acid daily, only one animal showed a pronounced reaction; and of the remaining 9, 6 showed not the slightest evidence of sensitization. The animals in this group were completely protected from scurvy. It would appear, then, that the minimum dose which seems, in our experiments, to exercise an inhibitory influence upon the sensitization to neoarsphenamine, as shown by group V, is definitely higher than that which is sufficient to protect against gross scurvy.

Those animals in the less protected groups which failed to become sensitized were frequently precisely those which exhibited the most florid symptoms of scurvy and were cachectic. It is possible that their refractoriness to sensitization was based upon a different mechanism (cachectic energy?), *i. e.,* upon a mechanism which was not operative in Group V.

7833 P

Spectographic Determination of Lead in the Blood Serum.

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The chemical methods available for the quantitative determination of lead in blood, while accurate, require amounts of blood too large for routine clinical work. Spectroscopic methods are more sensitive and allow of the use of smaller amounts of material. They have, however, been employed for qualitative estimations only.* [1, 2, 3]

We have developed a spectroscopic method for determination of lead quantitatively which using a simple procedure can estimate from 0.0005-0.01 mg. of lead with an error of ±50%.

In principle the method consists of comparing either photometrically or visually, the intensity of lead lines from the unknown sample

* Since the completion of our work an article appeared on the "Quantitative Spectographic Determination of Lead in Urine," by Jacob Cholak, *J. Am. Chem. Soc.*, 1935, **57**, 104. The technique and apparatus used, however, is more involved and expensive than that described by us.

1 Rabinowitch, I. M., Dingwall, A., Mackay, F. H., *J. Biol. Chem.*, 1933, **103**, 707; *J. Biol. Chem.*, 1933, **103**, 725.

2 Shipley, P. G., Scott, T. F. McNair, and Blumberg, H., *Bull. Johns Hopkins Hosp.*, 1932, **51**, 327.

3 Blumberg, H., and Scott, T. F. McNair, *Bull. Johns Hopkins Hosp.*, 1935, **56**, 32.

with lines from admixtures of known amount of lead salt and the amount of sodium normally present in the quantity of serum used. The accuracy of the method is ±50% visually; this accuracy should be increased when using a photometer.

Since lead is a common contaminant, all glassware used was Pyrex which was steamed for 2-3 hours previous to use. The silica crucibles were similarly treated. Only lead-free needles and syringes were employed.

Spectroscope. The instrument used was the Hilger 4 E quartz spectroscope loaded with Eastman 33 plates. The spectrum produced by this instrument gives a long range of wave lengths extending well into the ultraviolet and permits the use of a large number of lines from which to draw conclusions.

Sodium Chloride Solution.—NaCl is recrystallized several times until free of lead. 8388 mg. is made up to 1000 cc. with redistilled water. 1 cc. of this solution contains 3.3 mg. of sodium.

Lead Chloride Solution—$PbCl_2$ is recrystallized several times. 13.4 mg. is dissolved with 1 cc. of lead-free redistilled nitric acid and then made to 1000 cc. 1 cc. of this solution contains 0.01 mg. lead.

Preparation of standards. To 1 cc. of NaCl solution in a silica crucible amounts varying from 0.1 cc. to 1.0 cc. of the $PbCl_2$ solution are added. The solutions are evaporated and the solid residue flashed. The concentration of lead in these mixtures corresponded from 0.1 mg. Pb^{++} to 1.0 mg. Pb^{++} in 100 cc. of solution. This was the range encountered in lead poisoning cases. The normal concentration of lead was not measured accurately. It is definitely below 0.1 mg. per 100 cc.

Treatment of the serum. I cc. of serum is placed in a silica crucible that is steamed for 2 hours previous to its use. The whole is dried in an electric oven at 110° for 3 hours and then ashed in a muffle furnace for 24-48 hours at 500°C. and then flashed.

Flashing. One set of H. S. Brand pure Acheson graphite electrodes 10 mm. in diameter and of convenient length are prepared for each determination. The electrodes are ground, the lower one for receiving the ash, flat—and the upper one to a fine point. They are supplied by a 110 volt direct current through a 4 amp. resistance. (See Fig. 1.)

The flashing is conducted in the following way: Roughly, one quarter of the crucible contents are placed on the electrodes with a platinum spatula, the electrodes are placed in juxtaposition, the current turned on, the electrodes are drawn apart and allowed to flash

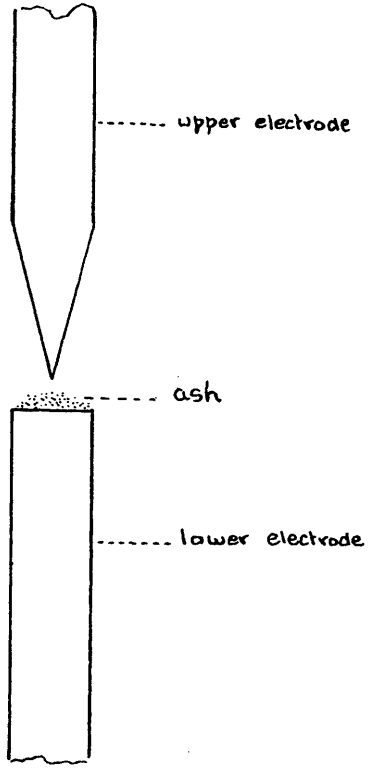

FIG. 1.
Diagram of electrodes and ash.

for 15 seconds. A second and third quarter are added in the same way. After the last portion has been added the flash is made to burn continuously for 10 minutes. (This time is found sufficient to volatilize the whole sample.)

The plates are then developed in Kodak D-61 for 12 minutes at 18°C., rinsed, fixed, washed and dried.

Determination of lead in serum. The concentration of lead in the serum is estimated by comparison of the intensity of lead lines against the standard spectra. An estimate of the concentration of lead is made by choosing those standard spectra whose lead lines are nearest in intensity to that of the unknown. In such manner the values are within ±50% when the comparison is made under a 25-fold magnification. The use of a photometer should increase the accuracy.

Results. Blood which showed no lead line on preliminary flashing gave satisfactory results on addition of known amounts of lead. Patients with clinical symptoms of lead poisoning showed concen-

trations in blood varying from less than 0.1 mg. to more than 1 mg. of lead per 100 cc. Normal values are definitely below 0.1 mg. per 100 cc. of serum.

TABLE I.
Sample Determinations of Lead in Human Blood Sera.
Values expressed as mg. Pb^{++}/100 cc. Serum.

Diagnosis	Subject	Lead Found
Lead Poisoning	Adult	.7
" "	"	.3
" "	"	.1
" "	"	1.0
" "	"	.2
" "	"	.5
Normal	Child	.03 appr.
" + 0.5 mg. Pb in 100 cc.	"	.5
" + 0.3 mg. Pb in 100 cc.	"	.3
" + 0.2 mg. Pb in 100 cc.	"	.2

7834 C

Demonstration of Hemorrhagins in Snake Venom by Means of the Chicken Embryo.

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The venoms of various species of snakes differ in their content of toxic principles such as hemorrhagins, hemolysins and neurotoxins. While the hemolysins and neurotoxins can easily be determined, a reliable method for the demonstration of hemorrhagins has been lacking.

In the course of experiments with the chicken embryo it occurred to us that such a preparation might lend itself as a suitable test object for the study of hemorrhagins in venoms.

Fertilized chicken eggs are placed in an incubator at 42°C. for 3 days. The egg is opened preferably at the small pole; one-third or half of the shell is carefully removed and the egg poured into a beaker. Then, the beaker is covered with a watch glass and kept in the incubator at 37°-38°C. The body of the 3-day-old chicken embryo is surrounded by a vascular network of 3-4 cm. in diameter (Fig. 1).

A 1% solution of moccasin venom *(Ancistrodon piscivorus)* in physiological saline was prepared. Serial dilutions were then made up to 1.0 cc. with physiological saline. These were placed in the