metabolic pathways had been elucidated. The pathway from tryptophan to serotonin (5-hydroxytryptamine, 5-HT) and onwards to melatonin had been clarified and the significance of such compounds to depression, mood and the regulation of sleep patterns was reasonably well understood. Serotonin, a product of tryptophan metabolism, was one of the first classes of neurotransmitters to attract the attention of autism researchers as potentially being relevant to the aetiology and pathology of the syndrome. Whilst interest in the role of tryptophan and serotonin to the autistic syndrome continues, the level of research into these important compounds has waned in recent years.

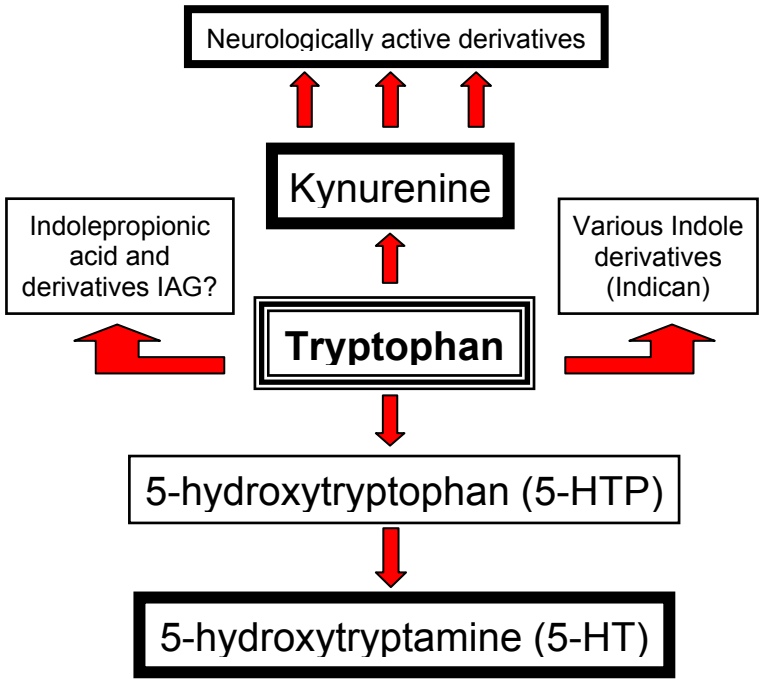
Metabolism of Tryptophan (See Figure 1)

Tryptophan ($C_{11}H_{12}N_2O_2$) is a neutral essential amino acid important for human growth. Tryptophan in humans cannot be synthesised *de novo* and is instead derived from dietary sources such as poultry and dairy products. Deficiency of dietary tryptophan enhances the development of the vitamin deficiency disease, pellagra (E52, ICD-10) as a consequence of the important role for tryptophan in the formation of nicotinic acid (niacin). The main human pathway for tryptophan metabolism, in terms of quantity, leads through kynurenine and on to, amongst other compounds of relevance, quinolinic and xanthurenic acids and nicotinamide (See Figure 1b). It has been estimated that, in humans, over 80% of tryptophan will be metabolised along this pathway [1].

The other route of significance, in humans, sees tryptophan being converted by the enzyme *tryptophan hydroxylase* to form 5-hydroxytryptophan (5-HTP) and onwards to 5-hydroxytryptamine (5-HT) better known as serotonin (See Figs 1a and b). Serotonin is a major neurotransmitter in the enteric nervous system (ENS) as well as in the central nervous system (CNS). A proportion of serotonin is further converted to melatonin, affecting sleep patterns. Serotonin is also reabsorbed from the neuronal cleft by the nerve cells and used again. Other pathways exist but their clinical relevance remains unexplored.

One method formally employed in the treatment of depression was the use of dietary tryptophan. The use and availability of tryptophan (for depression) has been massively

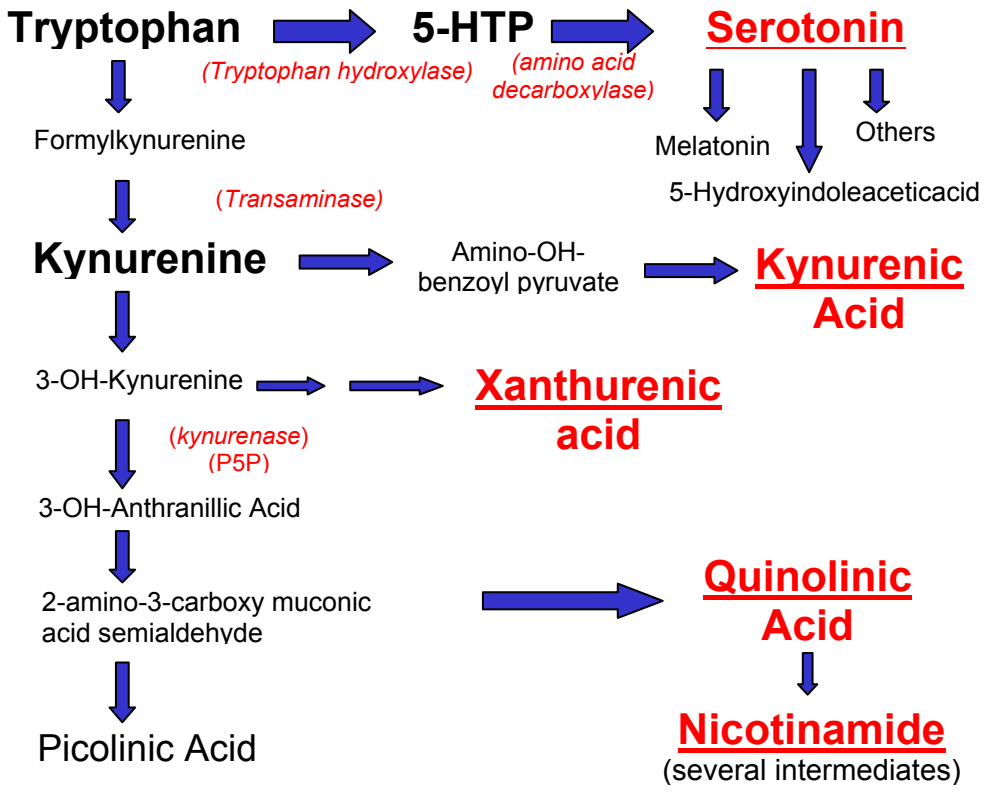
Figure 1(a & b) The metabolic pathways of tryptophan.



curtailed as a result of a number of deaths resulting from a single batch of contaminated compound (eventually found to be due to a toxin, present as a consequence of genetically engineered bacteria used in the production process).

The almost simultaneous (4 days later) appearance of the Selective Serotonin Reuptake Inhibitors (SSRIs) such as Prozac was fortuitous, especially for the drug manufacturers. A series of SSRI drugs has appeared at regular intervals since that time. Recently, their safety and efficacy has been questioned, as a result of reports suggestive of their propensity to encourage suicidal thoughts in some users. They continue, however, to be useful for some higher functioning individuals within the autism spectrum.

Figure 1b



Reichelt [2] isolated peptides that act as selective serotonin uptake stimulators to the platelets from the urine of some children with autism. It is assumed that the same effects occur in the serotonergic nerve endings of the CNS – yet another way in which serotonergic transmission will be inhibited in subjects with ASDs. The intention of all pharmacological intervention is to increase the availability of serotonin at the synapses and, consequently, transmission through these systems based within the CNS. It must be borne in mind that the majority of the serotonin in the body (around 90%) is not in the CNS but in the gastro-intestinal tract, where its functions are beyond the scope of this presentation. Their role has been extensively explored and discussed by Gershon [3].

The drug "Eltoprazine" was used for some years, on an experimental basis, to increase serotonergic transmission by effectively increasing the release of serotonin at the synapse. It did this by inhibiting the presynaptic receptors, stimulation of which would otherwise inhibit release of the transmitter. The manufacturers (Duphar) ceased commercial manufacture of this medication some years ago, possibly due to a perceived lack of market. This was particularly unfortunate since it had shown promise in reducing aggressive behaviour, especially in children with ASDs [4].

Indolylacryloylglycine (IAG)

There are several other routes by which tryptophan is handled in the body. One of these (not shown in Figure 1) involves the conversion of tryptophan, via a number of intermediate metabolites, to Indolylacryloylglycine (IAG). Whilst examining the urine from people with autism (mid 1980s – mid 1990s), we became aware that one particular component was more abundant in the urine of people diagnosed with autism than in control populations. Initially, we believed it to be a peptide (short chain of amino acids) because the samples had been subject to an initial clean-up process specifically to isolate such compounds. It was not until 1997 that we identified this component as IAG.

In our studies we found that this component was present in all of the samples we studied (with or without ASDs) but that the level was considerably elevated in ASD subjects. For a variety of reasons, mostly connected with the technology employed in the analyses and the fact that we had little understanding of the source of this molecule, we deferred publication for a while [5]. It was not until 2003 [6] that we finally published results that demonstrated elevated levels in populations with ASDs. Although significant, these differences were less marked than we had at first observed.

In 2004, a group of psychiatrists from Newcastle, fronted by Alcorn, [7] published the results of our efforts to identify Autism merely by assaying the urines of subjects (3 groups: those with autism; those with non specific learning disabilities; and asymptomatic controls). All were male and of the same age and (more or less) geographical location and with similar dietary intake. We looked for levels of IAG and for evidence of urinary peptides and were statistically successful. Although this paper was not published until 2004 [7], the samples were taken in 1996-97 and assays performed (in duplicate) at that time.

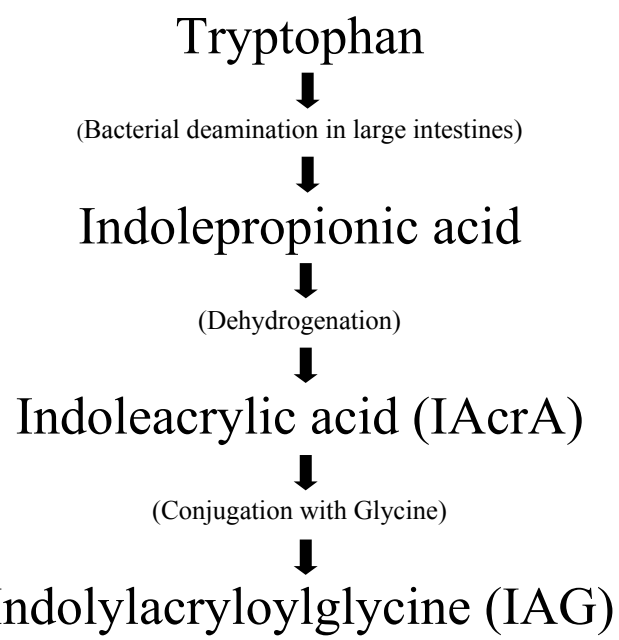
Although we calculated IAG levels by reference to urinary creatinine levels, we were unable, in this particular study, to demonstrate significant differences in IAG levels (at the $P< 0.05$ level). Since then, we have found [8] that creatinine levels are very variable in people with ASDs and are of doubtful use for this population. Our identifications, for the Alcorn study, were made on the basis of visual assessment of the profile and the relative proportions of IAG and peptides.

Since that time, other teams have attempted to replicate our original numerical data in terms of IAG but have failed to do so. Wright *et al* [9], in what appears to be a well-controlled study, found no evidence of differences in IAG levels and we are aware of a report, as yet unpublished, from South Africa that does not support our original findings. We are not able to explain these differences, since the methodologies are essentially compatible. At the same time, we find it difficult to conceive that we were so mistaken in the early days. We can see no errors in our methodology that would allow for such wide variations between test and control subjects.

Is it possible that the situation has changed? Is it possible that levels of IAG in asymptomatic individuals have increased as a result of increased environmental triggering? We believe that this is conceivable and are currently investigating this possibility.

IAG is not a novel compound. It has been reported before, as an unusual metabolite of tryptophan, where phenylketonuria (PKU) has been untreated. It is also very elevated in an unusual metabolic disorder called "Hartnup Disease" (HD). In HD, there is a problem with the absorption of tryptophan from the intestines. Intestinal bacteria begin fermenting dietary tryptophan into other metabolites that are absorbed. These are then converted, via indoloylacrylic acid (IAcrA), to IAG. It has frequently been reported that this conversion occurs in the liver but, given the reactive nature of IAcrA and the availability of glycine, it is more likely to occur in the gut wall (See Figure 2)[10].

Figure 2: Production of IAG.



Thus we have proposed that, in subjects with ASDs (and some other disorders), tryptophan is converted, via indole pyruvic acid and IAcrA, to IAG. IAG is probably the pharmacologically inert product resulting from the conjugation of IAcrA and glycine. IAcrA is a molecule that is both flat and very reactive and has the capacity to affect many aspects of bodily function. Since IAcrA is so reactive it will conjugate rapidly with glycine to form IAG and be excreted in urine. There may exist other significant routes for the formation of IAG but, if so, they are not recorded in the literature. Although IAcrA has not been isolated it is assumed to be present but, on account of its reactivity, it is unlikely to be found in the free state.

In particular IAcrA has the theoretical capacity to increase membrane permeability either by *replacing* the (flat) long chain fatty acids that make up the lipid elements of the membrane, or by inserting itself between these layers. There exists no direct evidence for this but in 1999, Bell [11] in an unpublished report, showed some modifications *in vitro* to cell membranes of carp with IAcrA but the quantities required may be physiologically unlikely. In a further report [12] showed how the levels of highly unsaturated fatty acids (HUFAs) in red blood cell membranes are affected by the presence of IAcrA. We suggest, therefore, that the presence of IAG represents a marker for permeability of the intestinal wall. It is possible that the relationship is a causative one, in which case it will also affect the blood brain barrier in the same way. It will immediately be realised that these hypotheses are very speculative but, at the same time, difficult to demonstrate *in vivo*.

Many groups around the world believe that IAG is a marker for permeability of some sort and use its presence in their own versions of diagnostic tests but we recognise that we are not in a position to understand precisely what processes are involved in the body. The relative contributions of the intestinal flora, the diet and the inflammatory situation of the intestines (which could result from infection or intolerance reactions) are not understood.

The Sources of IAG

One of the more problematic issues is the identification of the location of the source material for the IAG. In 1999, Marklova demonstrated that piglets devoid of intestinal bacteria (following treatment with neomycin) were able to produce comparatively small amounts of IAG (which appeared in the urine) [13]. However, as Hooper pointed out in his 2000 report on IAG, the quantities produced do not remotely equate to the levels identified in the urine of children with autism [14]. It is possible that children with autism may produce very much higher levels than is the norm but there are other, more plausible, explanations. There is no disputing the fact that intestinal bacteria (as described for Hartnup Disease – see above) will convert tryptophan into indolepropionic acid and that this will traverse the intestinal wall. The reported increased levels of IAG in ASDs could be consequent upon an abnormal intestinal flora. Alternatively, it could be totally epiphenomenal and consequent only upon the abnormally permeable intestinal walls that are characteristic of ASDs and many other disorders.

Hooper has discussed the relevance of IAG and intestinal permeability in a number of overlapping syndromes including multiple chemical sensitivities, Gulf War Syndrome and Myalgic Encephalopathy [14,15].

Interestingly, we have repeatedly observed that the levels of IAG do *not* appear to be elevated in the vast majority of children with ASDs where parents allege a causative involvement of infectious agents. These data, although presented to a number of journals, remain unpublished. It could be that the intestinal inflammation, triggered by infection, has resulted in the permeability. Alternatively, it is feasible that the cytokines produced by the inflammatory process will stimulate the enzymes in the kynurenine pathway. This would result in *reduced* levels of IAG when compared to levels in more typical forms of autism. Given the uncertainty about the location for the formation of these molecules, a degree of caution is required when interpreting such variations.

Why should people with ASDs produce elevated levels of IAG?

There are a number of possibilities (diet, abnormal intestinal flora, physical stress perhaps) that could be involved but there is an increasing awareness of a possible role for environmental pollutants and in particular organophosphate-based insecticides.

Our hypothesis, resulting from similar studies that we have performed on urines of people with Gulf War Syndrome and Sheep Dippers' Syndrome is that organophosphate (OP) pesticides are involved in some way. Organophosphonate products were originally developed, in the 1930s, as nerve gases for use in warfare. After World War II other derivatives were developed: organophosphates, which, when presented with a thiol group, are less toxic to man but more toxic to insects. In these cases, the OP products inhibit some of the enzymes called *cholinesterases,* which break down the neurotransmitter acetylcholine. It would seem impossibly unlikely that these would be the only enzymes that they would affect. OP insecticides will inhibit any enzymes with an active serine site[16], including the digestive enzymes *trypsin, chymotrypsin* and *di-peptyl peptidase IV (DPPIV)*; but many other enzymes of metabolic significance will be inhibited in a similar way.

In a number of studies, Pewnim [16] and Siefert [17] showed that OP pesticides would inhibit enzymes in the kynurenine pathway and have marked effects upon the metabolites. Our own studies [18] additionally demonstrated effects on the pathway leading to serotonin (by inhibiting tryptophan hydroxylase). We suggested that the increase in OP pesticides, including their equally active breakdown products, would cause an inhibition in these major pathways for tryptophan metabolism (kynurenine and 5-HT) and a consequent increase in

the production of IAG and intermediate compounds on this pathway. Thus, the increased environmental levels of OP pesticides that will have become apparent in the UK could be responsible for the increases in incidence of many disorders in humans. It should be noted that between 1979 and 1982, the older organo-chlorine pesticides (such as DDT) were phased out and replaced by the more toxic OP-based products. This change would appear to correlate closely to the reported increases in ASDs and some other disorders. We are currently investigating the possibility of an increase in the levels of other indole derivatives in the urine of subjects with autism.

Since it is not possible to avoid intake of OP pesticides through food and through drinking and bathing water without taking extreme precautions, we must assume that we all eat, drink and absorb such compounds. However, not everyone develops Autistic Spectrum Disorders, ADHD, Dyslexia, Asthma, Parkinson's disease, Gulf War Syndrome or other conditions for which OPs are sometimes blamed. Most of us have the genetic ability to break down OP pesticides using a number of genetically controlled systems. The enzymes paraoxanase 1 and 2 are known to be involved but sulphation and methylation are also involved. Deficiencies in any of these systems could result in elevated levels of OPs remaining in the body. It has been well documented that people with ASDs have inefficient systems for sulphation [19,20,21] and methylation and glutathione-based systems [22]. There is also preliminary evidence that at least one of the genes controlling paraoxanase production (PON1)[23] is aberrant in some subjects with ASDs and we are currently attempting to replicate this, using PCR techniques, on subjects from various parts of the world.

Thirty years ago such genetically controlled abnormalities would be unlikely to have a great effect on development because these pesticides were present only in minute amounts. Some of us parents of children with ASDs do exhibit certain behavioural traits that may relate to ASDs but these environmental triggers were barely present at that time. It must be borne in mind that these will not be the only enzymes affected by OP pesticides. They can also adversely affect the enzyme di-peptyl peptidase IV (DPPIV) that is responsible for the breakdown of peptides (some of which have opioid activity) into their constituent amino-acids. This could have considerable significance for people with ASD and related disorders.

We also believe that these same underlying mechanisms are involved in conditions such as Gulf War Syndrome and Sheep Dippers' Syndrome; but since these affect adults the developmental issue will be minor. We also suspect the involvement of similar mechanisms in many subjects with ME/CFS (although infections often play a big part too). Indeed, at present, we would be hard pushed to differentiate urinary profiles supplied by people with ASDs and with CFS/ME.

Effects of Supplementation with Tryptophan and derivatives.

It has been shown that a diet depleted of tryptophan is not beneficial for children with ASDs and that some symptoms are exacerbated [24]. Presumably, the existing lack of available serotonin (and other tryptophan derivatives) was exacerbated under these circumstances.

Supplementation with tryptophan would probably not be helpful in the majority of cases because the conversions along the important pathways are inhibited and tryptophan is likely to be converted along the IAG route, which would be unhelpful. Anecdotal clinical reports suggest that some children show benefits and others may get worse but no formal studies have been reported.

For this reason, and because tryptophan is a prescription-only drug*, we have looked at other methodologies. The active transmitter, serotonin, does not cross the blood brain barrier and so would be ineffectual in this respect. However, the precursor molecule 5-HTP *does* cross the blood brain barrier and reach the appropriate target areas. Some parents have reported impressive consequences, particularly with regard to sleep patterns; some physicians have been able to reduce the doses of e.g. risperidone (an anti-psychotic drug) by supplementing 5-HTP but, on the whole, the results have been less useful than would have been predicted.

The conversion reaction from 5-HTP to serotonin is dependent upon pyridoxine or, more particularly, the active derivative pyridoxyl-5-phosphate (P-5-P) and it has been shown [25] that people with ASDs make this conversion very inefficiently. A lack of available P-5-P would inhibit conversion to serotonin which would further affect the already depleted levels of available serotonin. P-5-P does not, however, cross the blood brain barrier, whereas pyridoxine does. It has been reported [26] that addition of high doses of pyridoxine to the diet will assist in producing beneficial effects. Nicotinamide (in the form of nicotinamide adenine dinucleotide (NAD)) is required for the conversion of pyridoxine to P-5-P. The hypothetical possibility of reduced levels of nicotinamide resulting from abnormal tryptophan metabolism (see below) could influence the rate of this conversion to P-5-P.

The Kynurenine Pathway and Autism

The kynurenine pathways remain virtually unexplored in terms of ASDs. We have found (and reported [18]) that OP pesticides will affect relative levels of some of the metabolites in this pathway (*in vitro*) but studies on individuals with ASDs have not been performed. The effects upon kynurenic, xanthurenic and quinolinic acids are likely to be significant but have yet to be explored in ASDs. In this paper we reported that the relative quantities of the neurotoxic metabolite, quinolinic acid, and the neuroprotective kynurenic acid were affected. This could have considerable clinical significance but the effects will vary considerably (*in vitro*) with the concentration of OP pesticide employed. We have still to determine the physiologically feasible concentrations and, for the time being at least, it would be too speculative to discuss these possibilities [27]. One of the elements of this pathway leads to the production of Vitamin B3 (= nicotinamide=niacin). The proportion of the required amounts of nicotinamide derived from this route is unclear. We do not, therefore, know whether or not disturbances in production by OPs or other agents will be of clinical relevance but this is being investigated. . Additionally, it is likely that nicotinamide produced by bacteria will be poorly absorbed through the intestines.

Although there is little but anecdotal evidence to suggest beneficial effects [28], for many years supplementary B3 has been given, usually in combination with other Pyridoxine and other B group vitamins, to people with ASDs.

Effects on Immune Function

It has been demonstrated, on a number of occasions, that there are irregularities in immune function in people with ASDs. The precise nature of these irregularities is unclear and is not consistent between individuals. There is a general tendency towards “allergic” responses and an apparent diminution in the ability to ward off infections caused by viruses and bacteria. There are a number of elements in the underlying biological mechanisms which could interact to cause such variations from the norm. Opioid peptides, OP pesticides residues, and the availability of sulphate will all influence the system. In 2002, Hooper discusses some of these issues, quoting from Mellor and Munn [29] and also refers to the ability, unique amongst amino acids, to stimulate T cell cloning and an increase in the ability to counteract infections

* Editor’s note: Tryptophan has been available OTC for supplementation use since last year in 250mg doses.

and malignant cells. The contributions of these elements will vary considerably between individuals and the interplay between these factors is likely to produce a very varied effect which is difficult to predict in any individual.

Conclusions

Attention has recently refocused on potential roles for tryptophan and its derivatives in the aetiology of autism. Understanding of these abnormalities could also provide clues for potentially useful interventions. There is the potential for involvement of the abnormal metabolite indoloylacrylic acid and consequences for the concomitantly reduced levels of serotonin, melatonin and nicotinamide. It is possible that organophosphate pesticides could, in genetically susceptible individuals (that is, those with genetically deficient or overstretched detoxification systems), persist and trigger these distortions in tryptophan metabolism.

Clearly, we are only beginning to appreciate the complexity of the interplay between the various factors which contribute to the symptoms of ASDs and related disorders. Some of the physiological consequences will be of major significance; some will be relevant to a limited number of individuals and others will be totally epiphenomenal. There are many reasons why there should be further research into the role of tryptophan and its metabolites and of the potential that an understanding presents for effective amelioration of symptoms.

About the Author

Paul Shattock is a Pharmacist who taught Pharmacy and related subjects at the University of Sunderland for 30 years. He took early retirement to establish and direct studies at the Autism Research Unit at Sunderland. He is the father of Jamie, aged 36, who is severely affected by Autism. He is the chairman of ESPA, a charity which provides residential and day services for 200 adolescents and adults with autism. He is the Honorary Secretary of the World Autism Organisation. His main interests revolve around a determination of the interventions that may ameliorate some of the problems of autism based upon an understanding of the underlying biological mechanisms.

References

1. Tanizawa K., Soda K., (1979) "The mechanism of kynurenine hydrolysis catalyzed by kynureninase" Journal of Biochemistry 86 1199-1209
2. Pedersen O, Liu Y., Reichelt KL., (1999) *"Serotonin uptake stimulating peptide found in plasma of normal individuals and in some autistic urines"* Journal of Peptide Research 53 (6) 641-
3. Gershon MD *"The Second Brain"* Harper Perennial, New York (1999)
4. Berney T.P. (2000) Personal Communication
5. Anderson RJ, Bendell DJ, Garnett I, Groundwater PW, Lough WJ, Mills MI, Savery D, Shattock PEG. (2002) *"Identification of Indoly-3-Acryloyl Glycine in the urine of people with autism."* Journal of Pharmacy and Pharmacology 54 (2) 295-298
6. Bull G., Shattock P., Whiteley P., Anderson R., Groundwater P., Lough JW., Lees G. "*Indolyl-3-acryloylglycine (IAG) is a putative diagnostic urinary marker for autism spectrum disorders* Medical Science Monitor. 9 (10) (2003) pp CR2-CR5
7. Alcorn A., Berney T., Bretherton K., Mills M., Savery D., Shattock P. (2004) *"Urinary Compounds in Autism"* Journal of Intellectual Disability Research 48 (3) 274-278
8. Whiteley P., Waring R., Williams L., Klovzra L., Nolan F., Smith L., Farrow M., Dodou K., Lough WJ., Shattock P. (2006*) "Spot urinary creatinine excretion in pervasive developmental disorders*" Pediatrics International 48 (3) 292-297
9. Wright B et al. (2005) Developmental Medicine and Child Neurology 47 (3) 190-192. *"Is the presence of urinary indolyl-3-acryloyl glycine associated with autism spectrum disorders?"*
10. Jepson JB. (1966) in Stanbury, Wyngarden and Fredrickson (Eds) "The Metabolic Basis of Inherited Disease", McGraw, Hill, New York 2nd Ed. 1283
11. Bell JG (1999) personal Communication

12. Bell J.G., Sargent J.R., Tocher D.R., Dick J.R., (2000) "Red blood cell fatty acid composition in a patient with autistic spectrum disorder: a characteristic abnormality in neurodevelopmental disorders" Prostaglandins, Leukotrienes and Essential Fatty Acids 63 (1-2) 21-25
13. Marklova E. (1999) *"Where does indoleacrylic acid come from?"* Amino Acids 17 401-413
14. Hooper M. (2000) *"IAG – a marker molecule for dietary intervention in overlapping syndromes"* The Nutritional Practitioner 2 35-36
15. Hooper M. (2002) "*Towards an Understanding of the IAG/Opioid Paradigm"* from the "Building Bridges" conference held at the University of Durham April 2002 Autism Research Unit, University of Sunderland 85-105
16. Pewnim T., Siefert J. (1993) *"Structural requirements for altering the L-tryptophan metabolism in mice by organophosphorous and methylcarbamate insecticides"* European Journal of Pharmacology 248 237-241
17. Siefert J., Pewnim T., (1992) Biochemical Pharmacology 44 2243-2250
18. Anderson RJ., Carr K., Cairns D., Lough WJ., Haavik J., Martinez A., Teigen K., Shattock PEG., Whiteley PR. (2002) *"Putting Tryptophan in the Spotlight"* Consensus in Child Neurology (Supplement of the J. Child Neurology) 35-38
19. Waring RH., Ngong JM., Klovzra L., Green S., Sharp H. (1997) *"Biochemical Parameters in Autistic Children"* Developmental Brain Dysfunction 10 40-43.
20. Waring R.,Klovzra LV. (2000) *"Sulphur Metabolism in Autism"* Journal of Nutritional and Environmental Medicine 10, 25-32
21. Alberti A., Pirrone P., Elia M et al. *"Sulphation deficit in "low functioning" autistic children: a pilot study"* Biological Psychiatry 46 420-424
22. James SJ., Cutler P., Melnyk S., Jernigan S., Janak L., Gaylor DW., Neubrander JA. (2004) *"Metabolic Markers of increased oxidative stress and impaired methylation capacity in children with autism"* American Journal of Clinical Nutrition 80 1611-1617
23. D'Amelio M., Ricci I., Liu X., D'Agruma L., Muscarello LA., Guarnieri V., Militeri R., Bravaccio C., Elia M., Schneider C., Melmed R., Trillo S., Pascucci T., Pugli-Allegra S., Reichelt K-L., Macciardi F., Holden JJA., Persico AM**. (2005)** ***"Paraoxanase gene variants are associated with autism in North America but not in Italy: possible regional specificity in gene-environmental interactions"*** Molecular Psychiatry 10 **1006-1016**
24. McDougle CJ., Naylor ST., Cohen DJ., Aghajanian GK., Heninger GR., Price (1996) *"Effects of tryptophan depletion in drug-free adults with autistic disorders"* Archives of General Psychiatry 53 (11) 993-1000
25. Adams JB., George F., Audhya T. "*Abnormally high plasma levels of vitamin B6 in children with autism not taking supplements compared to controls not taking supplements"* Journal of Alternative and Complementary Medicine 12 (1) 59-63
26. Audhya T, (2006) Personal Communication
27. Carr K., (University of Sunderland) 2006 Personal Communication
28. Kleijnan J., Knipschild P. (1991*) "Niacin and Vitamin B6 in mental functioning: a review of controlled trials in humans"* Biological Psychiatry 29 931-941
29. Mellor AL., Munn DH. *"Tryptophan catabolism and T-Cell tolerance. Immunosuppression by starvation?:* **Immunology Today 20** 469-473 through Hooper (2002) loc. cit. above