Methodology

Rats.

Procedure:

Study I:

Samples of blood were taken from the tail vein of six male and six female rats selected at random from each group for a hematologic examination before lead acetate was added to the diets and again after approximately one, two and three months and at three-month intervals for the balance of the two year period. A 24 hour urine specimen was collected from each of these rats prior to the blood sampling for routine urinalysis and to measure the concentration of delta-aminolevulinic acid. A second 4 hour sample was collected to measure the urine glutamic-oxalacetic transaminase activity. Six other rats were sampled for biochemical measurements in the plasma at the same intervals. Four to six rats were sacrificed after 3, 6, 12 and 18 months and the tissues analyzed for lead. At the end of the study, tissues from all survivors were analyzed.

Study II:

Blood was taken from [illegible] ten male and female rats selected at random in each group of 100 [illegible], lead acetate addition to the diet, at approximate time intervals of 1, 2, 3, 12, 18 and 24 months. Six rats were sampled for biochemical measurements in the plasma.

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at each of the intervals. Four to six rats were sacrificed at 12, 18, and 24 months and the tissues analyzed for lead. Though this feeding study was designed similar to Study 1, the time intervals for sampling did vary and are noted on the tables that follow.

Methodology:

Blood and Urine:

The methods used for both studies were identical. Erythrocytes and leucocytes were counted electronically with a Coulter Counter; hemoglobin was measured by the cyanmethemoglobin method using certified reagent[1] and standard; hematocrits were measured in a capillary tube as microhematocrits. Blood films were prepared on cover slips and stained with Wright's stain for differential leucocyte counts (100 cells) and for counting the number of stippled cells (50 fields) and number of nucleated red blood cells (per 100 leucocytes).

The analysis of urine consisted of a measure of the 24 hour volume; the concentration in milliosmoles per liter was determined using a Fiske® Osmometer; protein was measured quantitatively by the method of [illegible] et al.[2]; urine glutamic oxalacetic transaminase activity by the method of Reitman and Frankel[3]; delta-aminolevulinic acid by the method of Mauzerall and Granick[4]. Sugar was measured semi-quantitatively with Clinitest® tablets. All

specimens were tested for occult blood with Occultest®[5] tablets; those giving a negative test were pooled and the sediment examined microscopically. The specimens giving a positive test for occult blood were examined separately.

Alkaline phosphatase activity was measured on blood taken from the tail using a modification of the method of Huggins and Marin.[6] Glutamic-pyruvic transaminase was measured spectrophotometrically using the method of Wroblewski and La Due[7] adapted to a micro scale.

Lead:

The tissues were placed in individual plastic bags and frozen on solid carbon dioxide immediately following removal from the animal and then stored at -4°C. At the time of analysis, the samples were brought to room temperature, weighed into 35 milliliter Kjeldahl flasks and digested with 5 milliliters of a concentrated sulfuric-nitric acid solution (1:2) followed by 2 milliliters of a sulfuric-perchloric acid mixture (1:1). After the digestion was completed, all nitrate and perchlorate were driven off by heating. The samples were diluted and an aliquot [illegible] analysis by atomic absorption spectroscopy. [illegible] [illegible] with a chelat[illegible]

agent without pH-adjustment and analyzed according to the methods recommended by the Perkin-Elmer Corporation.[8] Tissues taken at interim periods were pooled by groups; sample size and dilution were chosen to obtain one to ten micrograms of lead in the final aliquot. Livers from the animals remaining at the end of the experiment were analyzed individually and the kidneys were combined within groups in order to obtain an adequate sample.

Bones were stripped of soft tissues (cartilage caps removed), crushed, extracted in a Soxhlet with an ethyl alcohol-diethyl ether solution (1:1), dried and weighed into flasks. Digestion and analysis were similar to soft tissues. Blood and urine were analyzed by the methods described by Berman[9]. Samples were pooled by groups at the interim sacrifices and analyzed individually at the end of the experiment.

Feed and feces were dried, ground through a 20 mesh screen and analyzed by the method described for soft tissues. [illegible]

Standards containing 1 to 10 micrograms of lead per milliliter were prepared from lead nitrate in 1 normal nitric acid.

One milliliter of each was chelated and extracted into methyl-isobutyl ketone without pH adjustment and aspirated into the flame. These standards were used for all digested tissue as there was no appreciable difference between standards prepared in 1 normal sulfuric acid and those prepared in 0.1 normal sulfuric acid. Standards for blood and urine samples were prepared according to Berman.

The limit of measurability for digested samples was approximately 0.1 microgram per gram, while that of blood and urine was 0.1 microgram per 10 milliliters. Only a single analysis of each sample was performed, but samples containing added lead were included with each batch of tissues. When recovery of added lead was less than 55%, the entire batch was re-analyzed.

The lead content of the fat, muscle, spleen and testis was low in comparison to the minimum measurable levels and had no consistent relationship to dietary lead. For this reason, fat was not analyzed after six months; muscle, spleen and testis were not analyzed after eighteen months.

(6)

Dogs.

Procedure:

Study I:

Samples of blood and urine were collected three times before the test began, often one, two and three months and then every three months for the remainder of the two years. Blood was taken by venipuncture for the hematologic and biochemical measurements. A 24-hour urine specimen was collected on the day preceding or following the blood sample. Two dogs, one male and one female, were sacrificed at the end of one year. All but one female survived the second year. At the end of the test period, the remaining animals were sacrificed and tissues were taken for histologic analysis.

Methods:

The hematologic examinations of the blood, made on a portion of the sample collected, was the same as described for the rats.

The urinalysis consisted of a measure of the specific gravity, a semi-quantitative measure of urobilinogen[10] and a test for acetone[11] and bilirubin[12] as well as the routine analyses described for the rats. Urine glutamic-pyruvic transaminase, however, was not measured.

The biochemical measurements made on the blood included a measure of glucose using

Boehringer reagent[13], urea nitrogen by the method of Karr[14], cholesterol by the method of Reasom, Stone and McGarrah[15], alkaline phosphatase by a modification of the method of Bessey, Lowry and Brock using Boehringer reagent[16], glutamic-pyruvic transaminase by the method of Wroblewski and La Due.[17] Delta-aminolevulinic dehydrase was also measured after 6, 9, 12, 15, 18 and 24 months using the method of Borsiger et al[18]. Prothrombin time was measured using the Lab-Tek Prothrombin system[19] and the total protein with the biuret reagent[20]; albumin-globulin ratios were determined after electrophoretic separation of the proteins using the dye elution technique[21].

Samples of blood were taken for lead analysis and stored in plastic tubes at -4°C until analyzed. Urine and feces samples were collected as described previously for rats. Tissues from individual animals were collected at the terminal sacrifice and kept frozen until analyzed. Lead was determined by atomic absorption spectrophotometry following the same procedures described for the samples from rats.

References

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