ISOLATION FROM LIVER OF TETRAETHYLLEAD AFTER ITS INHALATION

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Several investigators have obtained volatile lead from certain tissues of subjects intoxicated with tetraethyllead (TEL) and have assumed that this volatile material is indeed TEL and that TEL, as such, is present in the tissue (Norris and Cettler, 1925; Kehoe and Thamann, 1931; Mortensen, 1942; Bikhovskaya, 1948; Kröller, 1949). Certain potential defects in the isolation and analytical technics open these assumptions to question (Kehoe and Thamann, 1931; Calingaert et al., 1939; Calingaert et al., 1940; Calingaert et al., 1948; Heap et al., 1951; Looper et al., 1954).

To clarify the problem, a qualitative analytical method more specific for TEL was developed and applied to analysis of liver tissue from rats exposed to TEL. The description of this method and the results of this application are the subjects of this report.

METHODS. Exposure of rats to TEL was made in this way. Carworth rats were encased individually in wire mesh and immersed up to their necks in glass jars which were filled with water and set in a water bath. Two-tenths ml. of TEL was placed in a shallow glass cup which was clamped to one of the jars. A twenty liter glass jar was inverted over them, forming a water-sealed chamber. Air was at once partially evacuated until only four to five liters remained. Sodium hydroxide was added to the water bath and oxygen was bubbled into the inverted jar, keeping the gas volume fairly constant and the water level just below the heads of the rats. Light was kept at a minimum by placing the entire apparatus in a dimly lighted fume hood and by using an infrared heater to increase vaporization. The heater was turned on for 3 minutes out of each 15 minute period. An attempt was made to maintain the air in the jar nearly saturated with TEL (8 to 9 mgm. per liter). After 4 to 7 hours the animals were killed by the addition of carbon monoxide.

The rats' livers were homogenized for 5 minutes in a chilled Waring Blendor. Aliquots were rinsed into liter bottles with 0.8 of their weight of water. Here they were mixed with 0.11 of their weight of a normal solution of sodium hydroxide. Redistilled n-pentane was added (37.5 ml. per gram of liver) and the bottles were shaken vigorously for 1/2 hour. They were stored overnight at -24 C. The pentane was decanted through Whatman No. 1 paper, the residue thawed and the pentane again decanted. This procedure of pentane extraction was repeated. Not all the

pentane-soluble lead was extracted from the homogenate.

Pentane extracts from 12 rats (approximately fifteen liters) were combined and concentrated to about 45 ml. volume by distillation in vacuo below 0 °C. in a dimly lighted room. The distillate was collected at -70 ° C. and set aside for lead analysis. The residual concentrate (45 ml.) was rinsed into a 250 ml. flask with pentane and the solvent removed below 0°C. with an aspirator. The residue was subjected to vacuum distillation (1.5 to 2 mm. Hg) at 30° C. for 2 hours while the distillate was collected at -70° C. on a cold-finger condenser, using an arrangement similar to that described by Quaife and Harris (1948). The condenser was then transferred to a special sample holder [modified from the design of DeTar and Gold (DeTar and Kazimi, 1955)] and there rinsed off with 5 to 6 ml. of pentane. All but 1 ml. of this pentane was removed below 0° C. with an aspirator. The infrared absorption spectrum of this pentane solution was measured in a cell of 1 mm. thickness with a model 21 Perkin-Elmer double beam spectrophotometer. A second distillation was made of the residue in the flask (0.3 to 0.6 mm. Hg) at 30° C. for 1 hour, and this second distillate was similarly processed. After their spectrophotometric measurement, these two final distillates were analyzed for lead by an unpublished modification of the high pH dithizone procedure.

Because earlier investigators suggested that tissues of animals intoxicated with TEL might contain triethyllead and diethyllead ions, it became important to rule out the

possibility that these substances were included in the analytical method just described. Both indirect and direct evidence ruled against this possibility. Thus the fact that triethyllead ions and diethyllead ions react directly with dithizone whereas pentane extracts of livers taken from rats immediately after treatment with TEL gave no such reaction is presumptive evidence that these compounds if present in liver were not removed by the extraction procedure. Another kind of evidence was provided by control experiments involving attempted recovery of TEL, triethyllead chloride and lead nitrate when added to liver homogenate. E.g., in one of these experiments, 17 gms. of liver homogenate were mixed with the usual proportions of alkali, water and pentane, together with 270 micrograms. of triethyllead chloride, 1600 micrograms. of lead nitrate and 525 micrograms. of TEL. Twenty ml. aliquots of extract were reacted with dithizone. No color appeared other than that slight amount regularly found in the reagent blanks. Sensitivity of the method used in this particular test permitted ready detection of 1 microgm. of triethyllead ion or of inorganic lead. Retention in alkaline homogenate of materials giving color with dithizone was less complete when livers were taken from rats a day after their exposure to TEL; whether this color was due to ionic organolead compounds remains to be ascertained.

Another check on the analytical method was the recovery of most of the TEL added to concentrated liver extract, demonstrating the feasibility of the latter part of the

experimental procedure [Fig. 1 (d)].

TEL of high purity was kindly supplied by Dr. George Thomson of Ethyl Corporation. Triethyllead chloride was synthesized (Heap and Saunders, 1949), its purity established by chloride analysis (found: 11.1 per cent Cl, theory: 10.75 per cent), and the absence of inorganic lead was assured by testing with ammonium sulfide.

Results

The infrared absorption of TEL in pentane [Fig. 1 (a)] was found to be characterized by maxima at 15.1, 10.5, 10.0 and 8.7 microns. These maxima could also be identified in a solution prepared from the extracts of 212 gms. of liver from 12 rats exposed to TEL [Fig. 1 (b)] but not in a solution similarly prepared from non-exposed rats [Fig. 1 (c)]. As determined by absorption at 15.1 microns, 0.5 mgm. of TEL was present in the first distillate and 0.2 mgm. in the second distillate. These corrected values were obtained by subtracting a constant value from the observed absorptions. This value was arrived at from 2 measurements, each on a distillate prepared from 200 gms. of liver of unexposed rats. They showed absorption comparable to 0.16 and 0.18 mgm. of TEL per ml.

The corrected values were confirmed by lead analyses of the same two final distillates which showed 0.45 mgm. and 0.14 mgm. of lead, respectively, equivalent to 0.71 mgm. and 0.22 mgm. of TEL. This accounted for only part of the lead found in the original pentane extracts of liver; of the remainder, 1.12 mgm. were found in the 14 liters of recovered pentane and 0.14 mgm. in the residue left after all distillations. The difference between the sum of these, 1.85 mgm., and the amount in the original extracts, which was 1.72 mgm., may be attributed to analytical error and accidental contamination. The following diagram pictures the distribution of lead in this experiment.

original pentane extracts
(1.72 mgm. Pb)

14 l. of distillate
(1.12 mgm. Pb)

45 ml. of concentrate

first distillate (0.45 mgm. Pb) | second distillate (0.14 mgm. Pb.) | residue (0.14 mgm.Pb)

Discussion

The smallness of the quantities of TEL present in tissues, with the resulting restriction of methods, raises a question of the validity of the inference that TEL was present in the liver. Are the spectra and the lead analyses sufficient evidence of the presence of TEL in the final solution, or could they be reasonably attributed to other sources?

Insofar as the spectrophotometric findings are concerned, it is evident that the absorption maxima present in the known TEL solution were also present in the solution from the exposed rats. While the maxima differed in relative intensity in the two solutions, these differences were considerable only at wave lengths less than 10.5 microns. In the solutions from both exposed and unexposed rats the spectra in this region showed considerable absorption by materials other than TEL. The maxima, other than those related to TEL, failed to coincide in this region. This was not investigated further.

The discrepancies between the results as measured by infrared absorption (0.5 and 0.2 mgm.) and the results by lead analyses (0.71 and 0.22 mgm.) are attributed to these sources: (a) error in measurement of the final volume of solution in the special sample holder (DeTar and Kazimi, 1955) before the

spectrum was obtained (this could be in error by ± 10 per cent), (b) incomplete recovery of solutions from the infrared absorption cell, (c) the difficulty of evaluating corrections of the optical densities of the spectra for tissue "blanks," and (d) the limitations of the spectrophotometer.

There is a possibility of the interchange in liver tissue of ethyl and methyl groups on the lead atom. Such an interchange occurs in vitro (Calingaert et al., 1939 and 1940). That it may also occur in vivo is suggested by work on transmethylation (Cantoni, 1952).

The four methylated homologs of TEL in pure form all show marked absorption between 13 and 14 microns (A.P.I. catalog) where TEL is transparent. As demonstrated in Figure 1 (c) this absorption is also obvious in a solution of tetramethyllead in pentane. It was absent from the solutions obtained from exposed rats and, therefore, it is unlikely that the final distillates included significant quantities of methylated homologs of TEL.

A remote possibility of error was contamination of distillates with CO_2 and acetone. A solution of CO_2 in pentane showed a maximum only at 15.2 microns, but it showed no other absorption between 5 and 14.5 microns. A solution of acetone in pentane showed a number of maxima; none coincided with those of any of the liver extracts.

Explanation of some procedural details may be of interest. Rats, because they proved somewhat resistant to intoxication by

TEL, were chosen in preference to rabbits which developed pulmonary congestion and died within a few hours. The inhalation route was chosen to minimize the administration of non-volatile decomposition products of TEL, and to avoid arbitrarily localized deposits. Carbon monoxide was added during TEL exposure to avoid a terminal respiratory loss of TEL. Liver was taken for analysis because it was found to contain much pentane-soluble lead. Pentane [used by Kröller (1949)] was chosen as a solvent because it dissolves so little water [0.006 per cent w/v (Black et al., 1948)] and differs widely from TEL in volatility. The proportions of pentane, tissue, and water in the tissue extraction were chosen to derive a good dispersion on shaking with a minimal volume of emulsion.

Summary

Tetraethyllead (TEL) has been shown to be present in liver tissue of rats which have inhaled TEL vapor. TEL was extracted by pentane, concentrated by low temperature vacuum distillation, and identified by infrared spectra and lead analyses. No homologs containing methyl groups were detected in the concentrates.

Figure 1. Infrared spectra of: (a) a solution containing 3.6 mgm. of TEL per ml. of pentane, (b) a pentane solution prepared from livers of rats intoxicated with TEL, (c) a pentane solution prepared from livers of rats not exposed to TEL, (d) a pentane solution prepared through redistillation of the residual grease from (c) to which had been added 3.2 mgm. of TEL, and (e) a solution containing 10 mgm. of tetramethyllead per ml. of pentane.

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Isolation of Tetraethyllead

A list of items for the index:

Tetraethyllead, isolation from liver

Liver, isolation of tetraethyllead from

A condensed title running page headline:

ISOLATION OF TETRAETHYLLEAD