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LETTER TO THE EDITOR

Can humans metabolize arsenic compounds to arsenobetaine?

Dear Sir,

We appreciate the concern expressed by Dr Edmonds in his letter about the correctness of our suggestion that humans are able to form arsenobetaine when appropriately challenged, for instance by trimethylarsine.[1] Our study had been conducted because high concentrations of total arsenic compounds had been observed in the urine of one of our co-workers, who occasionally synthesizes organic arsenic compounds needed as standards. To obtain information about potential sources for this arsenic, the arsenic compounds were quantified in urine samples before, during and after synthesis of trimethylarsine from arsenic trichloride and methylmagnesium iodide in dibutyl ether. The preparation of approximately 100 g of trimethylarsine requires one week. During the addition of the Grignard reagent to the stirred solution of arsenic trichloride in dibutyl ether the reaction mixture is cooled in an ice–water bath and the assembly is kept in a well-ventilated hood. Exposure to trimethylarsine during this procedure is minimal, because the reaction mixture is kept at ~0 °C and the door of the hood needs to be opened only infrequently to adjust the stopcock of the dropping funnel and to replenish the ice for cooling the reaction flask.

On 10 October a graduate student distilled the trimethylarsine/dibutyl ether mixture from the reaction flask and on 11 October he distilled the trimethylarsine/dibutyl ether mixture to obtain pure trimethylarsine. Whenever trimethylarsine is prepared or distilled, the very distinct odor of this compound is noticeable in the laboratory in spite of all work proceeding in a well-ventilated hood with the doors of the hood closed to effect optimal ventilation.

Our urine-donor was not involved in the distillation of trimethylarsine on 10 and 11 October. However, he inhaled the laboratory air whenever he passed through the laboratory to his office, which is adjacent to the hood in which the synthetic work was carried out. The exposure to trimethylarsine or any other arsenic compound cannot have been excessive, because the concentrations of the arsenic compounds in the urine from 10 to 12 October were only slightly elevated. Our urine-donor distilled trimethylarsine himself on 12 and 13 October. Because the distillation has to be watched closely, the argon stream through the distillation unit must be adjusted and receiving flasks must be changed, the potential for exposure to trimethylarsine during this operation is much greater than during the time when the Grignard reagent had been reacted with arsenic trichloride.

Edmonds finds it unlikely that a worker, presumably taking all reasonable precautions when handling a toxic substance and carrying out the operation in an efficient fume hood, could inhale enough arsenic to excrete 130 μg As in his urine.

A person inhales ~360 liters of air per hour. To complete two distillations requires approximately six hours. If trimethylarsine, a good complexing agent, was completely retained (not an unreasonable assumption, when molecular oxygen, a weak complexing agent, constitutes 20% of the inhaled air and 2% of the exhaled air), then the ~2000 liters of air breathed in during six hours must only contain 130 μg As (~2 μmol). Each liter of this air would have to have only ~1 pmol of trimethylarsine. The partial pressure of trimethylarsine at this concentration is 1.7×10^{-5} Torr (vapor pressure 298 Torr at 25 °C).[2] Because the distillation is carried out under a slow stream of argon, receiving flasks must be changed and the distillation apparatus disassembled (operations that deliver trimethylarsine with a vapor pressure of ~300 Torr at room temperature to the air in the hood), the concentration of 1 pmol/l^{-1} is very likely not only to be reached but also exceeded, at least temporarily. Edmonds proposed that the arsenic excreted during the period 10–17 October came from the diet (mineral water, medicine, tonic, contaminated foodstuff, wine, grapes or other fruit, health food, fishy component to a meal) or from hand-to-mouth contamination. During the time urine was collected, the urine-donor recorded all the food he had consumed. The food items are not expected to contain arsenobetaine, with the exception of the codfish eaten on 6 and 19 October. The food items consumed were analyzed neither for total arsenic nor for arsenic compounds.

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Our co-worker was aware of the importance of arsenic compounds in the diet. He recorded all the food he had consumed during the study. The mineral water and wine that were part of the meals were the same throughout the study. On 10 October he had coffee and white bread with butter and jam for breakfast, a breakfast typical for the study period. Lunch consisted of apple slices dipped in batter and fried, and dinner of green salad with a few slices of bread. On October 12 a vegetable plate was served for lunch and yoghurt for dinner. He did not take any medicine and did not drink any tonic water.

Edmonds suggests for our co-worker an intake of 50 μg As as inorganic arsenic on 10 October. This amount of arsenic cannot have been delivered with the constituents of breakfast, because breakfast had not been changed during the study period. If the arsenic had been contributed by the apples consumed at lunch, the apples (∼250 g eaten, wet mass) must have been extraordinarily rich in arsenic (∼200 μg kg^{-1}, wet mass). The salad eaten for dinner is also an unlikely source for 50 μg As. Arsenic concentrations in Styrian salad samples are ∼100 μg As kg^{-1} (dry mass). Under the assumption that salad contains 90%, water, 250 g fresh salad can supply only 2.5 μg arsenic. The diet record did not reveal any food components as likely sources for 50 μg As. The 80 μg arsenic suggested to have come from the diet on 12 October (small fish meal, fishy component to a meal) is also unlikely to be diet-derived. Inspection of the diet record provided no indication of the consumption of arsenic-rich or arsenobetaine-rich components. Intake of arsenic by hand-to-mouth contamination is always a possibility, although a very unlikely one, because all of our co-workers wash their hands after working with arsenic compounds before they lick their fingers.

Considering all the possibilities of arsenic exposure and the olfactory fact that the odor of trimethylarsine was noticeable even outside the hood in which trimethylarsine was prepared, the exposure to trimethylarsine in the laboratory is at least as likely as the suggested intake with food. Edmonds states that our suggested conversion of trimethylarsine to arsenobetaine is not supported by previous work. The high concentrations of arsenic in the consumed food that are needed to account for an intake of 50–80 μg As per day are also not supported by previous work.

The pattern of arsenic compounds excreted during the period during which trimethylarsine was prepared is as strange to Edmonds as it is to us. Why the concentrations of arsenate in the urine during the period 9–12 October increase four-fold, and the concentrations of methylarsonic acid and dimethylarsinic acid five-fold over the background concentrations, and why the concentrations of arsenite remain at background, whereas the concentration of arsenobetaine decreases to background, cannot be explained, because sufficient knowledge about the pharmacokinetics of arsenic compounds in the human body does not exist.

Edmonds states 'Arsenobetaine was excreted, starting on 13 October, in a pattern which indicates a single dose on 12 October. Indeed, the pattern of excretion was almost identical to that following the first fish meal (that taken on 6 October). So, was arsenobetaine built up in the body then suddenly excreted starting 13 October even though the patterns of excretion of the other arsenic compounds (all supposedly metabolites of trimethylarsine) were changing? This seems also unlikely.'

A single dose of arsenobetaine on 12 October from the diet or any other source is extremely unlikely. The rather massive dose of trimethylarsine on 12 October is a fact. The high concentration of arsenobetaine in the morning urine of 13 October does not require a build-up in the body. During the six days preceding 13 October the concentration of arsenobetaine in the morning urine decreased practically to background on 12 October. This decrease can be attributed to the excretion kinetics of arsenobetaine introduced into the body through fish consumption on 6 October. A contribution to arsenobetaine in the body from the postulated conversion of trimethylarsine from low-level exposures to arsenobetaine prior to 12 October is not unlikely but cannot be proven from the available data. However, another plausible cause for the excretion of 33 μg As (0.44 μmol arsenobetaine), other than the conversion of trimethylarsine to arsenobetaine, cannot be given. The concentrations of arsenobetaine in the morning urines from 13 to 19 October decrease, producing an excretion pattern similar to the patterns observed after fish consumption. This similarity cannot be taken as unequivocal proof that arsenobetaine excreted on 13 October comes from the diet.

If arsenobetaine is produced from trimethylarsine in the body and the overall rate for this conversion is rapid compared with the rate of excretion (half-life of arsenobetaine in the body ≃30 h) the excretion pattern should be independent (as observed), irrespective of the source of arsenobetaine (ingested or synthesized in the body). That organisms are capable of making arseno-

betaine from organic arsenic compounds, and ultimately from inorganic arsenic compounds, is an indisputable fact. Whether trimethylarsine is one of the precursors of arsenobetaine in biological systems is not known, but it is worthy of consideration. Chemically, such a reaction is very reasonable.

The excretion patterns for arsenate, methylarsonic acid and dimethylarsinic acid show that the concentrations of these compounds in the morning urine are elevated above background after fish consumption and also after workplace exposure to trimethylarsine. These arsenic compounds are constituents of fish and will also be excreted through the urine. Should dimethylarsinic acid, methylarsonic acid, and perhaps even arsenate not only be derived from diet and excreted unchanged, but also be formed by oxidation/reduction and methylation reactions of less methylated arsenic compounds (reaction sequences experimentally proven for the human organisms, with dimethylarsinic acid as the most highly methylated arsenic compound) and perhaps even by demethylation of more highly methylated arsenic compounds such as trimethylarsine, trimethylarsine oxide and arsenobetaine (reaction sequences not experimentally proven in the human organism), the perhaps small concentrations coming from demethylation processes to the excreted arsenic compounds will be difficult to ascertain. If, however, the exposure to a highly methylated arsenic compound such as trimethylarsine also leads to the excretion of arsenate, methylarsonic acid and dimethylarsinic acid, the capability of the human body to demethylate arsenic compounds must be questioned. We raised this question to alert the interested scientific community but never stated that we had proof of such processes. Demethylation of arsenic compounds is known in microbial systems. Because the methylation of trivalent arsenic compounds (the only arsenic compounds that can be methylated) is an oxidative process (conversion of trivalent arsenic to pentavalent arsenic, e.g. arsenite to methylarsonic acid), the demethylation should be a reductive process. The reduction–methylation sequence in producing methylated compounds should be joined by a demethylation–oxidation process in removing methyl groups from arsenic compounds. Much needs to be learned about the mechanism of the demethylating reactions.

Edmonds is surprised that trimethylarsine oxide was not present in the urine. The HPLC–HHPN–ICP–MS method we used for the identification and quantification of arsenic compounds in the urine is capable of baseline separation of arsenite, arsenate, methylarsonic acid, dimethylarsinic acid, trimethylarsine oxide and arsenobetaine. The retention time for trimethylarsine oxide under the conditions employed is 240 s. Signals for this arsenic compound were not present in any of the chromatograms of the urine samples. Consequently, trimethylarsine oxide could not have been present in the urine in concentrations above 1 μg As l^{-1}. The results of Yamauchi *et al.*,[3] from experiments in which trimethylarsine was administered (p.o., i.p. in olive oil) to mice and hamsters and trimethylarsine oxide was identified in urine, are not proof that a conversion to arsenobetaine or a demethylation cannot occur in the human body. The p.o. and i.p. doses of trimethylarsine to the animals were orders of magnitude higher than the inhalative dose to our co-worker. The trimethylarsine–arsenobetaine pathway could have become saturated, and most of the trimethylarsine would be oxidized to trimethylarsine oxide. Small amounts of arsenobetaine in the samples are probably not detectable by the GC–FAB–MS method. In addition, mice and hamsters do not have to metabolize trimethylarsine in the same way as humans.

In our paper we reported analytical results for arsenic compounds in urine and suggested as an explanation that the human body can do more than methylate arsenic compounds. We never claimed to have proof that the human body can convert trimethylarsine to arsenobetaine. We are also aware that our suggestion is not supported by the literature. However, new facts and new hypotheses necessarily will have to be reported sometime for the first time. Where researchers were constrained to publish only results that are supported by earlier papers, scientific progress would be slow or it would stop altogether. As an example, the report about arsenobetaine in terrestrial biota did not have any precedent in the literature. Before 1995, complex organic arsenic compounds such as arsenobetaine, arsenocholine and arsenic-containing riboses were thought to occur only in marine organisms. The most complex organic arsenic compound known in terrestrial biota was dimethylarsinic acid. In 1995 we reported that arsenobetaine is present in mushrooms.[4] This paper did not elicit any comment to the effect that arsenobetaine is unlikely to be a constituent of mushrooms because no one else had found it in any terrestrial sample. During the past three years arsenocholine and tetramethylarsonium cation were identified in mushrooms[5–8] and arsenoriboses were detected in water/methanol extracts from earthworms.[9] Fortu-

nately, researchers are free not to accept 'common knowledge' and published facts as revealed truth for the sake of the advancement of science.

We will be very pleased if our suggestions that humans can convert trimethylarsine to arsenobetaine and perhaps can demethylate methylarsenic compounds prove to be correct. The reaction trimethylarsine→arsenobetaine would be the short route from an arsenic compound with good complexing ability to an almost innocuous arsenic compound that can be readily and quickly excreted. The introduction of a C_2-unit into a methylated arsenic compound must occur in biota, otherwise arsenocholine and arsenobetaine could be present neither in marine nor in terrestrial organisms. Should our hypotheses turn out to be incorrect on the basis of further experiments, we will stand corrected but still have the satisfaction of having raised the issue and served as initiators of further experiments that will have advanced our knowledge about the fate of organic arsenic compounds in the human body.

We thank Dr Edmonds for having started the discussion about our new suggestions. Because of his concerns over the likelihood of our explanations for the analytically secure results, we were forced to explore in more detail our experimental protocol and were led to develop new experiments. The 'self-correcting' process of science has begun that will ultimately cleanse our science of all incorrect 'facts', hypotheses and suggestions, and deepen our understanding of the cycling of arsenic compounds in nature.

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