10/22/56
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Problem: What happens to tetraethyllead (TEL) in the body ?

Work of previous investigators:

No publication bearing directly upon this problem has appeared during the past year, other than our paper in the Journal of Pharmacology and Experimental Therapeutics for August, 1956. Earlier work is cited in my previous reports.

Plan of attack:

Two main objectives are evident:

(1) reactions of TEL *in vivo*, - their rates, products and mechanisms, and

(2) distribution of TEL and of its decomposition products among tissues under various conditions.

To approach these objectives requires the development of analytical methods for TEL and for its decomposition products.

Progress, 1953-55:

Our work has been concerned primarily with development of analytical methods for detection of TEL, of triethyllead ion and of diethyllead ion in tissues. Methods of measuring TEL in water and in air were developed. These were applied in the determination of the solubility of TEL in water. Several technics were devised for the extraction of organolead

Progress (continued):

compounds from tissue without extraction of inorganic lead. Their application gave evidence that both TEL and triethyllead ion occur in liver after TEL inhalation. All this is detailed in earlier reports.

Progress, 1956:

The evidence suggesting that TEL can be isolated from tissue was pursued. Without elaborating the matter, suffice it to say we obtained fairly conclusive evidence of the presence in liver of TEL after its inhalation. This was published in the Jour. Pharm. & Exptl. Therap. in Aug., 1956.

Rather than follow the problem further in that particular direction, we now attacked the question of what organolead decomposition products of TEL may also be found in the body. In addition to the interference of such compounds in analyses for TEL, these decomposition products are of interest because intoxication by TEL may depend upon their formation and yet not a single decomposition product of TEL has ever been identified as occurring in the body or excreta. Could one or more such compounds, particularly those containing lead, be found in considerable quantity in tissues, our understanding of intoxication by TEL would be notably advanced.

Progress (continued):

Various clues we had uncovered pointed to the presence of triethyllead ion in tissues. There was less indication of diethyllead ion. We sought to extract triethyllead ion as a salt, the chloride, from livers of rats intoxicated with TEL. Fractionation of the extract proceeded through a series of steps, each of which had to be worked out and tested. That fraction which contained organolead salts was brought into aqueous solution, the salts were converted to hydroxides, the hydroxides to derivatives that would lend themselves to further isolation procedures. As derivatives, the benzoates proved most useful of all the compounds we synthesized and tried. The benzoates then had to be freed of considerable quantities of fatty material carried along by the processes of the isolation. A further series of steps yielded concentrated solutions showing a number of absorption maxima in the infrared. These maxima corresponded with those of solutions of triethyllead benzoate. The maxima of solutions of diethyllead dibenzoate, of TEL, and of benzoic acid were absent from the solutions as finally prepared from liver extracts. Now, ancillary evidence is being obtained that will, I trust, confirm and complete this identification.

Progress (continued):

In further exploration of the relation of TEL intoxication to cholinesterase activity, two experiments were done. Both suggested that cholinesterase activity is much less sensitive to the decomposition products of TEL than to inhibitors such as neostigmine. No inhibition of cholinesterase activity by TEL and its decomposition products as formed *in vivo* was found in qualitative histochemical tests of diaphragms of intoxicated rats. Nor did either triethyllead chloride or diethyllead dichloride, each in 10^{-5} molar concentration, decrease the cholinesterase activity of human plasma *in vitro*.

Proposal for continuation:

I suggest the work continue along these lines:

1. Completion of evidence for occurrence of triethyllead ion in liver after inhalation of TEL vapor.
2. Search for diethyllead ion in liver after inhalation of TEL vapor.
3. Development of technics for measurement of TEL and of its organolead derivatives in tissues.
4. Application of the quantitative technics to the problems noted above under "Plan of attack."