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THE PHYSIOLOGICAL EFFECTS OF SMALL AMOUNTS OF LEAD: AN EVALUATION OF THE LEAD HAZARD OF THE AVERAGE INDIVIDUAL

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The amount of lead absorbed and retained in the body even in fatal poisoning is usually less than a gram and hence might be spoken of as small. It is not our purpose, however, to add to the great numbers of papers and numerous reviews (Tanquerel des Planches, 1839; Meillère, 1903; Legge and Goadby, 1912; Oliver, 1914; Aub, Fairhall, Minot and Reznikoff, 1926) of the familiar picture of chronic plumbism. Here we are concerned rather with the more insidious effects of those quantities of lead to which the average individual living in modern industrial society is exposed. We must therefore:

1. Estimate as accurately as possible the magnitude of the lead exposure of the average individual;

2. Establish the quantitative relationship of this amount of lead to that which is recognized as likely to cause outspoken plumbism; and

3. Review what is known of the mechanism of the physiological action of small amounts of lead in the organism in order to evaluate the possible hazard of an exposure to lead insufficient to cause the commonly recognized signs and symptoms of poisoning.

While no entirely satisfactory conclusion can yet be arrived at with regard to any of these points, the purpose of the following discussion is to bring together the pertinent information which is available at present.

THE LEAD EXPOSURE OF THE AVERAGE INDIVIDUAL IN MODERN LIFE. *General approach to the problem.* The early recognition that lead causes poisoning has gradually brought about regulations which minimise the possibility that the average person in modern society will be accidentally exposed to gross amounts of lead. At the same time, however, certain modern practices make probable a constant exposure of

the general population to minute amounts of lead. Tremendous amounts of lead are used annually in insecticides and are added in the form of tetraethyl lead to gasoline used for motor fuel. As these products are used the lead is scattered in finely divided form over vegetation, top soil and surface waters. Such a dissipation of lead, as well as the modern custom of preserving a large fraction of our food in metal containers, must make it possible for the average individual to absorb small amounts of lead in dust-laden air and in the food and water consumed. The problem of estimating the significance of this small constant exposure may be attacked either from a study of the factors contributing to the total lead exposure or from a study of individuals who have lived for some time exposed to such sources in an average normal environment. The first approach would involve determination of the lead content of typical diets, water supplies, samples of air, etc., while in the latter the attention would be turned to a study of the lead in the excreta, blood and tissues of persons without industrial exposure to lead.

Chemical methods. Whatever the approach, the first requisite is a chemical method which is delicate and at the same time specific. Minute amounts of lead must be separated from relatively huge amounts of other material and then accurately measured. There are several methods advocated for the detection of small amounts of lead. These include two variations of the measurement of lead separated as chromate from a solution of ashed material. One is the titration method described by Fairhall (1922) and the other a colorimetric method based on a reaction between chromic acid and s-diphenyl carbazide described by Cazeneuve (1900). Great sensitivity has been claimed for the colorimetric method but Fairhall (1922, 1933) has thrown some doubt on its reliability. However, it has been extensively used in a somewhat modified form by Kehoe and his associates (Kehoe, Edgar, Thamann and Sanders, 1926) and others. The so-called dithizone test is another method for which great accuracy and specificity are claimed. The test is based on a reaction described by H. Fischer (1929) between lead and diphenylthiocarbazone. A colorimetric adaptation of this method is described by Bohnenkamp and Linneweh (1933) and a titrimetric procedure is advocated by Howitt and Cowgill (1937). The spectrographic method (Shipley, Scott and McNair, 1932) is also frequently employed for the detection and estimation of extremely small amounts of lead. Besides these quantitative methods, Fairhall (1923) has described a qualitative microchemical test based on the formation of crystals of complex potassium-lead-copper-hexanitrite which can be

recognized by their characteristic form. This test is extremely useful as a supplementary proof of the identity of small amounts of material which are indirectly measured as lead by other methods.

Lead in tissues and excreta of normal individuals. Our present information as to the magnitude of the exposure of the average individual to lead must be based on data gleaned from numerous sources in which the problem has been approached from various points of view and by the use of different chemical methods. Aub and his associates (1926), using Fairhall's method, found no lead in the urine and tissues of persons with no unusual exposure to lead. Even in the skeleton, where lead is most likely to be stored, none was usually detected except in individuals in whose history some unusual exposure to lead was revealed. These authors concluded that at that time in their locality (Boston) "the usual activities of daily life cause no retention of lead in the body." At about this same time Kehoe and his co-workers (1926) in Cincinnati, using the diphenyl-carbazide test, found lead present in the excreta of 65 normal persons and concluded that the general finding of lead in the urine must indicate that nearly everyone has absorbed some lead. Since that time numerous investigators, including Barth (1931), Weyrauch and Müller (1933), Pfrieme (1934), Maulbetsch and Rutishauer (1936) and Tompsett (1936), working with a variety of chemical methods and in different localities, have found lead present in the bones of practically all normal individuals without history of unusual exposure to lead. The concentrations reported ranged from about 0.5 to 10.0 mgm. Pb per 100 grams of ash. Usually the higher concentrations were found in the bones of older individuals and no consistent difference was noted between the lead contents of bones of city dwellers and of those who had spent their lives in rural communities.

More extensive surveys of normal lead exposure. In a typical modern American industrial city, Kehoe, Thamann and Cholak (1933a) studied the lead content of foods and of the excreta, body fluids and tissues of persons without industrial exposure to lead. It was found that the mean daily output of lead by normal adults was about 0.25 to 0.30 mgm. Most of this was found in feces, but the urine contained from 0.02 to 0.08 mgm. per liter. Lead was also found in the excreta of infants and children (Kehoe, Thamann and Cholak, 1933b). Human milk was found to contain from 0.0 to 0.05 mgm. of lead per liter. From studies of a large variety of foods, the authors conclude that the average daily consumption of lead by an adult is about 0.20 to 0.35 mgm., as compared to a daily intake of 0.10 mgm. by individuals living under

primitive conditions (Kehoe, Thamann and Cholak, 1933c). In persons in both primitive and modern communities, the daily excretion of lead was practically the same as the estimated daily intake. Some of the ingested lead must have been absorbed, since it was detected in the urine, blood and tissues. Even stillborn fetuses were found to contain small amounts of lead. The tissues of a child of two years were shown to contain approximately the same concentrations of lead as those of an adult, although the total present in the body was greater in the older individual.

It is of interest to compare these results with those of Tompsett and Anderson (1935), working at the Royal Infirmary in Glasgow. The latter authors used the dithizone method which was apparently carefully controlled. Analyses were made of the tissues obtained at autopsy from 20 persons without occupational exposure to lead. The ages ranged from 2 to 69 years. Lead was found in all the tissues analyzed. Stated in terms of milligrams of lead per kilo of fresh tissue, the following average concentrations were found: liver, 1.73; kidney, 1.34; spleen, 1.69; brain, 0.50; rib, 8.55; vertebrae, 7.09. The tissues of a child of two years were found to contain approximately as high concentrations of lead as did those of adults. Lead was also found in smaller but appreciable amounts in the tissues of 4 fetuses of 7 to 8 months' gestation. Analyses of blood from 3 normal persons and 18 patients (without exposure to lead) gave values ranging from 40–70γ with an average of 55γ of lead per 100 cc. The mean daily lead excretion of 10 patients was found to be 0.05 mgm. in the urine and 0.22 mgm. in the feces. This daily output was in close agreement with the estimated intake based on analyses of food and water. The average daily diet contained about 0.22 mgm., while the water had a lead content of 0.03 mgm. per liter.

Sources of "normal" lead. A few studies are available which furnish some idea of the relative importance of various sources which contribute to the total incidental exposure to lead in a given locality. Arranged in order of increasing importance as dietary sources of lead (Kehoe, Thamann and Cholak, 1933d) are listed: breadstuffs, meats, processed meats, ice cream, candy, leafy green vegetables and certain fruits. The relatively high lead content of vegetables and fruits is probably at least partly due to the general use of insecticides. Lendrich and Mayer (1927) found from 0.0 to 4.0 mgm. of lead per 3 apples (the sample uniformly chosen for analysis) raised in the United States. Much of this lead was on the outside of the fruit. No lead was found in apples

grown in Australia, where lead is not extensively used as an insecticide. Little data are available as to the importance of inhaled lead as a part of the total exposure. Bloomfield and Isbell (1933) report an average of 0.09 mgm. of lead per 10 cubic meters in 28 samples of air collected at congested street intersections and slightly higher concentrations in the air of industrial plants not handling lead, and in automobile repair shops.

General conclusions. When reviewed as a whole, the uniformity of results, obtained by numerous investigators in widely separated parts of the world and by means of a variety of chemical methods, leaves no doubt that the average individual in modern civilized communities continually ingests or inhales small amounts of lead. The closely agreeing results of Kehoe and his associates in the United States and of Tompsett and Anderson in Glasgow indicate that the magnitude of the average daily incidental exposure is probably from 0.2 to 0.4 mgm. of lead. Excretion more or less keeps pace with the intake, so that no marked increase in the concentration of lead in the body occurs with age. Some lead is, however, retained and may be detected in the blood and soft tissues, although the greater proportion of retained lead is found stored in the skeleton and teeth.

QUANTITATIVE RELATIONSHIP OF AVERAGE GENERAL EXPOSURE TO LEAD TO THE EXPOSURES WHICH RESULT IN OUTSPOKEN PLUMBISM.

Estimates of amounts of lead causing poisoning. Sollmann (1922) estimates that the intake of lead which usually eventually causes clinical plumbism is about 10 to 20 mgm. per day for an adult (0.2 to 0.4 mgm. per kilo) but suggests that smaller amounts probably do some harm. Numerous other investigators place this estimate much lower—as low as 1.0 mgm. per day for adults. Wright, Sappington and Rantoul (1928) report occasional poisoning in persons taking as little as 0.1 mgm. of lead daily in drinking water over a period of years, while poisoning was common in individuals taking 1.5 mgm. per day. Studies which measure only one of the possible incidental sources of lead may result in erroneously low estimates of the amounts of lead which produce signs of plumbism. It is evident, however, that attempts to predict the danger of a given exposure to lead solely on the basis of the amounts involved results at best in only a rough estimate.

Factors which influence individual susceptibility. Whenever the toxicity of lead compounds is investigated a wide difference in individual susceptibility is noted. Some persons can work with impunity for years at the same lead trade in which others quickly develop signs of poisoning.

Even when experimental animals are carefully selected with regard to uniformity of age, sex, previous nutrition, etc., and are exposed to the same amounts of lead there is a wide variation in the time of appearance and the severity of symptoms of poisoning. On the other hand, when single tissues such as blood cells, frogs' muscles, intestinal strips, etc., are exposed to lead for the purpose of studying some particular action of the metal, the results are much more uniform. Under these simpler conditions there is usually a consistent quantitative relationship between the amount of lead present and the intensity of the effect produced. Individual differences in susceptibility are, therefore, probably due not so much to an essential difference in the resistance of tissues to the effects of lead as to variations in the factors which influence the amount of soluble lead to which the tissues are exposed. Such factors include the absorption, storage, mobilization, and excretion of the lead which gains entrance to the body; and each factor is important in determining the danger of a given exposure to lead to each individual.

Absorption of lead. Fat-soluble organic compounds such as tetraethyl lead are readily absorbed through the skin (Flinn, 1926), but for the type of lead to which the ordinary individual is exposed only the gastro-intestinal and respiratory tracts need be considered as portals of entry.

Even on heavy exposure, by no means all of the lead taken into the gastro-intestinal tract is absorbed. Both the solubility of the compound ingested and the possible formation of non-diffusible compounds with other materials present in the gastro-intestinal tract influence the completeness of absorption. The portion which is absorbed is carried in the portal blood to the liver, where much is removed from the blood and held back from the general circulation, as evidenced by the higher concentrations of lead in the liver of animals receiving lead by mouth than when similar amounts are introduced by other routes (Minot, 1924a). Some of the lead which reaches the liver is excreted in the bile and probably never reaches the general circulation; some, however, is distributed to the other tissues of the body.

Minot (1924b) has stressed the more rapid entrance of lead to the general circulation when it is introduced into the respiratory tract. Blumgart (1923) has shown that lead need not reach the lungs but that ready entrance to the blood stream is afforded to lead in the upper respiratory passages. Lead absorbed from the respiratory tract enters the systemic blood direct[illegible] and so is promptly distributed throughout

the organism. Hence, although the amount inhaled may be much less than that ingested, it is potentially more dangerous.

Distribution of retained lead. Once in the general blood stream, the distribution of lead follows a similar pattern whatever the portal of entry has been. All the tissues receive some lead. Higher concentrations are usually found in bones, liver, kidney and spleen than in other organs. Excretion more or less keeps pace with retention in the soft tissues of the body, so that there is no great increase in the concentration of lead even during periods of continued absorption. The bones store lead and continued absorption results in a progressive increase in the concentration in the skeleton and teeth.

Biochemical behavior of lead in the body. There is not complete agreement as to the biochemical behavior of lead in the organism. The extensive studies of Fairhall and Shaw (1924), Aub and Reznikoff (1924), Brooks (1927), Bischoff, Maxwell, Evans and Nuzum (1928) furnish rather convincing evidence that in whatever form lead is absorbed it is converted to inorganic phosphates. According to the findings of Fairhall and Shaw (1924), as interpreted by Aub and his co-workers (1926), lead is probably transported in the blood stream as the more soluble di-lead phosphate and is stored in the bones as the very insoluble tertiary phosphate. Maxwell and Bischoff (1929a) and Kehoe and Thamann (1933) hold that other forms of lead more active than the phosphate are present in blood. Work by Maxwell and Bischoff (1929b) indicates that lead is carried in the blood as the diphosphoglycerate, while Jowett (1932) presents evidence that lead forms a complex inorganic phosphate containing calcium and chlorine. As Aub (1935) points out, it is possible that several compounds of lead exist in so complex a chemical medium as blood plasma.

Whatever the form in which lead is transported, it is the lead in the blood and body fluids which can do injury to body cells, while lead stored in the bones is relatively harmless. The deposition of insoluble tertiary lead phosphate in the bone is, however, not an irreversible reaction any more than is the deposition of calcium phosphate in this tissue. Fairhall and Shaw (1924) showed that under conditions of slightly increased acidity, tri-lead phosphate is readily converted into the much more soluble di-lead salt. The close analogy between the distribution, storage, mobilization and excretion of calcium and of lead in the organism has been clearly demonstrated in extensive studies by Aub and his co-workers (Aub et al., 1926; Hunter and Aub, 1927;

...uer, Aub and Albright, 1929; Aub, Robb and Rossmeisl, 1932). ...heir findings have also been confirmed by many others, most beautifully perhaps by Behrens and Baumann (1933) in their work with radioactive lead. Factors which favor the deposition and storage of calcium in the skeleton also favor the storage of lead, while the same conditions which tend to dissolve calcium from the bones and bring it into the blood stream serve to bring about the solution and redistribution of stored lead. Thus the state of calcium nutrition, the acid base equilibrium, parathyroid activity, etc., all play a rôle in determining whether lead retained in the body is harmlessly stored in the bones or is allowed to circulate in the blood stream as a menace to the more vulnerable tissues of the body. The participation of so many variable factors obviously makes it impossible to predict the danger to an individual of a given exposure to lead.

Excretion of lead as a criterion of lead hazard. The amount of lead excreted, especially that appearing in the urine, furnishes a somewhat better criterion. Fecal lead is a mixture of unknown proportions of lead which has never been adsorbed and that which has been excreted by way of the intestinal tract. Urinary lead must have been absorbed and hence is of more significance. Kehoe, Thamann and Cholak (1933e) conclude, after an extensive study of persons industrially exposed to lead, that exposures are probably safe (i.e., will not cause clinical plumbism) if they do not cause a mean excretion of more than 0.6 mgm. of lead per day in the feces or more than 0.15 mgm. in the urine. The same authors found that lead poisoning could be expected in persons with a mean daily excretion of more than 1.10 mgm. in the stools or more than 0.21 mgm. in the urine. Similarly, Litzner and Weyrauch (1933) conclude that the urinary output of lead must be significantly higher than 0.10 mgm. per day before the assumption is made that the blood and tissues contain unusual amounts of lead. Meyers, Gustafson and Thorne (1935) place their estimate somewhat lower and believe that significance should be attached to the consistent presence of more than 50 micromilligrams (0.050 mgm.) of lead per liter of urine. All of these estimates of the amounts of urinary lead which should serve as a warning of possible danger of clinical plumbism are considerably higher than the amounts reported in the urine of persons without unusual exposure to lead.

Lead in blood as a criterion of hazard. Since the really important point is the concentration of lead to which the more vulnerable tissues of the body are exposed, a knowledge of the concentration of lead which

a person's exposure maintains in his blood would be the most useful criterion of the danger involved Such data involve the measurement of extremely small amounts of lead in a volume of blood which it is practical to withdraw from large numbers of people for chemical analysis. Whatever available method is adopted, gross errors are probably often introduced when slight traces of lead are detected and measured in 10 to 20 cc. of blood and the final result then calculated in milligrams of lead in 100 or 1000 cc. One should be conservative in accepting the figures now available as an accurate expression of the actual concentrations of lead present in the blood. Despite these reservations, a comparison of the concentrations found by the same investigators by the same chemical procedure in the blood of normal persons, as compared to the amounts detected in the blood of those industrially exposed to lead or of those with recognizable symptoms of plumbism, serves as a useful guide in evaluating the danger of the usual exposure to lead. Such figures are presented in tabular form.

Lead found in blood of persons with varying exposures to lead

AUTHOR	METHOD USED	DEGREE OF EXPOSURE	LEAD IN BLOOD
			mgm. per 100 cc.
Kehoe, Thamann and Cholak (1933a)	Diphenyl carbazide	Modern American life	0.0 to 0.02
Kehoe, Thamann and Cholak (1933f)	Diphenyl carbazide	Significant industrial exposure	0.04 to 0.05
Kehoe, Thamann and Cholak (1933f)	Diphenyl carbazide	Patients with lead poisoning	0.09 to 0.36
Litzner and Weyrauch (1933)	Adaptation of electrolytic procedure	Normal life	0.01 to 0.02
Litzner and Weyrauch	Adaptation of electrolytic procedure	Early symptoms of poisoning	0.04
Litzner and Weyrauch	Adaptation of electrolytic procedure	Unmistakable symptoms	0.06
Litzner and Weyrauch	Adaptation of electrolytic procedure	Frank lead poisoning	0.07 to 0.10
Tompsett and Anderson (1935)	Dithizone	Hospital patients—no unusual exposure to lead	0.04 to 0.07
Tompsett and Anderson	Dithizone	Case of lead poisoning	0.380
Wexler and Sobel (1935)	Spectrographic	Normal life	Definitely less than 0.10
Wexler and Sobel	Spectrographic	Clinical plumbism	0.10 to 1.0
Myers, Gustafson and Thorne (1935)	?	Normal in vicinity of New York City	0.006
Myers, Gustafson and Thorne	?	Subchronic intoxication	0.024

While there is considerable lack of agreement in the concentrations of lead found in normal blood, where sufficiently complete data are

...ible, there is a reassuring difference in the amounts found in the ...d of normal persons and in those with early signs of lead poisoning. ...s than this cannot be said until the actual amounts of lead involved ...more definitely fixed by extensive studies in which small aliquots ...be same sample of blood are analyzed by several different methods ...these results compared with the analysis of much larger amounts ...he same material.

Conclusions as to danger of poisoning from present average exposure. ...e can conclude from the data available that the daily lead intake of ...e average individual at present is considerably less than the intake ...hich has been generally found to be dangerous. This exposure also ...sults in a lower concentration of lead in the blood and less excretion ...n the urine than is usually associated with early signs of plumbism. These conclusions must be taken, however, as applicable only to the approximate period in which the studies were made. Only time can tell whether a continuation of modern industrial and agricultural practices involving the use of lead are going to increase gradually the magnitude of the lead exposure of the average individual.

PHYSIOLOGICAL ACTION OF SMALL AMOUNTS OF LEAD. The clinical signs and symptoms, as well as the pathological lesions which have been observed in chronic lead poisoning are too varied, inconstant and non-specific to furnish even a working hypothesis as to the fundamental actions of lead responsible for the deleterious effects produced in the organism. There is, therefore, even less chance that a study of these superficial manifestations will give any clue to the possible insidious harmful effects of amounts of lead too small to cause outspoken plumbism. A more useful approach is the study of the chemical and physiological actions of lead on simpler forms of living material and on those systems involved in the more constant signs and symptoms of chronic lead poisoning.

Effect of lead on growth of plant and animal tissues. Studies of the action of lead on fundamental biological processes are still rather fragmentary but suggest that further work may yield information of fundamental importance. Plant as well as animal growth is retarded by the addition of small amounts of lead to the nutrient medium (Bell, 1924; Dilling, 1926; Hammett, 1928; Hammett and Wallace, 1928). Concentrations of lead nitrate as low as 10^{-4} to 5×10^{-4} were shown by Hammett (1928) to have a definite slowing effect on the rate of root growth. The degree of retardation increased with increasing concentrations of lead. Hammett (1929a) showed conclusively that a hin-

drance of cell proliferation was the basis of the retardation of growth in length of seedlings. He was able to show that mitotic nuclei have the greatest affinity for lead, although the metal was demonstrated in both the nuclei and cell walls of roots (Hammett, 1929b; Hammett and Justice, 1929). Hammett (1929c) described tests which indicate that lead enters into combination with an organic sulphydryl compound analogous to if not glutathione itself. Lead was shown to retard the growth of chick embryos and here again the most pronounced effect was in areas of intense growth and rapid cell division (Hammett and Wallace, 1928). Dilling (1926) showed that lead concentrations higher than $\frac{N}{20,000}$ generally inhibited germination of frog spawn, while lower concentrations greatly lowered the percentage of eggs which germinated. Tadpoles which did develop were 40 per cent behind controls in size at the end of a month. Similarly plaice embryos living in sea water with 1 part in 250,000 of added lead showed 20 per cent retardation in growth as compared to controls at the end of six months. Because of the marked effect of lead on rapidly proliferating tissue which is rich in lecithin, Bell (1922, 1924) advanced the theory without convincing chemical evidence that lead combines with lecithin. High hopes were raised for a time that because of the predilection of lead for embryonic and rapidly growing tissues it would be of great value in the treatment of cancer (Bell, 1922, 1924). Dramatic results have been reported in some instances (Bell, 1926). However, further experience has shown little or no specific affinity of malignant tissue for lead.

Effect on enzyme activity. There is some evidence that lead interferes with normal processes in living tissue through its action on enzymes. Preti (1908) found that small amounts of lead hastened and that higher concentrations hindered the rate of formation of certain nitrogenous cleavage products in autolyzing liver brei. An increase in urinary nitrogen has been noted in animals following the administration of non-toxic doses of lead and in persons with lead poisoning (Preti, 1909; Tscherkess, 1925). These changes were interpreted as evidence of the action of lead on intracellular enzymes. Corran and Lewis (1928) report an increase in lipolytic activity in serum after the administration of lead. Recently with the use of more exact methods Dolowitz, Fazekas and Himwich (1937) have studied the effect of lead on oxidation, dehydrogenation and glycolysis in some of the tissues more commonly affected in lead poisoning. Oxidation studies were carried out in the Warburg respirometer, while dehydrogenation and glycolysis

were measured in Thunberg tubes. Brain, kidney, liver and testis were studied. Very small amounts of lead caused a marked decrease in oxygen consumption in all four tissues. Both increased and decreased respiratory quotients were observed in different experiments. In brain tissue lead caused an almost complete abolition of hydrogen transfer and a decided inhibition of glycolysis. These authors believe that through its action on enzymes lead causes profound metabolic changes in tissues which may be the underlying cause of varied manifestations of lead poisoning.

Effect on formation of immune bodies. The influence of lead on the rate of formation of immune bodies is another indication that lead profoundly influences the natural response of body tissues as a whole. When rabbits were given sufficient lead to cause poisoning (Bickert, 1929a, b, 1930 and 1931) the production of hemolysins and agglutinins was markedly increased, while the formation of precipitins was retarded. The formation of antitoxin against diphtheria was more rapid in animals receiving lead than in controls. It must be noted, however, that sufficient lead to cause poisoning was administered in these experiments and no conclusions can be drawn from these observations as to the effect of incidental small amounts of lead on immunity.

Effect on red blood cells. The common occurrence of anemia accompanied by the presence of stippled cells in the blood has made the red cell a favorite tissue in which to study the action of lead. The circulating red cells show rather marked morphological changes in lead anemia. Mayers (1926) has listed these changes in the order of frequency with which he observed them in 381 lead workers without acute symptoms of poisoning:

(1) Anemic red cells	71 per cent
(2) Anisocytosis	44 per cent
(3) Polychromatophilia	40 per cent
(4) Stippling	39 per cent
(5) Poikilocytosis	23 per cent
(6) Nucleated red cells	8 per cent

So-called "stippling" which is so valuable a diagnostic sign in lead anemia is due to the presence in red cells of discrete basophilic granules. The change is not an immediate specific effect of lead on red blood cells, since it is occasionally seen in other types of anemia and cannot be produced by the exposure of red cells to lead in vitro. Stippling does not occur in the blood of all species following the administration of lead.

For example, punctate basophilia is not observed in cats with lead poisoning but is a fairly constant feature of the intoxication in man, rabbits and guinea pigs. There is not complete agreement as to whether stippling is an evidence of degeneration or regeneration of erythrocytes. The change is most readily produced in young cells. Key (1924), who investigated the problem extensively, concluded that a stippled cell is an altered young red cell undergoing degenerative changes which need not necessarily be due to lead but which may occur when young red cells are exposed to lead in the circulating blood. Key was unable to find stippled cells in the bone marrow even when they were plentiful in circulating blood. Young and Osgood (1935), on the contrary, observed stippled cells in the bone marrow of animals poisoned with lead and regarded their presence as evidence of blood regeneration. Similarly Sellars (1921) interprets stippling as a result of regeneration under somewhat abnormal conditions.

The development of severe anemia accompanied by a high reticulocyte count indicates that lead in some way increases the destruction rather than hinders the formation of red cells. Studies of the marrow by Speransky and Sklianskaja (1928) support this view as they find a marked increase in erythroblasts in marrow counts in lead poisoning.

Considerable work has been done with regard to the nature of the injury due to lead. Aub, Reznikoff and Smith (1924a, 1924b) demonstrated that when 1 cc. of washed red cells are exposed to 0.01 mgm. of lead in the form of chloride they undergo definite and consistent changes. The cells decrease in volume, become much more resistant to hemolysis in hypotonic saline, lose their normal stickiness and at the same time become more subject to destruction by trauma. In other words, the cells lose their ability to swell and are more rigid and brittle and less resilient and durable. Ørskov (1935) found that the shrinkage in volume of red blood cells exposed to lead is accompanied by a rapid loss of potassium into the surrounding medium. Bicarbonate must be present in at least a concentration of 0.007 N in order for this action to take place and the optimum pH is from 6.3 to 6.6. Under these conditions lead in as low a concentration as 1:25,000,000 causes a marked increase in the permeability of red cells to potassium. In some experiments the cells had lost 40 per cent of their volume and 80 to 90 per cent of their total potassium content within 10 minutes. After these changes the resistance of the cells to hypotonic saline is greatly increased. The same reactions apparently occur in vivo, since Henriques and Ørskov (1936) found a decrease in volume and potassium

content of red cells in rabbits following the administration of lead. These phenomena cannot, however, be readily demonstrated in the blood of all species. For example, they appear in the blood of man and rabbits but not in the blood of cats and goats. The close parallelism between the species in which the effect of lead on red cells can be demonstrated and those in which anemia and stippling are prominent signs in lead poisoning points to the fundamental importance of these changes in the injurious action of lead.

When red cells are exposed to lead which has been previously mixed with serum the effects described by Aub, Reznikoff and Smith and by Ørskov are no longer produced. It has been shown that 0.01 mgm. of lead is completely inactivated by admixture with 0.2 cc. of serum. The inactivating substance in serum has been shown to be inorganic phosphate (Aub and Reznikoff, 1924; Ørskov, 1935). At first thought it would appear that in vivo red cells would be protected by the phosphate of plasma from the action of any amount of lead likely to be in the blood stream. However, it has been shown that lead affects red cells in living animals. Lead introduced into the blood stream comes in contact simultaneously with cells and plasma and reacts with both. Thus, although fewer cells would be injured by the same amount of lead in the presence of plasma, some cells would be affected, however small the quantity of lead present.

According to Aub and Reznikoff, lead probably combines with the inorganic phosphate of both serum and cells as follows:

$$2PbCl_2 + 2B_2HPO_4 \rightarrow Pb_3(PO_4)_2 + 4BCl + 2HCl$$

Lead would thus be transformed to the very insoluble and inactive tertiary phosphate with the liberation of free acid. They postulate that the injury to red cells is due to the locally increased acidity resulting from a collection of acid at the cell membrane. Maxwell and Bischoff (1929) disagree with this interpretation, since they could induce similar changes in red cells with lead compounds which did not cause the liberation of free acid. They believe that lead diglycerophosphate as well as the inorganic tertiary phosphate is formed when lead is brought into contact with erythrocytes. The diglycerophosphate is a much more active compound than the inorganic phosphate and its formation may account for the injury to red cells.

Whatever the mechanism is, exposure to lead serves to shorten the life of erythrocytes in circulating blood. The more rigid brittle cells are less able to stand the wear and tear of circulation and are destroyed

more quickly than are normal cells. Key (1924) believes that they disintegrate more by fragmentation than by hemolysis, as he found debris and hemoglobin-containing fragments in the livers and spleens of poisoned animals. The destruction of cells in the peripheral blood stimulates the marrow to form new cells. When regeneration is sufficiently rapid young forms appear in the circulating blood. The simultaneous occurrence of cells injured by lead and young cells poured out to replace those which are lost accounts for the blood picture seen in lead poisoning.

Effect on white blood cells. The white cells have received much less attention than the erythrocytes in studies of lead poisoning. Fine (1923) demonstrated that the phagocytic power of leucocytes is greatly reduced by their exposure in vitro to lead chloride in the concentration of 0.02 mgm. of lead per cubic centimeter. The mechanism by which this effect is produced has not been explained. Clinically the total white count is usually within normal limits in lead poisoning. Ferguson and Ferguson (1934) and Shiels (1936) have stressed the value of the lymphocytic ratio as a prognostic sign in persons exposed to lead. An increase in the ratio of $\frac{\text{large lymphocytes} + \text{monocytes}}{\text{small lymphocytes}}$ occurs early in exposure and later falls markedly as symptoms of poisoning develop. The authors feel that a gradual fall in this ratio in the blood of persons constantly absorbing lead is a valuable warning sign of impending danger.

Effect on skeletal muscle. It is well known that the function of skeletal muscles is impaired in lead poisoning. Using isolated nerve muscle preparations from frogs, Reznikoff and Aub (1927) showed that fatigue is more rapid and complete and recovery slower in "leaded" than in normal muscles. Their work indicates that interference is with muscle function rather than with nerve conduction. Fatigued muscles were shown to be much more susceptible than resting muscles to the action of lead—a difference which the authors attribute to the greater lactic acid content of fatigued muscle which increases the solubility and hence the reactivity of lead. An increase in the permeability of "leaded muscles" was indicated by the more rapid diffusion of inorganic phosphate into the surrounding Ringer solution. In view of Ørskov's findings in red blood cells, it would be interesting to know whether muscles also show an increased permeability to and subsequent loss of potassium. To our knowledge, no such studies have been made. Since potassium plays so significant a rôle in normal muscle function, a loss of this elec-

...te from muscle cells might be of tremendous significance in the ...ired function following exposure to lead. Very recent work by ...man (1938) shows a lowered level of phosphocreatine in the resting ...cles of frogs exposed to lead. Following contraction in such muscles ...re is a marked interference with the resynthesis of phosphocreatine. ...appears possible that there may be some correlation between the ...normalities reported by Steiman and the loss of inorganic phosphate ...ported by Reznikoff and Aub. At any rate, interference with the ...rmal metabolism of so important a constituent as phosphocreatine ...ust be an important factor in the ready fatigability, weakness and ...lsy seen in muscles as a result of exposure to lead.

Effect on smooth muscle. The frequent occurrence of colic and constipation in persons with chronic plumbism has turned the attention of many investigators to the action of lead on smooth muscles. It has long been a moot question whether smooth muscle is affected directly or through peripheral or central nervous mechanisms or by means of a combination of these factors. Aub, Fairhall, Minot and Reznikoff (1926) have critically reviewed much of the older controversial literature on the subject which need not be repeated here. In clinical lead colic or in the condition produced in animals by the injection of lead, increased tone and hypermotility of the intestinal tract can be demonstrated (Wassermann, 1916; Aub, Fairhall, Minot and Reznikoff, 1926; Grünberg, 1928). On the other hand, when intestinal strips are exposed to lead in vitro, the effect most consistently noted is inhibition of contractions usually with a simultaneous increase in tone (Smith, 1926; Dilling, 1926; Grünberg, 1928). In strips containing nerve elements Dilling showed that the effect of lead persisted after atropine and after nicotine and so concluded it could be of neither vagal nor ganglionic origin. He found that strips of uterine muscle behaved similarly to intestinal muscle when exposed to lead in vitro. Both Smith and Dilling were able to demonstrate this response with nerve-free rings of circular intestinal muscle and concluded that lead must affect smooth muscle directly.

Somewhat at variance with the observations of these authors are the carefully controlled experiments of Grünberg (1928) which definitely appear to implicate the autonomic system in the effects produced by lead, at least under the conditions of his experiments. He first studied the effect in vitro of lead on various types of smooth muscle. He included in this study smooth muscle from the intestines of various species, pregnant and non-pregnant uteri, etc. He found everywhere a

close parallelism between the action of lead, the action of adrenalin and the effect of sympathetic stimulation of the tissue concerned. In those tissues in which sympathetic stimulation caused contraction, lead caused contraction; while in those tissues to which the sympathetic is inhibitory, lead also caused a cessation of contractions. Lead injected into the mesenteric artery had the same effect on intestinal muscle as when applied locally in vitro. When injected in this manner lead is carried to the intestinal tract directly and is prevented by the liver from reaching the general circulation. Small doses of lead injected into the portal vein had no effect on intestinal movements. On the other hand, lead introduced into the general circulation always caused intestinal contractions. This effect persisted even when the blood supply to the intestines was cut off by ligation of the aorta at the diaphragm, but failed to occur if the vagi were sectioned in the neck. From these and numerous additional experiments, the author concluded that lead has two effects on intestinal activity: 1, it acts locally to inhibit movements by the stimulation of sympathetic endings; and 2, it acts centrally to cause intestinal contractions by stimulation of the vagus nerve.

Aub et al., Dilling and Grünberg all agree that the constipation which is so common in chronic plumbism is due to the inhibition of intestinal movements by the local action of lead. Aub attributes the griping pain of colic to a reflex constriction which approaches a non-motile hypertonic area, while Grünberg interprets colic as increased contractions due to the stimulation of the vagus center by lead.

Vascular smooth muscle apparently shares the common effect of lead on this tissue. The action has been demonstrated in vitro (Siccardi and Dozzi, 1914), but it is still a matter of discussion as to how significant a rôle the effect of lead on vascular muscle plays in either the functional disturbances or pathological lesions of lead poisoning. Certain investigators (Goadby and Goodbody, 1909; Legge and Goadby, 1912) have contended that the most specific lesion of lead poisoning is vascular damage resulting in hemorrhage. Others (Orphüls, 1915) have failed to produce vascular lesions in experimental poisoning. The pallor of saturnism, which is frequently out of all proportion to the degree of anemia, is often attributed to the constriction of the blood vessels of the skin. Hypertension is frequently present in persons with chronic lead poisoning and is thought by some to be caused by the action of lead on the vascular system. High blood pressure is also common in adults without lead poisoning, so it is difficult to know in a given case

whether lead is a causal factor. Only extensive statistical studies can give significant data on this point and the results of even such studies are somewhat conflicting. Vigdortchik (1935) studied blood pressure in 1437 lead workers and in 1332 persons not industrially exposed to lead. When the mass of data was statistically analyzed according to age groups, length of exposure, etc., the author came to the conclusion that prolonged exposure to lead increases blood pressure. This view found support both in the percentage of persons showing moderate or marked hypertension and in the average level of blood pressure in each age group of lead workers as compared to those not exposed to lead. Belknap (1936), on the other hand, in a careful analysis of data obtained over a period of years in 81 men heavily exposed to lead, concluded that there was no significant difference in the blood pressure of these workers and of normals of the same age groups. Petroff (1930) found hypertension during the early stages of experimental poisoning in animals followed later by normal or low blood pressure. During the period of hypertension he also observed tachycardia, hyperglycemia and increased cholesterol in the blood—all of which changes are compatible with increased sympathetic activity. He believed that much of the conflicting data with regard to the effect of lead on blood pressure in man may be attributed to the fact that various stages of the intoxication have been indiscriminately studied. Teleky (1937), after an extensive review of the literature, came to the conclusion that it has not yet been scientifically demonstrated that high blood pressure accompanies the signs of early lead poisoning in man, but emphasizes the need for further studies of the effect of prolonged absorption of small amounts of lead.

Judging from the similar response of all types of smooth muscle in vitro, it appears reasonable to expect that vascular tone would be affected, provided that a sufficient concentration of lead was present in the blood stream. This would be equally true whether lead acts directly on vascular muscle or through stimulation of sympathetic endings.

No studies are yet available which are concerned with the chemical reactions of lead with smooth muscle. It is interesting to speculate whether an increased permeability to certain inorganic ions with a subsequent change in electrolyte equilibrium may occur in smooth muscle as well as in red cells and skeletal muscle. Such changes, if present, might well be an essential factor in the functional disturbance of smooth muscle in view of the marked influence which inorganic cat-

ions are known to have on both smooth muscle and autonomic nerve activity (Howell, 1905; Zondek, 1922).

Effect on nervous system. Pathological lesions have been demonstrated in the brain, cord, meninges, autonomic ganglia and peripheral nerve fibers of individuals with lead poisoning. The presence of lead in brain, cord, and spinal fluid leaves little doubt that it is responsible for the abnormalities seen. Objective manifestations which indicate impaired function of the nervous system range from sluggishness, poor memory and irritability, to paralyses and the violent convulsive seizures of lead encephalitis. Aub, Fairhall, Minot and Reznikoff (1926) have given a critical review of the older literature and for more recent studies the reader is referred to Lehmann, Spatz and Wisbaum-Neuberger (1926), McKhann (1932), Villeverde (1933), Grünberg (1930) and Blackman (1937).

Outspoken pathological changes or gross functional disturbances of the nervous system are, however, manifestations of severe lead poisoning and a detailed description of them would add little to the present discussion. Here we must look for more subtle manifestations of impaired function and for actions of lead which may influence the normal physiology of nervous tissue. McDonald (1930), Rawkins (1931) and Porritt (1931) report such symptoms as apathy or irritability, lack of confidence, unfounded fears and inability to concentrate in persons with long exposure to small amounts of lead and without the more common signs of plumbism. They report a return to normal following removal of the source of lead. The possible causes of symptoms of this nature are so numerous and varied that a very extensive study would be necessary in order to establish any general causal relationship between their appearance and the absorption of lead.

The pathology in outspoken lead encephalopathy, according to many investigators, gives evidence of severe circulatory disturbance and vascular injury. Such observations may indicate that the prolonged absorption of smaller amounts of lead might result in less marked but still significant abnormalities in the blood supply to the brain. Dolowitz, Fazekas and Himwich (1937) have already been quoted as reporting a reduction in oxygen consumption as well as qualitative changes in metabolism in sections of brain tissue exposed to small amounts of lead. Since nervous tissue is extremely susceptible to oxygen lack, it is reasonable to suppose that impaired function might result from continuous slight interference with normal oxygen supply or utilization. There is also again the possibility that lead may cause

permeability changes in nerve cells analogous to those demonstrated in other tissues. Such an effect could hardly fail to impair the optimal functioning of highly specialized nervous tissue.

Effect on various other organs. Other organs in which changes in morphology or in function have been noted in outspoken plumbism and in which the insidious effects of small amounts of lead might affect the well-being of the individual, include the kidney, liver, sex glands and other glands of internal secretion. Much more will have to be known, however, about the exact nature of the action of lead in various tissues before slight abnormalities can with any degree of surety be attributed to the action of lead.

Possibility of acquired tolerance. The usual concept is that, while there is a wide variation in susceptibility, there is no true acquired tolerance to the injurious effects of lead. Legge and Goadby (1912), however, make the observation that individuals exposed to lead in industry for long periods appear to acquire a certain degree of immunity. Persons who develop signs of poisoning early in industrial exposure in some instances can continue in the same work without further difficulty. Weller (1926) in experimental studies in guinea pigs demonstrated the production of increased tolerance to lead. He first established the amount of lead which would produce meningo-cerebral symptoms in 100 per cent of experimental subjects. The survivors of the first attack were then given a rest period and later a larger dose of lead. By repeating this procedure he brought some animals to the point of tolerating four times the original dose without the production of symptoms. More experiments with smaller amounts of lead in various species are needed before any general conclusions can be drawn. Observations of this type, however, suggest that continued exposure may decrease the danger of small amounts of lead.

Need for new criteria for recognition of slight early injury from lead. From the entire foregoing discussion it is apparent that the recognition of the harm done by the small amounts of lead absorbed by the average individual is bound to be difficult. The investigations which give evidence of the marked influence of lead in great dilution on various isolated tissues incline one to the view that the continuous absorption of any amount of lead must result in less than optimal conditions for the organism as a whole. At present, however, we do not know what to look for in the average individual as subjective or objective manifestations of this slightly unfavorable condition. From the available

estimates of the present lead exposure and the amount absorbed by the average individual, it appears doubtful that the usual recognized signs and symptoms of plumbism are to be expected in more than an occasional highly susceptible person. There may conceivably be many instances in which lead is contributing to constipation, or to a tendency to anemia, to ready fatigability of muscles, to hypertension or to impaired nervous and mental function. There are, however, many other possible causes of exactly the same abnormalities. Data on the amount of lead present in the blood and urine of persons both with and without symptoms of this type may eventually make possible a correlation between their appearance and the absorption of lead. To be useful for this purpose the chemical data must be accurate and the studies must include statistically significant numbers of individuals both with and without any recognizable deviation from normal.

PRESENT MOST URGENT PROBLEMS. Probably the question of greatest concern at present is whether modern practices are going to result in a gradually increasing lead exposure for the average individual. Again, it appears that the most satisfactory answer to this question is to be afforded by studies, repeated at intervals, of the lead in the blood and urine of large and representative groups of persons in both rural and urban communities. There is no doubt, as was stated at the beginning of this paper, that the continued absorption of amounts of lead which are still relatively small result in outspoken poisoning. It is important, therefore: 1, to keep informed as time goes on as to the margin of safety which we have between the lead exposure of the average individual and those amounts generally recognized as dangerous; and 2, to establish a more delicate set of criteria than we have at present for the recognition of early slight injury from lead.

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