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REVIEW

Sulphur Metabolism in Autism

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Abstract

Purpose: *Previous studies in autistic children have shown that they have reduced levels of plasma sulphate as compared with age-matched control children and the aim of this study was to see if this reflected increased urinary sulphate loss.*

Design: *Outpatient-based survey of autistic children and matched controls.*

Materials and methods: *The children in the study were elected on the basis of ICD-10 criteria and a diagnosis of autism. Use of a behavioural questionnaire allowed children with autism to be divided into 3 subsets. Urinary excretion of sulphate, sulphite, thiosulphate and thiocyanate was measured in 232 autistic children and compared with values from 68 age-matched controls.*

Results: *Autistic children excreted higher levels of sulphate, sulphite and thiosulphate, but reduced levels of thiocyanate.*

Conclusions: *The significance of these altered parameters is discussed with respect to catecholamine metabolism, mucin formation, gastrointestinal hormone activation and sulphur anion metabolism.*

Keywords: sulphate, autism, diet, phenols, amines, protein, gastrin, secretin, gastrointestinal tract.

INTRODUCTION

Autistic children are socially withdrawn, often with obsessive, repetitive behaviour patterns and hyperactivity or self-injury. Generally, the problems become noticeable around the age of 24 months when affected children regress, losing speech and the capacity to interact with carers. In recent years, some of the underlying biochemistry has become clearer and it is now possible to explain why many children with autism are sensitive to particular foods in their diet. Autism is a heterogeneous condition, so that there can be no single explanation for the clinical findings. Nevertheless, in a recent study, it was found that plasma sulphate levels were greatly reduced in autism, with mean values of 0.55 nmol mg^{-1} protein compared with age-matched controls (4.9 nmol mg^{-1} protein). This decrease in plasma sulphate was found in 92% of the children investigated, who had all met ICD-10 criteria for diagnosis as having an autistic disorder spectrum [1]. Inorganic sulphate can be absorbed from the gastrointestinal tract, using a sodium ion (Na^+)-linked transporter but it seems probable that only about 20% of the required supply is obtained this way in man [2]. A number of foods, particularly bread, beer and dried fruits, are sulphate-rich, and can help to supply the anion but high levels of sulphate in the gut, such as can occur if $MgSO_4$

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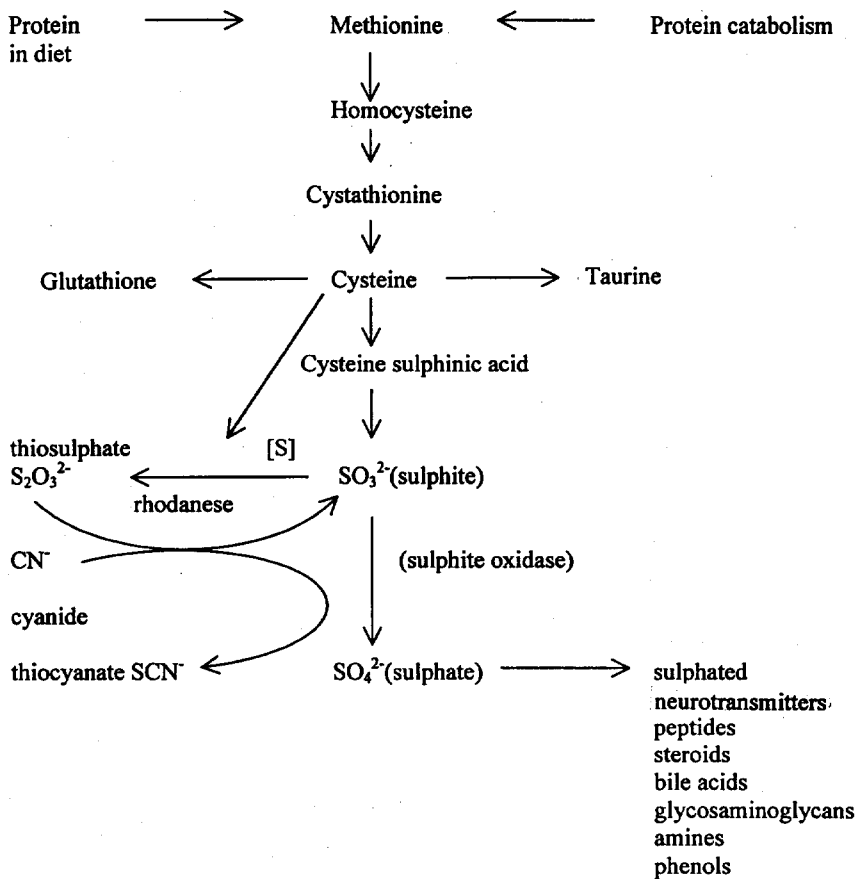

FIG. 1. Metabolic pathways of sulphur compounds.

(Epsom salts) is ingested, are usually accompanied by diarrhoea [3]. *In vivo*, therefore, oxidation of cysteine and methionine is the major source of sulphate (see Figure 1).

The initial (and probably rate-determining step) is carried out by the enzyme cysteine dioxygenase and leads to the formation of cysteine sulphinic acid. This undergoes transmination and degradation to give sulphite (SO_3^{2-}) ions which can be oxidized to sulphate (SO_4^{2-}) by the enzyme sulphite oxidase. The study was therefore carried out to find whether the low levels of plasma sulphate were due to an increased urinary excretion of the anion and whether the levels of other sulphur-containing anions were altered.

DESIGN

Children in the study were elected on the basis of ICD-10 criteria and had been diagnosed as autistic by paediatric developmental psychiatrists. Age-matched controls came from local primary school children in the West Midlands area. Early morning specimens of urine from 232 autistic children and 68 age-matched controls were collected, corrected for creatinine content and frozen at $-70°C$ until analysis. In a separate study, parents of 100 autistic children were asked to fill in a short behavioural questionnaire, as were the parents of 80 age-matched control children.

TABLE 1. Excretion of urinary protein and anions in autism

	Autism ($n = 232$)	Controls ($n = 68$)
Age (years)	7.6 ± 2.4	8.5 ± 3.7
Protein $\mu g\ ml^{-1}$	103.2 ± 89.9*	64.5 ± 27.5
Sulphite	106.9 ± 162.9*	2.1 ± 6.3
Thiosulphate	130.8 ± 148.1*	18.6 ± 25.0
Thiocyanate	6.4 ± 16.9*	44.0 ± 101.0
Sulphate	6819.0 ± 6712.3*	3030.8 ± 1461.0

Anion excretion is given in nmol ml^{-1}, mean ± SD* $p < 0.001$ (Wilcoxon rank sum test).

MATERIALS AND METHODS

Inorganic sulphate [4], sulphite [5], thiocyanate and thiosulphate [6] were estimated in urine by standard colorimetric methods. The answers in the questionnaires were sub-setted by standard cluster analysis methods.

RESULTS

As can be seen from Table 1, autistic children had increased urinary excretion of protein, which may be due to some renal dysfunction. They also had high urinary sulphate; this anion is normally largely resorbed in the proximal tubule of the kidney by co-transport with Na^+ ions [7]. This process relies on the presence of sulphated proteins in the renal tubule membranes and hence, as sulphate levels decrease, the synthesis of these biocomponents is less adequate and the resorption of the anions therefore becomes less effective. An increased loss of sulphate in urine may partly explain the low plasma sulphate levels, since once the process had started there would be a tendency for it to continue on a slow but steady downward spiral.

As sulphite oxidase is a molybdenum-containing enzyme, it was thought that the raised sulphite excretion might decrease on supplementation with molybdenum. However, this only occurred in 36% (14/38) of the children so treated. Reduction in urinary sulphite was associated with improvement in clinical symptoms as reported by parents and carers. Autistic children also had higher levels of thiosulphate and lower levels of thiocyanate ions than controls.

Cluster analysis of the behavioural questionnaire showed three main groups. Firstly, there was a subset who had problems (sweating, thirst, cravings, urination) probably caused by hypothalamic dysfunction, as has been previously noted in autism [8]. Secondly, there was a set who had problems (handed-ness, nightmares, epilepsy) which might be the result of the frontal lobe damage which has also been described in autism [9] while the third group had family backgrounds of allergy, attention deficit disorder, hyperactivity, autism and auto-immune dysfunction [10]. This last group were reported by parents and carers as being the most likely to respond to casein and gluten-free diets. If these children had inherited a tendency to over-react to endogenous or exogenous peptides and proteins, then this finding is not unexpected [11]. Potentially, sub-setting autistic children by biochemical and behavioural parameters may be helpful in the future in recommending the appropriate treatments.

CONCLUSIONS

Sulphite and Sulphate in Autism

It is probable that some children with autism require extra molybdenum as co-factor for

sulphite oxidase to function, while others may have a mutated version or one that is inhibited by endogenous metabolites. It is known that the total deficiency of sulphite oxidase leads to neurological dysfunction [12]. Partial sulphite oxidase deficiency, however, does not seem to have been linked with clinical problems, although high sulphite levels in the diet are associated with an increase in asthma and allergic reactions in susceptible individuals [13]. The raised levels of urinary thiosulphate and reduced thiocyanate may also suggest a reduction in rhodanese activity. This enzyme detoxifies cyanide ions by combination with thiosulphate to form thiocyanate (see Figure 1) and has been relatively little studied. Cyanide ions are toxic; they inhibit the processes of oxidative phosphorylation and cellular oxidation reducing the adenosine triphosphate (ATP) supply *in vivo*. As brain tissue is very energy-demanding, the lower levels of ATP, the main supply of chemical energy, can lead to cell damage and death so that low levels of cyanide ions act as chronic neurotoxins. Again, mutations in this enzyme or inhibition of its activity may be part of the aetiology of autism.

Sulphate provision appears to be anomalous in autism. In some cases of autism, S-oxidation of cysteine has been shown to be defective *in vivo*. Using the probe drug S-carboxymethylcysteine, a cysteine derivative which is also a substrate for oxidation by cysteine dioxygenase, it was found that many autistic children excreted reduced or only trace amounts of the S-oxide metabolite. Cysteine dioxygenase is known to be polymorphic in man, about 30% of the population being "slow" S-oxidizers and a further 2% being essentially "nul" S-oxidizers. In a small study on 48 autistic children, 45.8% (22/48) fell into this last category [1] so that *in vivo* oxidation of cysteine is often impaired in autism. The genetics of this pathway have been studied in depth [14,15]; the gene(s) for slow S-oxidation appears to be inherited on an autosomal recessive pattern and the phenotype has been associated with a number of disease states, especially rheumatoid arthritis [16] and allergy [17] which are more common in the family backgrounds of autistic children. In a study in this laboratory of 47 patients with severe food and chemical allergies, 44 had low S-oxidation capacity and reduced plasma sulphate, a pattern like that often found in autism. Reduced S-oxidation and sulphation appear also to have a clinical link with autoimmune dysfunction, correlating with the suggestion that this is a factor in many cases of autism [18].

Sulphotransferase Enzymes

Sulphotransferases (ST) are a superfamily of enzymes which transfer a sulphonate group ($-SO_3^{2-}$) onto a number of substrates using PAPS (3′-phosphoadenosine-5′-phosphosulphate) as a co-factor. As sulphate is often in relatively short supply, this controls the availability of PAPS and so the extent of sulphation [19]. Many exogenous and endogenous compounds are substrates including amines, phenols, neurotransmitter catecholamines, peptide hormones, proteoglycans, steroids and bile acids [20].

Some of the symptoms in autism, such as the stereotyped behaviour, mood swings and hyperactivity, suggest an imbalance of neurotransmitter amines in the central nervous system. Catecholamine levels in autistic children are known to be different, usually higher, than those of controls [21,22]. The major route of inactivation of compounds of this type is via the formation of sulphated conjugates. These metabolites are pharmacologically inactive and also rapidly excreted but as the sulphate supply is the rate-limiting factor in their formation, low sulphate levels can lead to increased concentrations of neurotransmitter amines and to prolonged effects on the central nervous system [23]. Similar effects can occur if the enzymes involved are less active. Sulphation of amines and phenols is catalysed respectively by the M-ST and P-ST sulphotransferases (both isoforms will use the alternative substrate at higher concentrations) which occur in many tissues, especially the brain, liver, platelets and gastrointestinal tract [24]. These enzymes catalyse sulphation of both endogenous and exogenous amines; dietary neurotransmitters (such as serotonin in

bananas, phenylethylamine in chocolate and tyramine in cheese) are normally inactivated in the gastrointestinal tract by the sulphotransferases which are present. However, individuals with reduced enzyme activity are less able to form the inactive metabolites and are susceptible to the central nervous system effects induced by the free amines. For example, patients with migraine usually have low P-ST activity and are readily affected by dietary "triggers", especially by those which contain amines [25]. Compounds such as flavonoids (present in red wine and citrus fruits) inhibit the enzyme *in vitro* and presumably also *in vivo*, leading again to headache in those who are less resistant [26,27]. It is fairly common for children with autism to have a family background of migraine and their hyperactivity may improve when chocolate, bananas, orange juice, vanillin and food colourants such as tartrazine are removed from their diet [28]. In a small study, children with both autism and a parent with migraine were found to have low sulphotransferase levels, but this is not necessarily generally true [1]. Nevertheless, a combination of low enzyme activity and low sulphate availability greatly reduces the capacity for detoxification of amines and phenols, both endogenous and exogenous. Many drugs are detoxified via the sulphation pathway and if this is ineffective, adverse reactions can occur. In a recent investigation it was shown that after oral administration of paracetamol, urinary excretion of the sulphated conjugate was reduced in autistic children, as compared with age-matched children of similar IQ, although the glucuronidation pathway was unaffected [29].

Gastrointestinal Function

Sulphation capacity also affects the gastrointestinal tract. The mucins which line the gut, providing both protective and adhesive properties, are sulphated glycoproteins which rely on the degree of sulphation and the addition of negative charges to maintain their structures in an extended configuration [30]. Reduced sulphation has been associated with inflammation and gut dysfunction, coupled with a breakdown in the protective properties of the mucins and an increase in permeability [31]. Many children with autism also have digestive problems and 9 out of 20 (42%) in one survey had increased gut permeability when challenged by the mannitol/lactulose test [32]. This finding has led to the idea that a "leaky" gut may be more permeable to dietary proteins and peptides. Groups working in two separate laboratories have found diet-derived peptides in urine from children with autism [33,34] which were attributed to incomplete hydrolysis of casein and gluten proteins from milk and wheat respectively [35]. Peptides of this type can show "opioid" activity, crossing the gut wall and penetrating the blood–brain barrier to act on the central nervous system [36]. They may be responsible for the social withdrawal, insensitivity to pain and altered responses to sensory stimuli which are commonly described in autism. Use of naloxone and other opiate antagonists has been reported to reduce the levels of self-injury, again suggesting that "opioid excess" may be a factor in autistic behaviour [37]. As would be expected from this work, behavioural problems in autism have been found to decrease in some cases if a gluten-free, casein-free diet is introduced, although the time interval required for complete improvement can be as long as 24 months [38,39]. Randomized, controlled studies are difficult to carry out, so that the results should be interpreted with caution. However, the positive findings reported can certainly be seen to be in agreement with ideas on peptide transfer across the gut, into the blood stream and then into the brain. Anecdotally, many parents find that a strict gluten-free, caesin-free diet is the most useful therapeutic approach for their autistic children.

Gastrointestinal Hormones

Normally, proteins would be expected to be hydrolysed to amino acids (rather than peptide fragments) in the gastrointestinal tract and it is not clear why this pathway might not occur in autism. Several factors may be responsible. Reduced peptidase activity has been

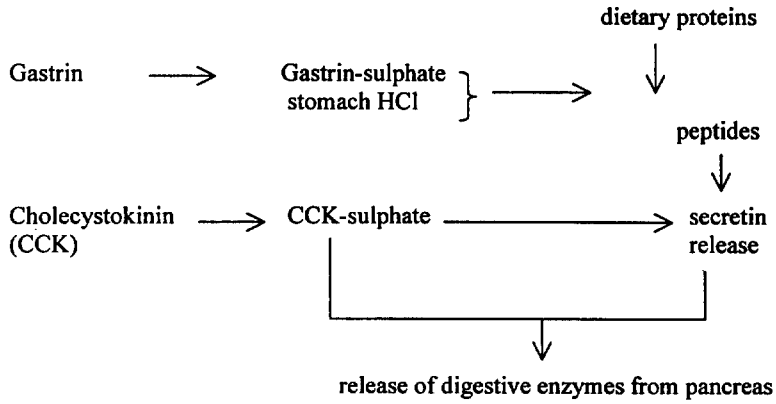

FIG. 2. Interactions of gastric hormones and enzymes.

suggested; as these enzymes are zinc-requiring [40] and autistic children often have low zinc levels, this may be a partial explanation. Another possibility is the interaction between sulphation and the digestive peptide hormones. Gastrin, which is secreted in the stomach, is more active when the molecule is sulphated on a tyrosine residue. This is also true of cholecystokinin (CCK), a related peptide which is active in both brain and the gastrointestinal tract [41]. The secretion of CCK, together with production of peptides liberated by the influences of gastrin and gastric hydrochloric acid on dietary proteins induces release of another peptide hormone, secretin. The combination of sulphated CCK and secretin allows release of the digestive enzymes from the pancreas (Figure 2). These include amylases, lipases, and peptidases such as trypsin and chymotrypsin which break the peptides down to their constituent amino acids [41]. This is essentially a cascade process which can be blocked at any stage; as autistic children often have achlorhydria and low sulphate levels, the indirect consequence may be that there are reduced levels of secretin and reduced hydrolysis of dietary proteins. Recently it has been reported that some children with autism may respond to infusions of secretin [42] although this is currently still controversial. Addition of bromelain or digestive enzyme capsules to the diet can also have beneficial effects and may avoid the adverse reactions which can occur with secretin infusions. It is possible that sulphated gastrin could be given with therapeutic benefit but this does not yet seem to have been tried.

Diet and Clinical Practice

Currently, the most useful advice that can be given to parents of autistic children is to try a gluten-free, casein-free diet for at least 6 months, also removing chocolate, bananas and citrus fruit from the food supply. Parents/carers are best placed to notice any behavioural changes and studies are currently being undertaken to provide statistical proof of the efficiency of this approach. Many children also respond to sulphate supplements, either given as trace amounts of magnesium sulphate spread throughout the day or even by dermal absorption via magnesium sulphate (Epsom salts) in bathwater. Supplements of molybdenum, zinc and vitamin B_6 may be helpful. Low serotonin levels are found in some cases of autism; and serotonin precursors such as tryptophan can sometimes lead to decreases in hand-flapping and an improvement in mood [43]. As autism appears to be a heterogeneous disorder there may well be no universal therapeutic recommendation although detailed knowledge of the individual biochemistry may eventually allow a more precisely targeted approach. However, some of the biochemistry underlying this complex condition is now

better understood, raising the hope that the alterations in neurotransmitter levels and in gut function may respond to dietary modulation of vitamin, mineral and protein intake.

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