November 4, 1938

Mr. Roger S. Hubbard
The Buffalo General Hospital
Buffalo, New York

My dear Mr. Hubbard:-

Please accept my thanks for your letter giving information on your analyses of urine samples from Wm. Guengerich, and discussing the significance of the results. I should like to give you our results, to comment on their significance and then to discuss the general problem of methods of analysis and methods of obtaining samples.

On July 28th we received from Dr. Hillenbrand a sample of blood and a sample of approximately 100 cc. of urine, both having been collected in our containers and according to our instructions, copies of which are enclosed. The result on the blood (0.035 mg. per 100 grams of whole blood) was quite within the normal limits, but that on the urine (0.047 mg. in a sample of 100 cc. equivalent to 0.47 mg. per liter) was not only at variance with the blood finding but also was so high as scarcely to be bona fide. Despite the prescribed precautions, samples are sometimes contaminated, and when collected in industrial plants they are not uncommonly so. It is our custom, therefore, in all cases in which the initial results are quite high, and especially when the blood and urine differ materially in their significance, to check them by obtaining additional samples, especially a sample of urine of large volume. In this instance this procedure resulted in the following data; a sample of urine of 3480 cc. contained lead to the extent of 0.02 mg. per liter, while a sample of feces contained 0.08 mg. of lead or 0.06 mg. per gram of ash. These results justified the conclusion that the high result on the previous small sample of urine was due to contamination of the sample, and we so reported our conclusion to Dr. Hillenbrand. Subsequently when further question was raised we obtained additional samples of blood, urine and feces, these giving results as follows: 0.025 and 0.03 mg. per 100 grams of whole blood, 0.05 mg. per liter of urine in a sample of 3180 cc., and 0.08 mg. in a sample of feces, equivalent to 0.09 mg. per gram of fecal ash. The combined results in my opinion remove all doubt as to the general level of lead excretion of this man, putting it within normallimits. The magnitude of the lead exposure, as indicated by these results, is in keeping with

the facts as to the occupational history of this man, and considering the atypical character of his clinical picture, with respect to lead, I believe lead can be excluded as an etiological factor.

As to methods of analysis employed by us, I am sending you separately the papers of Cholak and Hubbard from this laboratory. For a considerable period of time, and until we could satisfy ourselves as to the sensitivity and accuracy of the spectrographic method developed by Cholak and the dithizone method as adapted by Hubbard, we relied upon a chemical method developed by Edgar, Thomann and Cholak of this laboratory and described in the Journal of Industrial Hygiene for September 1933. It contains several modifications of Fairhall's method which served to make it more accurate and uniform in its application as well as specific with reference to lead. Fairhall criticized our modifications largely because he was offended by our comments on his method, but the fact is that he had never described his own improvements of the original method in such detail as to permit precise reproduction of his procedures, and therefore he had little justification for his irritation at us. That irritation has since been dispelled, I believe, now that he and his associates in the Public Health Service are using both a dithizone method and a spectrographic method similar to Cholak's, and are finding them quite satisfactory and far superior to the chromate method. We recognized years ago that the chromate method was not adequate for handling blood and small samples of urine, and it was primarily for that reason that we were compelled to develop more sensitive methods for carrying on certain phases of our experimental work.

You are quite right in your feeling that the chief criticism leveled at the chromate method referred to its loss of lead and its consequent low results. Nevertheless we found it impossible to avoid occasional high results in urine samples, and frequent high results on feces due to the presence of other metals, without the incorporation of a sulphate step based on the work of the Australians, Avery, Hemmingway et all, as well as a special procedure when bismuth occurred.

Not only are significant errors associated with the use of the chromate method as described by Fairhall, under the most favorable circumstances, but gross errors can be introduced, and in our experience are introduced, by the slight variations in technique which arise from infrequent and irregular performance of analyses, and by variations in the analytical environment and in reagents employed. The last two factors may result in gross errors. A plumber repairs a pipe line and the samples handled in the laboratory for that day or for several days yield high results. A dust is raised in cleaning or in moving objects in the laboratory and the analytical results go wrong. The glassware is handled with less than the usual precision and errors arise. By

reason of sad experience we have found it necessary to eliminate one factor after another, and now we change nothing in our analytical laboratories without stopping the work and cleaning thoroughly thereafter. We have had to exclude outside soot by filtering the air supply of our laboratories, and we have had to discontinue smoking in such laboratories. Dust is avoided with the utmost care. Glassware is washed in accordance with a specially prescribed technique, and C. P. bichromate is used in the cleaning solution instead of the customary technical grade. Reagents are also specially purified to a known purity as determined by spectrographic analysis.

The dangers of error associated with analytical procedures in the hands of even a careful and experienced man are very great, but they are as nothing compared with the opportunities for error in the collection of samples. I am sure you would be utterly astonished at the opportunities for contamination which we have discovered in some fifteen years of work with thousands of samples, but I shall not bore you with these details. Suffice it to say that the analysist is wasting his time and misleading his clinical colleagues if he does not provide chemically clean containers and detailed instructions for the avoidance of contamination in the collection of samples. Nothing that we have been able to do in hospital practice has enabled us to escape occasional gross contamination of samples of urine. Blood samples taken with hospital syringes and needles, cleaned and sterilized in the usual way will always be seriously contaminated. (We use a specially cleaned pyrex tube, supplying with it a special all-stainless steel needle sterilized by boiling in triple-distilled water and encased in a second correspondingly cleaned pyrex tube ready for use.) Urine samples collected in hospital urinals and poured into a bottle as in the case of ordinary 24 hour samples, will usually be grossly contaminated. Here our experience enables us to say that results in the urine over 0.30 mg. per liter are probably the results of contamination, since in the most serious cases of lead exposure and intoxication one rarely finds results in excess of this level. We have found a very few results as high as 0.50 mg. per liter, mostly in children, but figures above this range do not occur except as the result of massive doses associated with acute and usually fatal intoxication. Results of this order of magnitude or greater are the effect of contamination of the sample either in its collection or analysis. I have no hesitation, therefore, in saying that the results obtained in your laboratory on August 29th and September 24th are fantastically and impossibly high, as was also our result of 0.47 mg. per liter in a 100 cc. sample collected at the plant. Such concentrations simply do not occur except under the most unusual circumstances. Even your later samples, however, yielded high results. Our samples of blood on October 21st yielded 0.025 and 0.03 mg. of lead per 100 grams,

and our urine sample on October 24th yielded only 0.05 mg. per liter, as contrasted with your result of 0.12 mg. per liter on the same date. This is in spite of the fact that our analytical methods are more accurate and more sensitive than yours at its best, i.e. there is little or no inherent loss associated with the application of our methods. There must have been some factor of contamination therefore, or some failure on the part of the analytical procedures.

I have gone to some length to discuss the details of this case, but I should be glad to consider further any other points which may be raised. By reason of our experimental work and its implications, I have been much more interested in such cases than clinicians in general would be, and moreover, I am greatly disturbed by the increasing reliance on certain laboratory data, the significance of which are not adequately appreciated by our clinical colleagues.

I should also be glad if you would send us the aliquot of the sample you have analysed. We will analyze it spectrographically and by the dithizone method. Incidentally we will be glad to determine the lead, copper, aluminum and silver quantitatively, and bismuth as well if it is found to be present under our conditions of combustion.

Cordially yours,

RAK:is

Robert A. Kehoe, M.D.