

IN SEARCH OF NON-INDUSTRIAL SOURCES OF ORGANIC FLUORINE
IN NORMAL HUMAN BLOOD FROM THE GENERAL PUBLIC.

Synopsis:

The existence of two fractions of fluorine in human and animal blood , one inorganic and the other organic, has been established by two diverse studies carried out independently by Taves at the University of Rochester, New York (1) and by Venkateswarlu, Singer and Armstrong at the University of Minnesota (2). Perfluorooctanoic acid or a similar compound was reported to be present in a cut from the organic fluorine extracts from normal human blood (3).

However, the presence of organic fluorine compounds of non-industrial origin in normal blood has not been precluded. In other words, organic fluorine from natural sources could be present in normal blood. For example, certain bacteria can convert inorganic fluoride into organic fluorine and so can certain plants. Through this means organic fluorine from natural sources (i.e. of non-industrial sources) could enter the food cycle and consequently show up in normal human blood.

Further, there is evidence that rats exposed to sources of inorganic fluoride show in their blood two fractions of fluorine. One of the fractions is definitely inorganic (responds to the fluoride ion electrode). The precise nature of the other fraction is not clearly understood so far. It could be a very complex form of inorganic fluoride (that does not respond to the fluoride ion electrode) or organic fluorine in which fluorine is covalently bound. There is some evidence that it is probably organic in nature. However, I consider that the evidence is not definitive, because the analytical methods employed in these investigations have certain flaws. If we can confirm the presence of organic fluorine in this unknown fraction by using sound methods (which are now available), we would be generating some valuable information. As a result, the present perception that organic fluorine in normal blood perhaps is exclusively of industrial origin could change for the better.

It is in the interest of 3M to strengthen the evidence of non-industrial sources of organic fluorine in normal human blood. 3M would be making a significant contribution to the knowledge of sources of organic fluorine in blood. This would be important from public health and environmental perspectives as well.

It could cost approximately \$20,000 to carry out this work, mostly at the University of Minnesota under the supervision of Dr. Robert H. Ophaug. (Dr. Ophaug is a former colleague of mine when I was at University of Minnesota Medical School; he is a well recognized investigator of fluorine metabolism).

(A proposal submitted by Venkateswarlu Pothapragada, Specialty Materials Division, Analytical Laboratory)

Introduction

Ever since the establishment of the existence of two fractions of fluorine (inorganic and organic) in human and animal blood (1,2) there has been considerable interest in trying to understand the nature and origin of organic fluorine in blood. Perfluorooctanoic acid or a similar compound was the only compound so far reported to be present in a concentrate of one of the several cuts of organic fluorine obtained from a large pool of normal blood from several donors(3). The so-identified fluorochemical could have come from just one or a few individuals and probably the fluorochemical is not present in the blood samples of all donors. Unfortunately, this finding, implicating industrial fluorochemicals as the source of organic fluorine in normal human blood, has virtually killed all interest in looking for non-industrial sources of organic fluorine in human blood. The presence of other organic fluorine compounds, both natural and synthetic, in blood serum should not be precluded.

Non-industrial sources of organic fluorine

Organic fluorine in foods:

The evidence for the presence of organic fluorine in foods we consume is not definitive; nevertheless, the presence of some organic fluorine in certain foods is nowadays being suspected.

Organic fluorine biosynthesis in plants, bacteria and possibly in mammals:

It is known that certain plants like "Gifblarr", *Dichapetalum cymosum* (*Chaillietia cymosa*), and *Acacia georginae* convert inorganic fluoride to organic fluorine. The microorganism, *Streptomyces Calvus*, also converts inorganic fluoride to organic fluorine. Is it possible that some mammalian systems also are capable of converting inorganic fluoride into organic fluorine?

If so, we have yet another source of organic fluorine right within our physiological system itself. There is some evidence that this might be so (4,5). Ophaug and Singer (4) raised rats on a low-fluoride diet with different levels of fluoride ingestion via drinking water, and Morris and Smith (5) exposed rats to different levels of HF gas in the chamber air. Both these investigators report a progressive increase in blood inorganic fluoride (which responds to the fluoride electrode) with fluoride exposure time and fluoride levels, which is to be expected. They also report a corresponding increase in yet another fraction of fluorine which is revealed only after ashing. Because ashing converts organic fluorine into inorganic fluoride, these authors and others suspect that this fraction could represent organic fluorine synthesized from inorganic fluoride by the rat (a mammalian system). The ashing of samples is carried out in a muffle furnace in platinum dishes with a fixative like calcium oxide to reduce loss of organic fluorine during the high temperature of ashing (500 - 600 °C). This is called the open ashing technique.

The problems of open ashing technique:

In normal human and animal blood samples (1, 2) and in rat blood samples from the above experimental studies (4 and 5), the concentrations of organic fluorine in blood are very low, i.e. below 0.1 ppm compared to more than 1 to 30 ppm F we encounter in some of our plant workers. As was emphasized by this author (6), at such low levels of organic fluorine, results obtained by open ashing are subject to two potentially serious sources of error, (a) loss of fluoride during ashing, which would give a false low value and, (b) contamination with extraneous fluoride from fluorochemical dust borne air or with traces of fluoride from the furnace brick walls, which would give a false high value. In the light of these facts, it is difficult to state that what the investigators observed was truly a new fluorine fraction in blood or it was an artifact due to extraneous fluoride contamination during the process of open ashing.

Overcoming the problems of open ashing:

It was to preclude these sources of error that the author of this proposal developed the oxygen-bomb technique and the sodium biphenyl method (6,7). Unfortunately Ophaug and Singer, as well as Morris and Smith used open ashing techniques. However, these individuals are very careful investigators and I have no doubt that they ran several blanks and took all possible precautions to validate their findings and conclusions. Since these findings are corroborated by two different groups of workers, I am prone to believe that there indeed (or perhaps) is a new fraction of fluorine in blood of these rats exposed to inorganic fluoride. This new fraction is definitely not free inorganic fluoride. Is it inorganic fluoride so complexed that it does not respond to the fluoride ion electrode? Or, is it an organic fluorine compound with a C-F covalent bond in which fluorine is revealed only after ashing? That is what we like to find out. This is the foundation of the present proposal.

Usefulness of the sodium biphenyl method:

At these low levels of organic fluorine spectroscopic methods are not sensitive. However, if we can demonstrate release of fluoride ions (which can be readily detected with the fluoride electrode) following decomposition of the unknown fluorine fraction in blood with the sodium biphenyl reagent, we would have good evidence of the presence of organic fluorine (i.e. covalent fluorine) in blood of these experimental animals.

The final phase:

When once the presence of organic fluorine in the blood of experimental rats is confirmed, we will develop experiments to elevate the levels of such compounds in the animal as well as to fractionate and concentrate the organic fluorine-containing compounds for characterization by spectroscopic techniques such as NMR, GC/MS etc.

The Present Proposal

The present proposal is to repeat some of the above studies (in which the rats were exposed to sources of inorganic fluoride at physiological levels) and look for organic fluoride in body fluids and tissues, employing methods for organic fluorine which are more reliable than the methods used before. In the new study, the open ashing technique would be replaced by the sodium biphenyl method.

Organic extracts of the samples (which are rendered devoid of inorganic fluoride) will be decomposed with sodium biphenyl reagent. Release of inorganic fluoride ions following this step would be a definitive indication of the presence of covalent fluorine in the samples. This, then, would constitute the evidence that organic fluorine compound(s) can be naturally biosynthesized in mammalian systems as well. And, therefore, organic fluorine in normal human blood may not be necessarily of industrial origin.

Following this, attempts will be made to concentrate the organic fluorine so revealed and characterized by appropriate spectroscopic techniques.

It is proposed that most of this work will be conducted at the University of Minnesota, under the supervision of Dr. Robert Ophaug, Department of Biochemistry, School of Dentistry, who was a former collaborator and colleague of mine before I joined 3M. If the presence of organic fluorine in blood of rats, ingesting physiological levels of inorganic fluoride, is confirmed Rick Payfer, Tom Kestner and other members of the SMD/Analytical Laboratory, will be involved in characterizing the organic fluorochemical(s). Since the levels of organic fluorine at physiological levels of exposure will be very low, the sodium biphenyl method (presently used to determine significantly higher levels of organic fluorine in blood) needs to be appropriately modified. Venkateswarlu Pothapragada will help Dr. Ophaug in this regard.

Following the approval by the Steering Committee, the necessary protocols will be prepared for review by the Fluorochemical Technical Advisory Committee.

References *

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