Biochemistry and Autism

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We started a long time back looking for biochemical causes of autism. It didn't seem like a purely a psychological phenomenon, which is what people were telling me. But what sort of things could possibly underlie autism? Well, a lot of parents told me at the time that their children suffered if they ate certain foods, and particularly they mentioned bananas, chocolate, cheese. This rang a bell, because we had just finished doing quite a bit of work on migraine, and most people find that bananas, cheese, chocolate are the absolute classic triggers for migraine attack.

We know quite a bit about why these foods trigger migraine. They are generally believed to inhibit enzymes which would normally inactivate neurotransmitter amines in the brain. And in the first group of autistic children that I looked at, most of their parents had migraine. So it seemed logical to start by looking to see whether something about neurotransmitter amines could be one of the underlying faults in autism. You can't do anything invasive to attach this problem, so you really have to look at things in a rather indirect way.

Quite a few researchers have said that children with autism tend to excrete aberrant levels of neurotransmitter amines in urine and that these are at high levels in the blood as well. But these amines are the ones that control mood and behavior, such as stereotypical behavior from excess dopamine in experiments with rats. In a few cases stereotypical behavior is seen experimentally in humans as well. And you can get nausea and vomiting. These are in fact some of the symptoms recorded in autistic children, so it seemed worth going on with the neurotransmitter amine hypotheses to see if we could clarify things any further. Why are these amine levels altered, if they are?

These compounds do their work in the synaptic gap between the ends of two nerve cells. The chemical, when released from a transmitting nerve cell, reaches a receptor across the synaptic gap on the receiving nerve cell. This is the way signals are transferred. What should happen once the neurotransmitter amine hits the receptor and sparks off an electrical signal, is that it should immediately be either sucked up back to where it came from or else be removed and inactivated biochemically.

Now, the major pathway in human beings for this inactivation is by adding on a sulfate group. This is done by sulfotransferase enzymes. So there are a number of possibilities for what could go wrong. If you have aberrant levels of brain amines in autistic children, there could be a problem with reuptake. There could be a problem with the sulfotransferase enzymes themselves. Or, you might find that there just isn't enough sulfate available to supply the biochemical inactivation.

Migraine patients are known to have very low levels of the sulfotransferase enzyme and also to have attacks triggered by foods known to interfere with enzyme function, so we tested a large number of foods to determine the compounds they contain. Sulfotransferase is inactivated by a lot of compounds in foods—certainly in test tubes, and probably in real life as well. These foods include flavonoids such as red wines and quite a lot of vegetables and fruits like grape fruit and orange juice. Many of the food triggers are the same in migraine and in autistic patients, so we assumed that there would be low levels of functioning sulfotransferase in autistic children.

What we seem to be looking at is that in at least some cases of autism there are problems both with low levels of the enzyme and with easy blockage of the enzyme. And there is a further complication in that compounds in quite a lot of these foods as in the banana, cheese, chocolate variety not only block the enzyme but

actually also contain a lot of the amine compounds that require the body to use enzyme.

But when we measured sulfotransferase levels in autistic children, the results did not meet our expectations. Some of them definitely do have low levels of the enzyme, but only about 12 %. These tend to be the ones whose parents have migraine indicating that the tendency for low sulfotransferase gets passed on to the next generation. So we had to go on to look at the levels of inorganic sulfate in autistic children. This turned out to be more striking. About 95 % of them have really very low levels of sulfate.

People haven't looked at sulfa chemistry until fairly recently because it is technically really extremely difficult to do. The compounds are difficult to assay. But sulfate is involved in a lot of chemical pathways in the body. It is used to inactivate neurotransmitters. Steroids, bile acids and a lot of drugs are removed from the body by first being linked with sulfate, and then compounds that show up are steroid sulfates and bile sulfates. Also, sulfate is linked into most of the body's connective tissue such as the gut wall. If the sulfate isn't there the connective tissues don't work properly.

Most of the sulfate in the body is derived not from foods but from oxidizing an amino acid called cysteine. There aren't very many results available, but so far as is known in human beings, probably only between 5 % and 20 % of the sulfate needed is absorbed across the gut wall. The pathway for breakdown of cysteine to get the inorganic sulfate is rather complicated, and if the process is not functioning properly there should be a lot of "knock-on" effects. So we measured the levels of cysteine in blood plasma of autistic children. In a control group the level is about 4.9 nanomoles per milligram of protein, but in the autistic group the level is only about 1/8 as much. That is a rather dramatic decrease and means there really isn't much sulfate available in plasma. (One of the problems with this particular assay is that the metabolism of cysteine varies according to the time of day. You have to take blood samples before breakfast and then again about 9:00. It is an irritatingly difficult thing to have to do with young patients.)

With low sulfate a lot of biologic components will not be sulfated as they should be. One of the knock-on effects will be low cholacystakinyne(sp?) or CCK, a hormone which occurs in both the gut and the brain. Without the sulfate group attached, CCK is non-functional, but it normally controls a large number of biochemical parameters such as appetite, and a quite a lot of enzyme secretions. And we suspect, though there isn't much work done on this, that since CCK is found in the brain it should effect mood and behavior.

The normal gut and gut wall contain sulfotransferase enzymes and massive amounts of sulfate, so that when you take in amine compounds in foods like banana, cheese, chocolate they are immediately sulfated. An amine in banana that could be particularly damaging is the neurotransmitter serotonin. If it is not sulfated in the gut and ends up being absorbed into the blood stream, it should effect the central nervous system and cause cerebral problems. Biochemists generally believe that the system evolved because man is descended from fruit-eating species that would not have evolved if they were high and dysfunctional on dietary serotonin. This sulfation of amines in the gut is necessary for survival.

Sulfate is also instrumental in forming the protective, slippery mucus lining along the wall of the gut. This mucus is made up of proteins with sugar residues and sulfate groups. Without the sulfate groups these mucines(sp?) are no longer really slippery, and problems occur. They no longer spread out as a thin lining all along the gut wall but instead end up as thick lumps with gaps between. This makes the gut much more permeable. Peptides, proteins not yet fully broken down into individual amino acids, can then get through and be absorbed into the blood stream. It has been shown in Italy that permeability of the gut is a factor in autism, and people who have looked at lack of sulfation in gut proteins have found that it is invariably

associated with inflammation, poor absorption and sometimes over rapid absorption. Poor sulfation definitely means GI tract problems.

Sulfation in the gut also makes it much more difficult for microorganisms to settle and colonize. We haven't worked on this, but several groups have reported in the literature. All bacteria looked at as well as candida, a yeast, tend to have a negative charge on their outside surface. They do not settle on properly sulfated gut walls, probably because of the negative charge of the sulfate. Sulfation seems to be vitally important in protecting against unwanted colonization.

Aberrant responsivity to drugs is one of the problems associated with autism. There may be non-reaction or over-reaction even to quite simple compounds. One that we have looked at is paracetimal(sp?) because it is relatively nontoxic and safe to experiment with. It is normally excreted from the body either as a sugar, that is, as a gluciaronide(sp?) conjugate, or linked to sulfate. Now, children for some reason normally produce much more of the sulfate link than adults do. But not autistic children. Their liver is not the problem. It removes many compounds normally, but the sulfation of paracetimal is greatly reduced. This backs up what we have found about cysteine and sulfate ratios. The strange responses to drugs appears to be due to near absence of this secondary method of removal of compounds from the body.

We find that a lot of the other sulfa containing anions like thiosulfate, sulfite and thiocyanate also appear to be out of balance in autistic children. Starting with sulfite we find higher levels than in control children. Sulfite is supposed to be converted to sulfate by sulfite oxidase, so perhaps one of the reasons sulfate is low is because of inadequate presence or functioning of this enzyme. We checked the possibility by supplementing diet with molybdenum, a trace metal vital for the functioning of sulfite oxidase, and sulfite levels returned to normal in about half of the autistic children tested. Elevated sulfite has been shown to make asthma much worse. Very high sulfite is associated with mental retardation and early death. But it looks as though moderate sulfite oxidase deficiency mostly causes excess excretion of sulfite in the urine. You expect it in general not to be good for the body.

We have looked at the whole cysteine pathway. It starts with the oxidation of cysteine to cysteine sulfinic(sp?) acid (by the action of cystine dioxogenase(sp?) enzyme) and then after many steps leads to sulfite and sulfate. Thiosulfate and thiocyanate are also produced along the way, and autistic children have elevated levels of thiosulfate. We don't really know why, but there could be a block in the enzyme called rhodamise. This is an enzyme found in primitive life forms, and so has been around for a long time and must be quite basic. It appears to exist for the purpose of removing cyanide, a normal low-level byproduct of body metabolism. We haven't looked at this yet, but if rhodamise is deficient, you would expect that there could be a build up of cyanide in at least some autistic children along with the elevated levels of thiosulfate. So there are a lot of candidates for suppressed enzyme function relating to sulfate in autism—sulfite oxidase, rhodamise, and perhaps the cystine dioxigenase at the top of the pathway.

And there are other possibilities for the depressed sulfate levels in autistic children. Their urine shows elevated levels of cysteine and of protein, which suggests a possible fault with the kidneys. Perhaps they are not recovering the protein in the kidneys and there is a leaky kidney problem as well as a leaky gut. The cysteine is really, I think, a bit of a mystery. Children with autism often have elevated levels of cysteine in plasma, and this is highly toxic. At high levels cysteine is known to cause damage to neurons and brain cells. We looked at the secretion of sulfate and bound up sulfates in urine and found a lot of it for autistic children. It just seems to "fall out." I have often suggested to parents that they try adding magnesium sulfate to bath water or perhaps tiny amounts to drink. This does seem to help a bit, but you can't get very much sulfate in this way,

and it just seems to fall out right away, anyhow. We don't really understand why this is, but there is a theory in the literature that if you do not recover sulfate it's partly a kidney function problem. The kidney proteins which should recover it may be nonfunctional, but we don't really know about this one yet.

So the one thing I think we really have found in autistic children is this lack of sulfate and that this is difficult to correct. It has a lot of knock-on effects like altered neurotransmitter function, changes in the workings of the gastrointerstinal tract and altered drug metabolism. Improper sulfation must be one of the factors in autism.

The "holy grail" that biochemists are looking for is a linking between clinical parameters of symptoms and behaviors with body chemistry anomalies like this lack of sulfate. We did a cluster analysis of 256 normal individuals versus 86 autistic children. Fortunately, two undergraduates were interested in helping us with this project. They found the normal volunteers from their friends and families. These were mostly children but included some college students up to age 20. We had parents fill out check-box questionnaires about a lot of possible symptoms and family characteristics and then did a cross-correlation analysis to look for clusters of answers. The number of possible question pairs is the factorial of the number of questions, so it takes a computer to do this, and the undergraduates did a nice job in completing the task.

Answers from the normal group generally showed little grouping or cross correlation except for two linked mini-clusters. People with diabetes and thyroid problems gave clinical histories that are linked. Medically this link has been known for a long time. There is an auto-immune component in both thyroid disease and diabetes. Questionnaires for the autistic children showed not only the thyroid-diabetes link but also three other distinct categories of symptoms. These were: 1) dyslexia in the family, eczema, learning difficulties and auto-immune problems; 2) poor temperature control, sweating and urination difficulties not linked with thyroid disease; and 3) aggression, sometimes fitfulness and reactions to general anesthetics as change in handedness. For the group size these were rather separate, uncorrelated groupings.

We don't know how to explain the third group, but the other two do seem to relate to sulfate deficiency. The second group could be showing malfunction or damage to the pituitary gland or the hypothalamus which surrounds it. Both depend critically on the amount of dopamine, so it is possible that if dopamine were not sulfated adequately there would be improper function of the pituitary and hypothalamus and then these autonomous system problems. The first group with auto-immune problems is far more clearly related to sulfate. Several studies have shown that low levels of sulfate in plasma seem to be associated with allergy in general. Exactly why is not entirley clear, but it is probably because sulfation is such a vital part of protein metabolism and recognition. Improperly sulfated protein might not be recognized as "self" and be seen instead by the body as "foreign," which is the basis of allergy reactions. So in two of the subsets you can see that problems with sulfation might underlie the symptoms. Sulfation doesn't explain everything, but it looks like a major factor in the puzzle of trying to explain autism biochemically.

We need to do more in the future with evaluating these clinical-symptoms questionnaires. A difficulty is that you don't know what questions to ask at first until you have done a trial, so then you have to go back to the beginning and start over again. And some of the questions have to be designed to give you a response that is "normal" from everybody. This statistically improves discrimination ability. We'll be doing more to measure sulfate levels. And we hope to collaborate with colleagues and be able to examine the same patients they have seen and look for more correlation between biochemical profiles and autistic symptoms.