March 26, 1945

Dr. O. H. Gaebler,
Henry Ford Hospital,
Detroit 2, Michigan.

Dear Dr. Gaebler:

Thanks for your letter of March 21, to which I reply chiefly for the purpose of correcting an error in my letter of March 15. Our rack blank is of the order of magnitude of 0.1 micrograms rather than the 0.01 indicated in my letter. I rather suspect that you or any other experienced person would elevate his eyebrows somewhat at this virtually impossible precision.

With reference to the end point of phenol red, it does not seem that this has ever given us any very great difficulty. Perhaps it would have been better to make the point a little clearer in the description of the method.

There is a new development in the Dithizone method recently called to our attention, which I think will simplify the procedure considerably. Inasmuch as an article from another laboratory is due to be written on this soon, I should try to bear it in mind and send you a reference to it or a reprint if the latter are available to us.

Cordially yours,

Robert A. Kehoe, M. D.

RAK ef

KE 0001330

Henry Ford Hospital

DETROIT 2, MICHIGAN

March 21, 1945.

Dr.R.A.Kehoe, Director:
Kettering Laboratory of Applied Physiology,
U. of Cincinnati,
Cincinnati, Ohio.

Dear Dr.Kehoe:

Thank you very much for your letter of March 15, and for the reprints which arrived this morning.

I am sure that my inquiry left you very much puzzled. This is due to the simple fact that I was well aware of your extensive work, but had never once bothered to look where it came from. So I was quite as surprised to get a reply from you as you must have been to be asked whether you had any data on the lead content of blood. Although a person who has to keep an eye on some 45 different procedures can not expect to contribute very much, some of my experiences may interest you from the standpoint of being a familiar story. So I am taking the liberty of mentioning them.

My interest in this subject was stimulated in 1938, by the paper of Willoughby and Wilkins, in the J.Biol.Chem. Through years of experience my routine has become somewhat rigid:- 1) Is the method one that we have the equipment, facilities, and "know how" to introduce; 2)Can we recover the substance to be determined regularly when we add it; 3)Do we get the normal values given by the author.

The method of W.&.W. was therefore tried out, and although I could work it, it did not seem to be the sort of thing that one could handle occasionally at unpredictable times. The dithizone solutions were always decomposed when suddenly wanted. So the matter was dropped. The review of 8 series of normals, including your earlier results, by W. &.W., also left me in doubt as to how valuable the method would be if gotten in shape.

In 1942, the paper of Bambach and Burkey, summarizing improvements in the method, interested me again. The reagents indeed proved to be stable. Then to my horror I found that I could not carry any known lead nitrate, 10 micrograms, through the procedure and recover more than 10 per cent of it! After some time I found that this was due to a difference between the author's interpretation of "alkaline to phenol red" and my interpretation. In texts such as that of M.B.Jacobs, one reads that lead is extracted by dithizone at pH anywhere above 7. But I found that in the presence

of the amount of ammonium citrate used in the procedure one does not extract lead at all quantitatively until the pH is 8.0 or above. Further experience showed that one can add a fair excess of ammonia before dithizone passes to the water phase in large amounts, and it was assumed that this is what the author did. Recoveries then were good.

Due to an emergency in a local plant, I had to get this method in hand for actual use suddenly a short time ago, hence my rather hurried letter. Fortunately I told the doctor to send me control bloods on cases having no known exposure to unusual amounts of lead. The controls ran 50 to 65 mcg.%, and explained why a large majority of some 20 workers had this level. I then furnished Pyrex tubes, washed with nitric acid and with lead free water, and stoppered with new corks from a tested lot. The next five controls were 25, 15, 19, 18, and 20 mcg.%.

One specification in your letter will probably not be achieved under our conditions,- limiting the blank to 0.01 micrograms. I may be influenced here by some years of attempts to get the blank in blood iodine determinations down to nothing. Then one day I read in a publication from a leading laboratory specializing on this subject that one should always work with reagents containing a detectable amount of iodine. In other words, the blank should not be near zero, but an amount that could be determined regularly and subtracted. So I did our lead determinations with reagents containing many times 0.01 micrograms per determination.* Four determinations were always run together,- a blank, two bloods, and a blank with 10 mcg. of lead added. The latter would correspond to a blood with 100 mcg.% of lead, since approximately 10 gm. of blood were analyzed. All four determinations are finally read, in a Beckman spectrophotometer, against pure chloroform as a blank. The readings of course indicate whether there has been contamination or loss. Present data indicate that the five controls mentioned at the end of the preceding paragraph might all have had the same normal value of 20 mcg.%. However, this does not invalidate values of 100 to 250 mcg.% that we are getting in cases in which other findings as well as clinical symptoms of lead poisoning are also present.

I want to thank you especially for the report of the committee of the American Public Health Association, and I am sure that the other reprints contain much that will be of interest to me.

Sincerely yours,

O.H.Gaebler

O.H.Gaebler, Ph.D., M.D.

* Less than 1.0 mcg.

KE 0001392

March 15, 1945

Dr. O. H. Gaebler,
Henry Ford Hospital,
Detroit 2, Michigan.

Dear Dr. Gaebler:

I am very glad to answer the questions raised in your letter of March 6, concerning lead determinations. I had rather thought that our publications on this subject were rather well known and readily available, but since you have not seen them apparently, I am taking the liberty of sending you a number of reprints, which I think will be useful, not only in connection with the method but with the interpretation of results.

I do not want to proffer too much unsought advice, but inasmuch as you have said in your letter that you have only recently begun to use the Dithizone method, I feel that a word or two might not be amiss. You may have had experience with other methods, but if you have not used methods of the sensitivity of the Dithizone method, you may not appreciate the difficulties. These difficulties are two in number; 1. the very great likelihood of the contamination of samples in the process of obtaining them, and 2. the serious risk of contamination of the samples by reason of contaminated reagents, laboratory equipment, and laboratory atmosphere. In general, concerning the first of these, it is fair to say that samples of blood and urine collected in hospitals by the usual means are regularly contaminated to a significant and serious degree. For this reason in our opinion, the analyst should always make certain that any samples that come to him have been collected according to his wishes by the most meticulously careful methods. No assumptions of any kind can be made, for under even the most ideal conditions occasional serious contaminations will occur. As to the second source of contamination, we have found it necessary to control all of the conditions in our Laboratory to the point where our blank is of the order of magnitude of 0.01 micrograms. Reasonably satisfactory work can be done under somewhat poorer conditions, but a larger number of dubious results will be obtained otherwise. Under these conditions we have established the limits of the normal lead concentration in the blood, tissues, and excreta of normal individuals. I think we can say now with very nearly absolute certainty that analytical results on the blood in excess of 60 micrograms per 100 ml. are abnormal. In the same way we find that urinary results while variable,

vary within certain rather strict limits, and we are able, therefore, to differentiate between conditions involving abnormal lead exposure and those of the usual type. There is nc possibility of interpreting analytical data without a broad background of observations on normal persons under a wide range of conditions. Our observations have supplied this information, and there should now be no question on this subject. In our feeling practically all of the contradictions that now exist in medical literature, are the result of the use of methods which are insufficiently sensitive on the one hand, or so highly sensitive on the other, that the most minute factor of contamination becomes serious.

Finally, may I invite your careful attention to the prolonged observations we have made on laboratory subjects and also on industrial employees, with the idea of determining the difference between safe and dangerous lead absorptions. From the medico-legal point of view, this is of the utmost importance, for there is much too much tendency among inexperienced persons to interpret evidences of lead absorption as evidences of impending or actual lead intoxication.

Since I am sure you are very much interested in this problem, I am sending you a copy of the report of a Committee of the American Public Health Association, of which I had the honor to be chairman. We have attempted in this report to clarify many of the most significant difficulties in connection with this subject.

Sincerely yours,

Robert A. Kehoe, M. D.

RAK ef

Reprints under separate cover.