June 26, 1937

Dr. Kenneth D. Blackfan
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Children's Hospital
300 Longwood Avenue
Boston, Mass.

Dear Doctor Blackfan:

I am complying with the further request in your letter of June 22nd by sending you herewith a reprint of Cholak's article describing his spectrographic method of lead analysis. We are in the process of preparing an article for publication which will describe the details of the "Dithizone" method of analysis which we use.

As to the methods of collecting samples, we have had all stainless steel needles manufactured for us by the Max Wocher and Son Company of Cincinnati. It is only necessary to specify that the needle be made entirely of stainless steel, and that the hub which is commonly used in the ordinary needle should be completely eliminated. This requires a special method of fabrication but one which is not difficult. Obviously these needles are somewhat more expensive than the standard brand. In obtaining blood samples, we prepare pyrex test tubes into which blood and spinal fluid can be drawn. These are washed carefully with cleaning solution, rinsed repeatedly with distilled water, and then rinsed at least three times more with distilled water, triple distilled from either a pyrex or a silica still, preferably the latter. These tubes are allowed to drain, stoppered with a clean cork which has never been used before, and covered with a clean filter paper which is held in place by a rubber band. The blood sample is taken directly from the vein into the tube by means of a special need and usually without a syringe. (I occasionally take samples in the laboratory making use of a pyrex syringe which has been sterilized by boiling in distilled water after which it is rinsed three times with triple distilled water, as previously mentioned. In general, however, I prefer to let the blood run directly through the needle into the tube.) When we have samples obtained by other doctors, we provide both the blood tube and the needle. The latter is cleaned as previously described, put into a clean pyrex tube,

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stoppered with cotton and sterilized in the autoclave so that it is ready for use without any further attempt at sterilization. After taking the sample, the blood tube is stoppered, the filter paper replaced carefully and the tube is kept in an upright position, if possible, so as to avoid contact with the cork. (We have had a number of samples sent to us through the mail in which this latter precaution could not be observed, and we have had no difficulties. However, there is the possibility of slight error in that the serum might be absorbed to some slight extent into the stopper.

Substantially the same technique is observed in the case of spinal fluid, the needles being prepared and sterilized in advance and the fluid being withdrawn into the tube prepared as in the case of the blood tubes.

In general, where it is feasible, we obtain a spot sample of urine at the same time the blood sample is taken. A small pyrex bottle is prepared in exactly the same manner as the blood tube and the subject is asked to void approximately 100 cc of urine directly into the container, after which it is stoppered with its glass stopper which is held in place by a filter paper covered securely by a rubber band. We have been able to obtain samples in this fashion even when they have had to be mailed to us from some distance. In general the results are satisfactory although, obviously, better results can be obtained in the laboratory. The great difficulty in connection with urine samples is that it is almost impossible to impress people with the necessity of having the sample voided directly into the container. Ordinary hospital equipment, if used as an intermediate container, will so contaminate the sample as to render it utterly useless, and misleading. Consequently in our work here at the General Hospital and the Childrens Hospital, we have insisted that the internes obtain the samples themselves and make sure that they are collected precisely according to instructions.

In general there is approximately a ten to one relationship in the concentration of lead in the blood as compared with the urine. That is to say, the concentration in the blood expressed in terms of 100 grams is approximately the same as the concentration in the urine expressed in terms of a liter. Obviously this relationship does not hold too closely if if we are able to obtain a blood sample and a small urine sample on a patient at the same time, and if these samples check so as to have approximately the same physiologic significance, we feel that in all

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probability neither sample has been seriously contaminated in the process of collection. If the samples do not agree, we feel disposed to insist upon the collection of additional samples, and with somewhat greater precautions. Moreover we do not attempt to draw refined conclusions from borderline cases. The lead content of both blood and urine may vary from time to time to such an extent that repeated observations may be necessary in order to give a certain significance.

In our experience spot samples of urine taken at different times of the day and on different days are subject to fairly wide variation. Consequently we do not like to draw too strong conclusions from a single spot sample. Where it is feasible, we try to obtain a sample which will represent the urinary output over a period of 48 to 72 hours. In such a sample the hour to hour variations will be ironed out and we have reason to believe that in such a sample we are dealing with an average secretory condition. A lead content in the urine of one tenth of a milligram or more per liter is obviously significant of a comparatively recent exposure to lead, and if the quantity is in ecess of two-tenths of a milligram per liter, we feel certain that we are dealing with significant lead exposure. Not all persons with this level of lead excretion show symptoms. A very large proportion of persons with symptoms show levels of this order of magnitude.

You will note that I am inclined to put much greater faith in the analytical results from more than one source than in a single sample. It is for that reason that I like to have a sample of urine and a sample of blood obtained substantially simultaneously, if this is possible.

I trust I have not indulged in any repetition of my former remarks to you, but I want to be quite clear as to my point of view and the reasons for it.

Cordially yours,

RAK:is

Robert A. Kehoe, M.D.

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