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U. S. PUBLIC HEALTH SERVICE
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IN REPLYING
ADDRESS THE SURGEON GENERAL
U. S. PUBLIC HEALTH SERVICE
Industrial Hygiene Division

September 16, 1946

Dr. Robert A. Kehoe
Kettering Laboratory of Applied Physiology
University of Cincinnati
Cincinnati 19, Ohio

Dear Dr. Kehoe:

As you are acting as summarizer and principal discusser of the papers presented at the Conference on Lead Poisoning in Boston on September 30, 1946, Dr. Carl Peterson has requested that copies of the papers to be presented be forwarded to you.

I am, accordingly, sending you a copy of the short discussion of analytical methods which I am presenting.

Sincerely yours,

Lawrence T. Fairhall

Lawrence T. Fairhall
Senior Scientist (R)
Chief, Chemical Section

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Encl.

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ANALYTICAL METHODS IN DIAGNOSIS

L. T. Fairhall

Industrial Hygiene Division
United States Public Health Service

It is not my purpose at this meeting to present any new method of lead analysis or any new procedure for the determination and detection of lead in biological substances, but rather to discuss certain phases of the analytical problems which are presented inevitably by the type of material being analyzed for lead.

In the course of the last quarter of a century, an almost endless variety of methods for lead determination with reference to the general problem of lead poisoning has been developed and this very healthy state of affairs has done much to clarify many of the obscure problems of lead poisoning. It is perhaps rather unfortunate that in general the tendency has been to emphasize ease of execution rather than accuracy of execution, with reference to these methods and, to my mind, this has been a detriment rather than a help. The main difficulty, of course, in the analysis of lead in body fluids and tissues has been the great disparity between the bulk of material analyzed and the minute amount of substance sought. When it is realized that the determination frequently revolves around a matter of millionths of a gram in 1,000 or 2,000 grams of fluid or substance,

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and that the total amount of other inorganic constituents accompanying lead in such material may amount to 50 or 100 grams, it is obvious that the separation and quantitative evaluation of these extremely minute amounts of lead is exceedingly difficult. However, the problem is further complicated by the fact that in addition to mere bulk of accompanying substances, lead is also accompanied by small amounts of other metals which are potentially difficult to separate from lead. It is indeed fortunate that the substance, diphenylthiocarbazone, commonly known as dithizone, was brought to light several years ago and that the singular properties of this substance with respect to many metals was so thoroughly investigated by Fisher. With the aid of this substance, it is possible to separate lead quantitatively, even though it is present in extremely small amounts, from a very large amount of material. It is true that a number of other substances are similarly separated, but the separation of these from lead is not too difficult a problem. It is unfortunate, however, that so much attention has been devoted to the further or final evaluation of lead by means of this reagent rather than to the effectiveness with

which lead is separated from other metals as described above. This is understandable because an extremely small amount of lead gives a pronounced and very deep color under certain conditions with this reagent and the facility with which colorimetric determinations are carried out in general makes such a procedure extremely inviting. To that end, long and elaborate procedures have been adopted in order to minimize the effects of interfering substances without too greatly affecting the precise evaluation of lead itself.

This brings up a point which I should now like to discuss more in detail. The analytical chemist is confronted in his laboratory work with a series of operations requiring the greatest care and delicacy of manipulation. Frequently this requires a great deal of time and an unusual, exacting attention to detail which is almost unique. In this sense, each analysis represents a technical achievement of high order and the intelligent analyst usually seeks means to shorten the arduous labor involved in the various steps of analysis by short-circuiting such steps in such a way that needless labor is avoided. It is this fact largely that is responsible

for the development of "new" methods of analysis. As the need for by-passing needless work has grown, particularly in the industrial field, technological advances have kept pace with the chemist and instruments have been developed which greatly simplify his work and perhaps produce a great increase in output.

At the same time, many of these instruments have permitted him to evaluate quantities of substances so small as to be undreamed of at an earlier day. We, for instance, have the quartz micro-balance, the spectrograph and the Geiger counter which respond to the presence of very small amounts of material indeed. Such instruments are of the greatest utility and have tended not only to extend the field of activity of the analyst but, in many cases, to extend accuracy of observation. It is unfortunate, however, that with the acquired facility of operation and the widening horizon of application, as well as the extreme sensitivity of such instruments, there is a tendency on the part of many to short-circuit operations without too close an attempt to find whether such short-cuts are valid; to substitute, in other words, for the

known delicacy of the instrument working under the best of conditions, the application of the instrument to procedures of uncertain accuracy. It is simple to do this and to shift the responsibility from the analyst to the instrument, but no known procedure can be carried very far without the analyst's assuming responsibility. The fact that an instrument of great delicacy and under proper circumstances of accuracy is used, does not in itself effect the true solution of an analytical problem. The analyst must still accept responsibility and the degree to which this responsibility is important rests, of course, upon the nature of his problem. As an instance of this, I can cite the application of the spectrograph and the polarograph to the determination of lead in biological substances.

About 20 years ago refinements began to appear in the quarts spectrograph which greatly facilitated its use and extended its range in the field of analytical chemistry, and quantitative applications of the spectrograph were rapidly developed. This has been facilitated by several means, perhaps the simplest of which is the comparison of the densities of a given line at various concentrations with reference to the density

of a comparison substance in known concentration. Very many modifications of such procedure have been introduced, depending on the nature of the material for analysis. While at first approach this seems to be a method of solution of many analytical problems and indeed is of the greatest importance to many analytical problems, there are phases of this procedure which have natural limitations. The arc spectrum, for instance, is in general most suitable and most sensitive for many applications of the spectrograph but there are factors, which, when closely investigated, indicate that frequently there are difficulties in securing true analytical values. The arc itself is not the simple thing that it first appears to be. There are gradations of temperature varying from the positive to the negative electrode and as a metal boils off, there are variations with time in the amount of metal at various points between the electrodes. At the temperature of the arc, which is around 6700°C, all the substances in the arc are partly ionized and, due to the electrical field, these ions wander in the gas column. The metal ions migrate to the cathode and are neutralized there, diffusing away as vapor. The result

is a concentration of neutral metal atoms and ions concentrated in a thin layer just above the cathode. This cathode layer effect is most notable when small amounts of substances are vaporized in the arc.

The types of lines emitted and their intensities are also affected by the presence of such easily ionizable substances as sodium chloride. It is apparent, therefore, that the direct current arc is far more complex than it is customarily believed to be and that exacting quantitative work requires consideration of these factors.

However, in spite of the known difficulties of application of the spectrograph to the determination of lead and perhaps in total disregard of them, I have heard of determinations made of urinary lead with a single drop of urine. While this may be impressive to some, it is to anyone familiar with the field, merely ridiculous. In spite of the many difficulties attached to the application of the spectrograph to determinations of this character, it is in the hands of the conscientious analyst, an instrument of the very greatest utility.

An interesting method for the determination of lead is that of the

dropping mercury electrode which was first proposed for this purpose by Shikata, a Japanese student of Heyrovsky's, about 1927. The polarograph utilizing the dropping mercury electrode has been applied to the determination of small amounts of a variety of substances in addition to lead for a number of years. Its application depends upon the interpretation of current-voltage curves obtained during electrolysis and it employs a dropping mercury electrode in order to provide a fresh cathodic surface continuously. By applying a small and steadily increasing voltage, a point is reached corresponding to the deposition potential of the ion sought. At this point there is a sudden current surge owing to the discharge of the ions at the cathode and dissolution of mercury at the anode. The voltage at the onset of the current surge represents a deposition potential characteristic of the ion. This current surge reaches a limiting value depending upon the ion concentration, and the proportionality between the total rise and the concentration of the depositing ion is determined by calibration against known standards. The application of the polarograph to lead analysis of body fluids is

invitingly simple and if one is content to accept instrumental readings without further checks, the analytical through-put of the instrument is large. With extreme care one can, however, obtain analytical values, say for lead in urine, comparable with those obtained by other means.

In order to attain the desired accuracy, however, great care must be used in the purification of mercury and reagents, the calibration of the instrument, and the interpretation of results, as well as to the constancy of salt content and acidity of the solutions analysed. Organic material must be absent. Extraordinary precaution must be taken to remove oxygen from the nitrogen gas used as a bubbler to sweep oxygen from the cell preceding electrolysis. I mention this latter since the impression appears to be somewhat prevalent that commercial nitrogen is sufficiently pure for this purpose. In fact the accurate determination of urinary lead with this instrument is more or less of a research job and is so time-consuming that it presents no advantages to the routine analyst whose interest lies beyond a quick value of any sort.

The colorimetric determination of lead by means of dithizone has

perhaps received more attention in recent years than almost any other method. It also has the advantage of rapidity and where the amounts of lead are sufficiently large, and in the absence of interfering substances, it is very useful for this purpose. The principle difficulties that one encounters for the determination of minute amounts of lead are first of all the presence of certain other metals and the tendency of dithizone to oxidize and give a slightly colored solution with certain oxidative substances. This becomes particularly pertinent in the determination of blood lead where an estimation is usually recorded in terms of 3 or 4 micrograms of lead in a 10 gram sample of blood. While lead is unquestionably present in the blood stream in extremely minute amounts, it is doubtful if it is present to the extent that a number of individuals have postulated as a result of measurements of this type. So far as I am aware, outside of our laboratory, no one has taken the trouble to compare the analytical values for the usual 10 grams of blood with a sufficiently large quantity of the same blood to fix incontestibly the value for normal lead. Instead, repeated "determinations" of lead made upon 10 gram

samples of blood have been used as the basis for fixing values for blood lead. It may be said that these values have been substantiated by spectrographic, polarographic and electrolytic methods and that they all are consistent. It is only when one attempts to make a serious study of lead in these concentrations of 3 or 4 micrograms per sample of blood and is confronted, not with the techniques of investigation, which are sufficiently simple, but with the vagaries of the material sought, and readings attributable to other factors, that he is likely to revise his opinion. I regard most reported blood lead values with considerable reserve and suggest that more investigation be made of blood with reference to its intrinsic lead content. Certainly, it should not be difficult to test this by separating lead quantitatively from one hundred times the usual amount used for analysis, if analyses can be made of 10 gram samples with the great accuracy claimed for this amount. Such analyses can be made with pooled lots of human blood, as we have done, or animal blood can be used, comparison being made between 10 gram and 1 or 2 kilogram amounts of the same blood.

I bring up this point because there has been a certain tendency to have a blood lead determination made in suspected cases of lead poisoning and to rest the diagnosis on the analytical report as received. In cases of this type, a child with an apparent high blood lead is in the unfortunate position of possible treatment for lead poisoning when diagnosis of disease of an infectious nature is difficult or obscure. If blood lead values are to be important in the diagnosis of lead poisoning, further investigation should be made of this subject in the interest of scientific accuracy.

I have referred to the question of blood lead somewhat at length because the disparity noted with reference to the actual amount present under ordinary conditions introduces an uncomfortable element in regard to data representing the amounts present in actual cases of lead poisoning.

One further point I should refer to in connection with chemical data. To the non-scientific mind, positive results frequently carry more weight than low or negative results, and the greater the values reported, the more dramatic the effect. Perhaps it might be as well if we look behind

the scenes more and study the source of technical errors. It would be unfortunate if in the field of biochemistry we have a situation analogous to that referred to by T. B. Smith in certain phases of commerce in which professional analysts are classified as "high" analysts and "low" analysts, the sellers favoring one class and the buyers the other.

A very great deal of responsibility is attached to the determination of lead as a diagnostic aid in lead poisoning. Instrumental readings of the greatest accuracy are obtainable from the modern apparatus available to the present day chemist. But the instrument itself cannot supply the intelligence necessary for accurate analysis. The conduct of an analytical operation should match in care the accuracy of the reading to which the instrument is susceptible. In other words, the chemist has a great responsibility in analytical work of this type, for so very much depends upon the outcome of his analysis. It is not sufficient to follow a routine procedure and to assume that, because he has followed the directions word by word, that his analytical results must be correct. A poor chemist may obtain poor results with a good procedure; a competent chemist can obtain

fairly accurate results with an indifferent method.

These observations are offered, not in a critical spirit, but in order to focus attention on certain points that frequently arise with reference to analytical methods used as diagnostic aids in cases of suspected lead poisoning. A large number of investigators have been and are exploring much of this field, and from their perserverance and resourcefulness it is to be hoped that many of the obscure chapters in lead poisoning will become more completely clarified.