Problem: What happens to tetraethyllead (TEL) in the body ?

Work of previous investigators:

In last year's report the pertinent work of earlier investigato was cited and the paucity of data was noted. Since then only one item of direct interest has been published. In "A Case of Poisonin with Tetraethyl Lead in Well Water" (Ostapenia, et al. '54), it is stated, "Analysis of the water gave 10-15 mg. TEL / liter." There is no indication of the method of analysis. These values are difficult to reconcile with my tentative finding of 0.4 mgm. of TEL per liter of water saturated with TEL.

Plan of attack:

At the beginning two main objectives are evident:
(1) distribution of TEL among tissues under various conditions, and
(2) reactions of TEL in vivo, - their products, rates & mechanisms.

To approach these objectives, analytical methods must be developed, (a) methods of measuring TEL and (b) methods of detecting and of measuring its decomposition products. No methods for TEL in water have been published. The few methods that have been applied to measure TEL in tissues are inadequate for present purposes; they lack specificity and they have not been demonstrated to recover TEL, as such, after its addition to tissue.

Progress, 1953 & 1954:

In 1953 analytical methods were developed in this laboratory for measurement of TEL in air and in water. These were applied in determining the air-water partition of TEL and the solubility of TEL in water.

In 1954 problems of tissue analysis and of tissue distribution were investigated. Use of pentane and other solvents for extractio of organolead compounds from tissues was explored. Concurrently, some of the extraction technics were applied to tissues of animals after their exposure to TEL. Evidence was obtained of the persistence of pentane-soluble lead in live animals for several days, of its accumulation in the liver, of its excretion into the G-I tract, and of its distribution among various organs and tissues

As necessary aids in the search for possible decomposition products of TEL formed in vivo, a number of triethyllead and diethyllead compounds had to be synthesized. Methods for their characterization in extracts of tissues had to be developed. For the first time, absorption spectra of several ethyllead derivatives were measured. These were used as analytical tools.

Upon making extracts of tissues of animals after their exposure to TEL and testing these extracts for presence of ethyllead degradation products of TEL, evidence was obtained that triethyllead ion may be formed in the body.

Progress, 1955:

This finding was of such interest that we followed it up this year by pharmacologic experimentation in mice. One implication of the finding is that TEL, in decomposing, forms a substance which is known to inhibit choline esterase activity. Other evidence - such as the "bloody tear" we have observed in rats - points toward this, too. Hence drugs protecting against such inhibitors might protect against TEL intoxication. In the fall of '54 there had just appeared an account of the protective action of curare, atropine and hexamethonium ion against several inhibitors in mice. We injected these three drugs into mice exposed to TEL. Their lives may have been prolonged but not sufficiently to lead us to pursue the work further then. Results suggested need for more detailed analysis of the problem.

One unexpected observation was of interest: mice quickly exhibit effects of very low concentrations of TEL in air (0.2 mgm. TEL / liter), reminiscent of the responsiveness of canaries to monoxide. No other animals have shown such obvious, high sensitivity to TEL.

Progress (continued):

Main progress this year, as proposed last year, has been in analysis of tissues. That is, technics of extracting, isolating, characterizing and measuring TEL and its organolead derivatives are being developed.

The principal developments have been:

(1) a method for extracting (from liver) unidentified organolead compounds formed in metabolism of TEL, without extraction of inorganic lead,

(2) a method of extracting TEL with only minimal quantities of other organolead compounds and without inorganic lead, and

(3) a continuing search for unambiguous proof of the presence of TEL, as a chemical individual, in tissues after inhalation of TEL vapor.

Incidental to these developments many observations have been made of the stability and instability of TEL and of a number of triethyllead and diethyllead compounds under various conditions of analytical and biological importance.

Progress (continued):

(1) A method for extracting unidentified organolead compounds formed in metabolism of TEL

Extraction of organolead compounds from homogenized tissues of an animal after exposure to TEL proved to be a slow process. For example, even after 54 hours of continuous extraction with pentane, appreciable amounts of lead (micrograms) were still being leached out of a gram of liver. By that time, decomposition of the liver was obvious.

I had the good fortune to hit upon a simple method of hastening this extraction. One can better than halve the required time by saturating the aqueous tissue homogenate with ammonium chloride. With this technic two-thirds of the [illegible] lead is removed from liver in 4 hours and 95% in 20 hours. Thus, we have a technic which permits assurance that extraction is exhaustive, i.e., that no more lead extractable under these conditions remains in the tissue residue. Curiously enough, only organic lead is extracted, for color tests of these extracts (with dithizone) show presence of no inorganic lead. Even if a thousand micrograms of inorganic lead are added to a gram of tissue, none appears in the extract.

Progress (continued):

(2) A method of extracting TEL with only minimal quantities of other organolead compounds.

A method of extracting TEL without its decomposition products was also developed. Previous work at the Ethyl Laboratories had shown that certain decomposition products of TEL can be washed from gasoline with alkali. But when alkali was added to tissue in the continuous extraction procedure, uncontrollable emulsions were formed. Again, we were fortunate in finding ways around this difficulty. The essential requirements are (a) that tissue be diluted with only a small volume of alkali and (b) that extraction by shaking be used rather than continuous extraction. The new technic successfully suppressed extraction of inorganic lead and of triethyllead ion when these known chemicals were added to normal liver with TEL. And when the technic was applied to the livers of rats after their exposure to TEL, less than 8% of the extracted lead, on the average, was found to be non-volatile. In other words, minimal contamination of extracts with unwanted lead compounds had occurred. An advantage of this shaking technic is that it can be carried out much more easily at lower temperatures than can continuous extraction. The number of successive extractions necessary for exhaustion of TEL from tissue is undetermined at present.

Progress (continued):

(3) A search for unambiguous proof of TEL in tissues

For several reasons we are attempting to identify TEL in alkaline tissue extracts:

(a) to learn whether TEL can actually be isolated from tissues (after exposure to TEL vapor) and identified as a chemical entity,

(b) to learn whether most of the lead is in volatile combination, as validation of our method for TEL in tissue,

(c) to learn whether volatile lead compounds other than TEL are present in tissue in demonstrable quantity after exposure to TEL, and

(d) to learn whether extracts can be freed of TEL sufficiently well to leave non-volatile organolead compounds uncontaminated in the residue for investigation.

For this work we found that rather large quantities of TEL - many milligrams - are needed for our analytical procedures, e.g., infra-red spectra. These quantities must, of course, be obtained from tissues of animals after their exposure to TEL. We had been exposing one rat at a time and extracting 0.5 to 1 milligram of lead from his liver. Now we tried rabbits. They were given TEL, some by inhalation and others by intravenous injection. But all died with quantities of TEL only one-tenth to one-twentieth as great as those lethal for rats. So we contrived to expose five rats at a time to TEL vapor. Extracts of their livers were pooled,

Progress (continued):

concentrated below 0° C., and combined with extracts from more rats. After concentrates containing several milligrams of lead had thus been accumulated, the yellow greasy material was vacuum distilled at 30° C.. Distillates were examined by infra-red spectroscopy. The spectra showed several absorption peaks corresponding to peaks in the absorption spectrum of TEL. Experiments designed to give confirmation are in progress.

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C. S

Proposal for continuation:

I suggest my work continue in the next year a

1. Search for proof of TEL in liver after inhalat
This is the work now underway.

analytical technics for measure

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