INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps.

ProQuest Information and Learning
300 North Zeeb Road, Ann Arbor, MI 48106-1346 USA
800-521-0600

UNIVERSITY OF CINCINNATI

May 29, 1951

I hereby recommend that the thesis prepared under my supervision by Paul Jones Whitaker

entitled The Absorption, Excretion and Retention of Ingested Lead by Human Experimental Subjects

be accepted as fulfilling this part of the requirements for the degree of Doctor of Industrial Medicine

Approved by:

Robert V. Kehoe

FORM 668—G. S. & T. C.—500—5-48

THE ABSORPTION, EXCRETION AND RETENTION OF INGESTED LEAD BY HUMAN EXPERIMENTAL SUBJECTS

A Study of Long-Term Experiments on the Lead Metabolism of Human Persons Subjected to Normal and Increased Levels of Ingestion

submitted to the
Graduate School of Arts and Sciences
of the University of Cincinnati

in partial fulfillment of the
requirements for the degree of

DOCTOR OF INDUSTRIAL MEDICINE

1951

by

Paul Jones Whitaker

B. S. University of Pittsburgh 1935

M. D. University of Pittsburgh 1939

Acknowledgments

The experimental work herein reported was done at the Kettering Laboratory in the Department of Preventive Medicine and Industrial Health of the College of Medicine, University of Cincinnati, Cincinnati, Ohio, from 1937 to 1946 by Robert A. Kehoe, Jacob Cholak, Donald M. Hubbard, Karl Bambach, Robert R. McNary and Robert V. Story.

The section on laboratory methods was edited by Robert A. Kehoe, under whose direction the entire experiment was performed. Dr. Kehoe's personal knowledge of the difficulties encountered in the performance of this long term metabolic study and the methods employed in overcoming these difficulties are exceedingly valuable to anyone interested in this type of experiment. The present writer became interested in the experiment in 1947 and had no knowledge of these procedural difficulties. Any discussion of laboratory methods by him necessarily would have been a mere listing of procedures, and for him to have done this would have meant the elimination of the important techniques herein described by Dr. Kehoe.

P. J. W.

TABLE OF CONTENTS

INTRODUCTION

Previous articles (1, 2, 3) have sketched the general methods and the preliminary results derived from a study of the effects of the oral administration of lead, as a solution of lead acetate, to two healthy human subjects over a long period of time. When these experimental observations had progressed to a point at which it was possible to visualize the character of the induced metabolic pattern, with respect to the rates of absorption, excretion and retention of lead in the body, the administration of lead to these subjects was discontinued. Originally, it had been intended to continue observations on these subjects, with respect to the intake and output of lead, during their return to normal levels of lead excretion, but in neither instance were the observations continued for the length of time required for this purpose. During the observations on these two subjects, it became apparent that a parallel study over a period of at least one year, employing a normal subject on a normal diet without any additional lead intake, would be highly advantageous as an extension and a refinement of previous studies on normal lead metabolism (2, 4, 5). Such an experiment was initiated, and when it had been completed, the same subject was employed to demonstrate the effects

of the daily administration of a relatively large oral dose of lead acetate (3.0 mg. of lead). During the observations on this third subject, it became apparent that it would be advisable to make similar observations on another normal subject on a normal diet without any additional lead intake. Accordingly, a fourth experimental subject was employed and his normal lead metabolism studied for a period of about one year. It then seemed desirable to determine experimentally, as nearly as might be feasible, the amount of lead which could be added daily to the diet of the normal human adult without inducing a demonstrable retention of lead on his part (3). This fourth subject was then further employed to demonstrate the effects of the daily administration of a very small oral dose of lead acetate. Since the last report (3), the observations on the last six months of the third subject's year of normal lead metabolism, the observations on his ingestion of a large daily dose of lead acetate, all the observations on the fourth subject and observations on the second subject for an additional 612 days have been made. It seems desirable now to review the entire procedure, to describe in some detail the experimental methods and to give the results in a comprehensive manner. The data, of necessity, must be given in greatly contracted form.

CONCLUSIONS

1. Each of two human subjects, while taking a normal diet of his own choosing, ingested approximately 0.25 mg. of lead daily in food and beverages.

2. Each of these subjects eliminated approximately 0.25 mg. of lead daily in his feces during the period of the normal diet.

3. During the period of the normal dietary regimen, each of these subjects eliminated approximately 0.03 mg. of lead daily in his urine, the urine having an average lead concentration of approximately 0.03 mg. per liter.

4. Over the period of approximately one year, during which a normally chosen diet was taken by these subjects, each eliminated more lead from his body in the feces and urine than was ingested in his food and beverages, the excess of output over intake being approximately 10 mg. for the period of a year, in both instances.

5. Each of four human subjects, to whom lead was administered orally in doses of 0.3 mg., 1.0 mg., 2.0 mg. and 3.0 mg. per day, respectively, eliminated less lead from his body in urine and feces than was ingested in food and beverages. The rate and the amount of lead thus retained depended mainly upon the rate of ingestion (dose per day) and the duration of the period of administration

but it varied also with other less obvious factors, among which, apparently, was a seasonal factor.

6. Each of three human subjects, while taking 1.0 mg., 2.0 mg and 3.0 mg. of lead daily in addition to that contained in his food and beverages, demonstrated an irregular but progressive increase in the urinary and blood lead concentrations during the period of lead administration, there being a progressive decrease in these concentrations for some time after the discontinuance of the administration of lead.

7. Lead retained in the bodies of these three subjects during periods of oral lead administration was slowly lost from the body after discontinuance of the lead administration. This loss occured in each instance by way of the urinary route, and in two instances, associated with the higher dosages, by way of the alimentary tract as well. The rate of such loss varied with the rate at which the lead had been retained in the body.

8. The amounts of calcium and phosphorus and all combinations of these elements in the daily diet of one subject had no measurable effect on the rates of absorption, excretion or retention of lead during the period of elevated intake of lead.

9. None of these human subjects showed clinical signs or symptoms of lead intoxication at any time, despite the

fact that amounts varying from 110 mg. to 118 mg. of lead had been retained in the bodies of two of the subjects during periods of two and four years respectively, while 55 mg. of lead had been retained in the body of another subject during a period of four months. The quantitative significance of these amounts, when retained in the body, can be realized more satisfactorily on the background of the available evidence which indicates that the normal American adult, with no occupational exposure to lead, has about 120 mg. to 180 mg. of lead in his tissues as the result of his normal intake of lead in food and beverages over periods of many years.

EXPERIMENTAL METHODS

MANAGEMENT OF EXPERIMENTAL SUBJECTS

All four subjects, members of the laboratory staff, were employed for the purposes of these experiments after being judged healthy by a detailed clinical study, consisting of a comprehensive medical history and physical examination, various dental and radiographic examinations and laboratory tests indicated for the determination of the organic functional status. A series of preliminary observations for determining the normal lead intake in food and beverages and output in the feces and urine was carried out on each subject. The minimum period of the

preliminary observations was twenty-eight days.

Each subject collected duplicate samples of all food and beverages consumed, all feces evacuated and all urine voided. The food was composited into daily samples for analysis and the beverages, except milk, which was treated as a solid food, were collected separately for measurement of the volume and analysis for lead. Each fecal evacuation was collected separately and the urine collected as a twenty-four hour sample from 8:00 A. M. to 8:00 A. M., except on a few occasions when samples were collected at more frequent intervals for determining diurnal variations in urinary lead excretion. One technically trained subject, I. F., weighed or measured all food and beverages consumed and collected duplicate samples of the same weight or volume. The other subjects collected their duplicate samples to the best of their abilities using only household measurements. Each subject was recompensed for the additional cost of collecting duplicate samples of food and beverages so as to encourage his adherence to his normal dietary habits. In addition, each subject was required to keep a diary, in which were listed all articles of the diet, all unusual occurences, and all deviations from the regimen adopted for experimental purposes.

On the first day of observation, and at weekly intervals

thereafter, with but few exceptions, duplicate 10 ml. samples of blood were drawn from an arm vein of each subject for parallel lead analyses by two methods.

A clinical examination was carried out weekly on each subject, at which time an interval history, pointed particularly at alimentary symptoms, was recorded, together with the subject's weight, temperature, pulse and respiratory rates, systolic and diastolic blood pressures, and grip, as measured by a hand dynamometer. A general physical examination surveyed the subject as a whole, particular emphasis being placed on the condition of the teeth, gums, nose and throat and the neuro-muscular system. Comprehensive hematologic examinations were performed at weekly intervals, and films for counts of reticulocytes and stippled erythrocytes were made daily from the blood of each subject.

The additional lead to be ingested by the subject was taken as a solution of lead acetate, one-third of the total daily dose being taken with each meal. A standard container was provided and was emptied in a uniform manner each time, so that the slight loss due to incomplete emptying was made as nearly constant as possible. A similar amount of the solution was added to the composite daily food sample by corresponding technique, thereby subjecting the total dosage of ingested lead from all sources to analytical determination rather than to calculation. On a few widely scattered

occasions, one or more doses of soluble lead was omitted by a subject by reason of error or inconvenience, and on these occasions, only the actual number of doses ingested was added to the composite daily food sample.

EXPERIMENTAL SUBJECTS

Subject M. R.

M. R., a white male, was born March 12, 1907 and judged to be healthy after the preliminary examinations. After twenty-eight consecutive days of observation to determine his normal lead intake and output, the subject began taking 1.0 mg. of lead per day in three divided doses of 0.33 mg., one with each meal. He continued on this dosage of lead, in addition to that contained in his freely chosen diet, for a period of 1477 days, beginning on February 18, 1937 and ending on March 5, 1941.

Some time after the inception of the experiment, observations on the intake and output of calcium and phosphorus were initiated and were thenceforth carried on regularly before, during and after a series of induced dietary changes.

During three weeks in October of 1940, the subject was permitted to relax somewhat while on vacation in the southern United States. During this period, each dose of lead was taken one-half hour before the corresponding meal, so as to

avoid conspicuous behavior in public. The urine was collected only once daily as a single sample of approximately 100 ml. around 4:00 P. M. Duplicate foods and beverages were not collected. Only the feces were collected in their entirety. This regimen provided for the recognition of any unusual lead intake in the food or any unusual excretory response while maintaining the daily experimental intake of lead. The data obtained for this period were in no wise unusual and could have been used to calculate the approximate lead exchange for the period. Instead, however, the period was treated as though it had not existed.

Other deviations from the customary daily routine occured on several occasions when samples of blood and urine were obtained at two-hour intervals throughout a twenty-four hour period, when sizeable samples of sweat were collected for analysis and when dietary changes were induced for twenty-eight day periods to measure the effect of calcium and phosphorus metabolism on lead metabolism. These changes in no way interfered with the daily performance of the experiment and are mentioned only for the sake of completeness in noting the exceptions to the otherwise highly uniform daily behavior.

The only other accidental factor, which might have exerted some influence upon the lead absorption and excretion of M. R., were the intercurrent illnesses suffered during the four year

period. He had several upper respiratory infections of minor severity, and on one occasion, a typical attack of food poisoning, with diarrhea and vomiting, which terminated in recovery in three days. In this latter instance, all the vomitus and fecal evacuations were collected for analysis with no interruption of the experimental observations. No changes in lead metabolism referable to these periods were demonstrated except that ingested lead traversed the alimentary tract with increased rapidity during the period of diarrhea. In August of 1939, the lower right third molar, and in April of 1940, the lower first and second right molars were extracted. Healing occured normally in each instance. All extracted teeth were saved for analysis for lead.

Subject E. B.

E. B., a negro male, was born January 6, 1906 and was judged to be healthy after the usual preliminary investigation. After a study of 56 days, during which the normal lead intake and output was determined, the subject was started on a daily dosage of 2.0 mg. of lead, taken as a solution of the acetate in three doses of 0.67 mg., one with each meal. This dosage was continued without interruption for 646 consecutive days from March 5, 1939 to December 12, 1940.

Observations on the intake and output of calcium and phosphorus were made throughout all the experimental period, including the period which followed the discontinuance of the

administration of soluble lead, but at no time was an attempt made to modify the subject's diet. There was no vacation or other interruption in the daily routine during the period of experimental lead administration. On a few occasions, a single dose of lead was omitted by error and a corresponding allowance was made for such an error by adding the proper amount of lead to the composite food sample for the day. The subject had several acute upper respiratory infections during the experimental period, none of which confined him to bed. No other illnesses or injuries developed to interfere with the experimental procedure.

After the conclusion of the period of lead administration, E. B. was observed for 908 more successive days, during which time the same procedure was followed except that no additional lead was added to that present in his chosen diet. At the end of this time, the subject was inducted into the Armed Services and he was unavailable for further study until three and one-half years later, when he returned for a twelve week study of his lead metabolism under normal conditions.

Subject I. F.

I. F., a white male, 34 years old, with considerable training and experience in laboratory procedures, was selected as a subject for a prolonged study of normal lead metabolism. Following extensive examinations to establish his status as a healthy man, the routine procedure was

established with the following exceptions: 1) all items of solid food were weighed accurately and the volumes of all liquid foods were measured, and duplicate samples of food and beverages were obtained on this basis, this being done in an effort to reduce the sampling error as much as possible; 2) the metabolism of aluminum, on the part of this subject, was being studied simultaneously, and a few dietary changes were induced for short periods from time to time, for the purpose of altering the aluminum intake, the diet being well-balanced and adequate during all such periods; 3) the intake and output of calcium and phosphorus were followed throughout the experimental period; 4) weekly observations were made on the blood serum phosphatase by the method of Bodansky.

These observations were continued for 364 days, beginning April 4, 1941, with no interruptions from intercurrent illness and with only minor deviations in routine in the form of omitted daily blood films and irregular dates of blood sampling and physical examinations, on two occasions, when the subject was on brief holidays. The collection of food, beverages and excreta continued without interruption during these periods.

Following this period of study, observations were discontinued for 41 days, after which, following a control study period of 14 days, the subject was given 3.0 mg. of lead daily,

in the form of a solution of the acetate, in three divided doses of 1.0 mg., one with each meal. This dosage schedule was continued without interruption for 113 consecutive days, the observations being made as before, from September 21, 1942 until January 12, 1943. Following the end of the experimental period of lead ingestion, observations were continued daily until June 28, 1943, at which time the subject left the laboratory to join the Armed Services.

Subject S. W.

S. W., a white male, age 31 years, was selected as the other subject for the study of normal lead metabolism, after very extensive examinations had revealed him to be an essentially healthy man, who had, however, certain recognized minor defects. The routine set up for subjects M. R. and E. B. was followed and observations were continued without interruption over a period of 347 days, from July 28, 1943 to July 9, 1944. The subject had frequent upper respiratory infections during this period, some of very minor severity, others of moderate severity. In March of 1944, the subject underwent nasal polypectomy. Daily observations on the lead metabolism were continued without interruption during the brief period of the subject's hospitalization and subsequent convalescence.

Following this year of study of the normal lead metabolism, S. W. was given a ten day vacation period during which no

observations were made, and then immediately placed on 0.3 mg. of lead per day in addition to that in his chosen diet. Again, the lead was administered as a solution of the acetate, in three divided doses equivalent to 0.1 mg. of lead, one with each meal. This dosage schedule was continued with only occasional deviations from the normal routine, including the omission of only one dose of lead, for 420 days, from July 19, 1944 until September 12, 1945.

LABORATORY METHODS

Technique of Blood Examinations

Except for brief periods, the blood examinations were made by one person whose long experience insured uniformly accurate results. Erythrocytes, leukocytes, and types of leukocytes were counted by the customary methods, and the hemoglobin was determined by the Haden-Hausser technique. Films for reticulocyte counts were prepared and stained by the standard dry vital-stain technique. The reticulocytes were counted in the manner described below for stippled erythrocytes.

Basophilic granulation of the erythrocytes (stippling) was studied by a method which has been employed in the Kettering Laboratory for many years, and has yielded comparative data on large and varied groups of persons (6). Blood films, one cell layer in thickness, were made carefully

on clear and chemically clean glass slides and dried in the air without fixation. They were dipped momentarily (not exceeding 5 seconds) in an undiluted stain consisting of 0.25 gm. methylene blue and 0.25 ml. of a 1% solution of sodium hydroxide in methyl alcohol, in 100 ml. of acetone-free methyl alcohol. The slides were then washed quickly in a profuse stream of distilled water and dried rapidly in a strong current of air. The stained films were examined with microscopic equipment selected for the sharpness of definition at 900 diameters and for the flatness of the entire field. Fields were chosen for examination that presented even distribution of the cells with a minimum of space between. Such fields contained approximately 250 erythrocytes. Each erythrocyte in 50 microscopic fields was carefully scrutinized, and only those showing definite basophilic granulation, whether fine or coarse, were counted. The results were expressed as the actual number found in 50 fields, or in terms of one million erythrocytes, on the approximately correct assumption that 12,500 erythrocytes were present in the 50 fields.

Methods of Sampling Materials and Analysing for Lead

Experience gained in the past years with a number of analytical methods for the determination of lead in biological materials (7, 8, 9, 10, 11, 12, 13, 14, 15, 16) has resulted in the development of techniques which are

especially suitable for the rapid handling of large numbers of samples without sacrificing specificity or accuracy. The details of the chemical (7, 12, 13), spectrochemical (8, 9, 10, 11) and polarographic (15) methods employed have been fully described elsewhere, and it has also been shown that certain of these methods may be used interchangeably without sacrificing accuracy or in any way altering the results of extended experimental studies (11, 14, 15). However, the methods of obtaining and handling samples of various materials and of maintaining a satisfactory level of analytical uniformity and precision in an experiment extending over a period of years, are quite as important as the analytical procedures themselves in relation to experimental results that have far-reaching significance. Accordingly, it seems advisable to describe such methods in detail and to demonstrate their adequacy for the purpose for which they were employed.

Blood

All equipment for drawing and handling blood samples was kept and prepared for use within a room, the air of which was freed of dust by continuous filtration. Within this room, 20 ml. of blood were withdrawn from an arm vein of the subject by means of a special all-stainless-steel needle and a Pyrex glass syringe, and immediately discharged into two

chemically clean, tared 100 ml. silica dishes, approximately 10 ml. in each such dish. The dishes were again weighed in order to obtain the weight of the blood samples. To each dish, 10 ml. of redistilled nitric acid were added and the contents were taken to dryness on an electric hot plate. Volatile materials were driven off and the contents were brought to a char by means of an adjustable electric heater. The combustion was then completed by permitting the dishes to remain three to four hours in an electric muffle furnace automatically maintained at 500° C. After cooling, the ash was moistened with a few ml. of triple distilled water, 1 ml. of redistilled nitric acid and 2 ml. of redistilled hydrochloric acid were added, and the dish was heated until solution was complete. One of the samples was analyzed spectrographically (10), while its duplicate was analyzed by means of the dithizone method (13).

Statistical treatment of the results obtained on 156 consecutive duplicate samples of the blood of Subject M. R. and 103 duplicate samples for Subject E. B. showed a practical correspondence between the sets of results by the two methods, mean values of 0.054 mg. and 0.058 mg. of lead per 100 gm. of blood (M. R.), 0.058 mg. and 0.059 mg. of lead per 100 gm. of blood (E. B.), being obtained for the spectrographic and dithizone methods respectively (11). The medians and modes for the two methods were also calculated

and the differences resulting from the use of the two methods were found to be as insignificant as those indicated above. A limited number of results was also obtained by means of a polarographic method (15), and while they were slightly lower than those obtained by the spectrographic and dithizone methods, the range of values was generally of the same magnitude as that obtained by the latter methods. The samples employed for polarographic checking consisted of the material remaining after spectrographic analysis.

Urine

The analyses of the urine were made spectrographically, and periodic checks by the dithizone or polarographic methods confirmed the reliability of the results. The differences between the values obtained by the methods rarely exceeded $\pm$ 0.01 mg. of lead per liter of urine and were generally of the order of $\pm$ 0.005 mg. of lead per liter. The spectrographic method was employed because aliquants of 100 ml. could be removed directly from these fresh clear samples and prepared rapidly for analysis by the methods described elsewhere (8, 9).

When check results by the dithizone method were desired, the entire 24-hour specimen (collected in a chemically clean gallon jug) was prepared as follows:

The urine was measured and placed in a Pyrex beaker of suitable size, 10 ml. of nitric acid for each 100 ml. of

sample were added, and the mixture was evaporated to small volume, transferred to a 500 ml. Pyrex evaporating dish, and evaporated to dryness. The residue was ignited to a white ash in an electric muffle furnace maintained at 500° C. The ignition was started at the mouth of the furnace, and the dish was gradually moved into the hotter portion of the furnace in order to avoid deflagration. When all danger of the latter was past, the furnace door was closed and the sample heated until the ash was white (30 minutes to one hour). The dish and contents were cooled, the ash was dissolved in distilled water and nitric acid, the solution rinsed into a 250 ml. volumetric flask without filtering, and diluted to the mark with distilled water. A suitable aliquant was then analyzed by means of the dithizone method.

Feces

The fecal sample was transferred from the container in which it had been collected to a tared 400 ml. Pyrex evaporating dish by means of hot water, and was heated to constant weight on the electric hot plate. The volatile materials were then driven off by heating at low heat for three hours on an electric heater provided with a three-way switch so as to give three temperature levels. The switch was then turned to the medium position (375 watts) and heating was continued for one-half hour. The sample was then

placed in an electric muffle furnace (500° C.) and heating continued until most of the organic matter had been burned out. Since seven muffle furnaces, each capable of holding four to five dishes, were available for this purpose, the usual practice was to load the furnaces with samples in the evening, removing and weighing them and recording the weights on the next morning. The remaining small amount of carbon was destroyed by treating the ash with a little nitric acid, evaporating to dryness and replacing the dishes in the furnaces for 10 to 15 minutes. The ash was then dissolved in 10 ml. of nitric acid, 10 ml. of hydrochloric acid (added to dissolve metastannic acid) and 20 to 30 ml. of distilled water, heating if necessary. The sample was rinsed (without filtering) into a 100 ml. volumetric flask, and when cool, sufficient distilled water was added to bring the volume up to the mark on the flask. A suitable aliquant corresponding to 0.1 to 0.15 gm. of ash was taken for analysis by the dithizone method (13).

The results obtained by the dithizone method were cross-checked by the spectrographic method. Fifty consecutive samples from one of the subjects gave respective mean values of 1.184 $\pm$ 0.060 mg. and 1.188 $\pm$ 0.062 mg. for the spectrographic and dithizone methods. Subsequent occasional checks on a limited number of samples showed equivalent uniformity in the results by the two methods.

Early work with fecal samples to which known amounts of lead had been added, especially in the range of 1 to 2 mg., frequently resulted in low recoveries of lead. Examination of the various steps showed that loss of lead may occur at two points in the procedure. First, spraying or volatilization of lead may occur if the sample catches fire during ashing. The method of volatilizing the combustible materials as outlined above has eliminated this danger. The procedure is admittedly somewhat time-consuming, but adequate drying equipment permitted the daily handling of enough samples to charge the furnaces each night. This equipment consisted of a series of electric heaters, capable of holding twenty-four dishes, confined in a metal hood. The heater elements consisted of two 375 watt coils connected to a three-way switch in such a way as to give three temperature levels.

A second source of loss was found in the occlusion or absorption of lead by the silica in the sample, which when removed by filtration and discarded, carried some lead with it. Acid washing failed to free the lead from the silica, and other procedures designed to remove the silica (hydrofluoric acid treatment or alkaline fusions) were not only tedious and time-consuming but also invariably tended to add lead to the sample. Since adsorbed or even particulate lead is easily removed by shaking with dithizone, the obvious answer to this problem was to avoid filtration and to extract

the lead from an aliquant taken from the solution (and suspension) of the ashed samples after thorough shaking of the latter so as to distribute the silica evenly within it. The elimination of the filtration step not only shortened the preparation of the samples considerably, but also eliminated an occasional source of contamination not usually recognized, in that filter papers from the same package may contain varying amounts of lead, which when added to samples of low lead content may cause appreciable errors.

Mixed Food Samples

The composite food samples were handled by a method designed to employ a minimum of chemical reagents, equipment and time. They were "homogenized" in a Sears and Roebuck "liquidizer", the measured volume of the fluids serving as the suspension medium and also to rinse out the liquidizer bowl and sample jars. The finely divided mixture thus obtained was then stirred into a tared four-liter Pyrex beaker and its weight was recorded. One-third of the sample was placed in a four-liter Pyrex beaker, the balance being retained for future analysis if such analysis should be necessary. The aliquot was digested by repeated additions of 100 ml. portions of concentrated nitric acid until a small amount of clear solution was obtained. The digest was then rinsed into a 500 ml. Pyrex evaporating dish and, after the

addition of 5 ml. of concentrated sulfuric acid, was taken to dryness on the electric hot plate. From this point, the procedures described in the case of the feces accomplished the evolution of the volatile material, the final combustion in the muffle furnaces, and the solution of the ash. In the case of the food, however, somewhat larger amounts of the reagents and water were required to effect the solution of the sample, and the final volume was adjusted to 250 ml. in a volumetric flask. An aliquot corresponding to 1/10 or 1/20 of the ash of a day's food was taken for analysis by the dithizone method (13).

As in the case of the fecal samples, cross-checks were obtained by means of the spectrographic method. The adequate correspondence of the results was shown by the respective mean values of 0.316 $\pm$ 0.011 mg. and 0.334 $\pm$ 0.011 mg. obtained on fifty consecutive samples by the spectrographic and dithizone methods.

The difficulties associated with the preparation of fecal samples were encountered with mixed food samples and the corrective measures employed for the former were also effective for the latter. In addition, the nature of the material introduced two other difficulties. First, the high content of carbohydrates produced large amounts of carbon, which at 500° C. had a considerable reducing action on the lead salts, converting some of the latter to metal and vaporizing it in the muffle furnace. The addition of

sulfuric acid evidently converted the lead of the sample to the sulfate, which did not reduce readily at 500° C., since attempts to recover amounts of lead (up to 2.0 mg. added to normal food material were always successful when sulfuric acid was added. Without sulfuric acid, the recoveries were frequently as low as 85 percent.

The other difficulty arose from the size of the sample. Since the weight of the latter was generally 1.5 to 2.0 kg. exclusive of the fluids consumed, attempts to digest the entire sample required excessive amounts of reagents and equipment, and sometimes resulted in delays of two weeks or more in the preparation for analysis. The use of aliquants effected a considerable saving in time, reagents and space, and the results proved to be as accurate as if the entire sample had been used. Comparative studies on 28 consecutive food samples, in which results were obtained on one-third portions and on the remaining two-thirds of the samples, gave regularly corresponding results and almost identical mean values (0.292 $\pm$ 0.014 and 0.287 $\pm$ 0.15 mg. respectively) and indicated that the samples were homogenous with respect to the distribution of lead within them. It is probable that considerably smaller aliquants could be used (200 to 300 gm.), as was done by Largent (17) in the determination of fluorine in mixed food material, but no attempt has been made to do so in analyses for lead.

The mixer used by Largent (17) was not satisfactory for

our purpose because it had tinned surfaces which might be suspected of containing appreciable quantities of lead. The apparatus employed consisted of a glass bowl carrying stainless steel knives rotated by means of a high speed motor, which engaged the shaft carrying the knives through an opening in the base of the bowl. The equipment as purchased was altered considerably before being found satisfactory, since the lock-nut holding the knives on the shaft, the bearing housing and the bearing were made of lead-containing alloys. When the housing and the lock-nut were made of stainless steel, and the bearing of cast iron (lubricated by means of graphite in oil), all sources of lead contamination were removed, as was proved by repeated tests of 1000 ml. portions of 5% nitric acid churned in the equipment.

It should be pointed out that all commercial acids contain lead, and that it was not feasible to redistill the large amounts used in the preparation of samples of food and feces. Acids were ordered from identified lots, the lead content of which was determined by analysis, and corrections were made for the amount of acid used in the preparation of the samples.

Methods of Analysis for Calcium and Phosphorus

Preparation of Samples

In the case of food and feces, calcium and phosphorus

analyses were made on aliquants of the samples prepared for lead analysis. The 24-hour urine samples were prepared separately. Their volume was measured and 10% of this amount of concentrated nitric acid was added to the urine and thoroughly mixed. An aliquant of 275 ml. was removed and placed in a Pyrex evaporating dish. The aliquant was evaporated to dryness, carefully ashed in the muffle furnace at 500° C., redissolved in dilute nitric acid, transferred to a 250 ml. volumetric flask, and diluted to the mark with water. 1.0 ml. of this solution then corresponded to 1.0 ml. of urine. Generally, 10 ml. were taken for phosphorus analysis and 100 ml. for calcium analysis.

Analytical Procedures

Calcium was determined in food, urine, and feces by permanganate titration of the precipitated calcium oxalate according to the usual procedure (18, 19, 20, 21). This method has stood the test of time and still compares favorably with more recent methods when sufficiently large samples are available for analysis, as was the case here.

Phosphorus was determined by alkalimetric titration of the ammonium phosphomolybdate precipitate according to the official volumetric method for fertilizer of the Association of Official Agricultural Chemists (22).

RESULTS

The Ingestion, Excretion and Retention of Lead by Normal Subjects on Normal Diets with no added Lead Intake

Observations on Subject I. F. were continued for 449 consecutive days, and on Subject S. W. for 361 consecutive days, while both subjects were on normal diets of their own choosing with no additional lead intake. The daily occurence of lead in the duplicate food samples collected during these periods is shown in Table 1. The mean daily values of 0.240 mg. for Subject I. F. and 0.217 mg. for Subject S. W. are in general accord with previously published results (1, 2), but are well below the level of 0.32 mg. for average American men (2). This lower level of lead intake may be due to two reasons: 1) laboratory workers do not perform hard physical labor and eat less than those who do, and 2) these subjects, put to inconvenience and, at times, embarassment, by the collection of duplicate samples of all ingested foods and beverages, tended to adjust to a very simple routine of eating and living, selecting diets which could be easily duplicated, so that while being recompensed for the added cost of duplicating their food and beverages and thus encouraged to adhere to freely chosen diets, the latter was not always done. That this ingested lead is not effectively absorbed but largely traverses the alimentary tract unabsorbed is shown in Table 1 and Figures 1 and 2. The mean daily alimentary lead

output, as shown in Table 1, is almost identical with the mean daily lead intake, as was also that case in all previous observations (1, 2, 3). A further study of Figures 1 and 2 shows that the lead content of the feces is a reflection only of the lead intake and the alimentation time of the individual, usually 24 to 48 hours. Each block on Figures 1 and 2 represents one days alimentary lead intake and output, and the troughs and peaks of the output follow by only one or two days the corresponding troughs and peaks of the intake. There is no recognizable seasonal variation in the quantity of lead ingested or passed in the feces.

Throughout the entire experiment, the alimentary lead output was measured in toto and calculated as mg. per 100 grams of dried feces and as mg. per gram of ash. These calculations were studied carefully for the entire period, but no correlation could be found between the total amount of lead in the feces and the amount per 100 grams of dried feces or the amount per gram of ash. This might have been expected since the lead content of the feces obviously bears no relation to the water content, and since lead represents such a minute portion of the mineral content. The only conclusion which could be reached regarding this portion of the experiment was that the only significant value with respect to the alimentary output of lead is the amount per unit of time; i. e., per day or week.

The daily occurence of lead in the urine, the daily concentration of lead in the urine and the daily volume of the urine of Subjects I. F. and S. W. are shown in Tables 2 and 3. Subject I. F., having a mean daily urinary volume of approximately one liter, had a mean daily urinary lead excretion of 0.0329 mg., while Subject S. W., with a mean daily urinary volume of approximately one and one-half liter, had a mean daily urinary lead output of 0.0397 mg. The mean daily lead concentration in the urine was only slightly higher in Subject I. F. Further study of these two tables reveals that larger volumes of urine removed larger amounts of lead from the bodies of these subjects, but the urinary lead concentration remained relatively unchanged. This is shown especially well in Table 2, Subject S. W., in whose case the range of daily urinary volume was much wider and more evenly distributed than that of Subject I. F. (Table 3). The larger volumes (Table 2) contain much more lead than do the smaller volumes, while the lead concentration remains almost constant. More will be said of this later, but suffice it to say here that one method of increasing lead elimination from the body is to increase the volume of the urine. A seasonal variation in both urinary volume and lead content of the urine, involving increased quantities in cold months and lesser quantities in hot months, was found, as was expected from previous observations of similar type (2, 3).

The retention of lead in the bodies of these two experimental subjects is shown in Tables 4 and 5 and in Figures 3 and 4. In both subjects, over the prolonged periods of the observations, more lead was eliminated from the body in the urine and feces than was taken into the body in food and beverages. This loss of lead from the body was a virtually continuous process, and was too great and too nearly uniform in rate to be the result of sampling errors, since the latter could scarcely exert an influence in one direction only. The curves as shown in Figures 3 and 4 are remarkably similar and seem best fitted to straight lines, although an obvious seasonal variation is present in both curves. The excess of lead loss over lead intake measures approximately 0.02 mg. per day, or 0.6 mg. per 28-day period in each subject. It follows, then, that some small amount of lead must have been absorbed regularly by each subject from some source other than the gastro-enteric tract. This source, in all probability, was the respiratory system. Both subjects lived in suburban Cincinnati but spent their working days in the laboratory building, the atmosphere of which was freed of particulate lead for the most part by filtration. Unpublished data of atmospheric pollution studies carried on in Cincinnati have demonstrated that lead is present in the atmosphere in suburban areas in concentrations of the order of 0.002 mg. per cubic meter. The atmosphere in industrial areas, and in

suburban areas during the winter months, contains greater quantities of lead, and it has been estimated that as much as 0.05 mg. of lead might be available for absorption by the respiratory system in a 24-hour period. Only a part of this lead need be absorbed to account for an otherwise unexplained factor of lead intake.

Blood lead determinations were made on both subjects at weekly intervals, with but few exceptions, and the occurence of lead in the blood samples is shown in Table 6. Subject I. F., with a mean of 0.029 mg. per 100 grams of blood, and Subject S. W., with a mean of 0.039 mg. per 100 grams of blood, fall well within the normal range for American men as previously reported (2). On six occasions, the blood samples of Subject S. W. were partitioned for the determination of lead in the blood cells and in the plasma. These results are shown in Table 7. It will be seen at once that by far the greater part of the lead (90%) is present in the blood cells, and only a small portion is in the plasma. This finding has been confirmed by similar observations on another subject (E. B.) and on numerous other persons.

Lead Ingestion, Excretion and Retention at Abnormal Levels

Subject M. R.

Subject M. R., who ingested 1.0 mg. of lead daily in addition to the lead in his normal diet, was studied for 1477

consecutive days. Observations made during this experimental period have been reported in some detail in previous articles (1, 3), and a complete review of this phase of the overall experiment would be out of place here. Some of the more important features will be mentioned briefly for the sake of completeness, or for comparison with later observations. At this time, it should be noted, however, that there was an irregular but progressive increase in the urinary and blood lead concentrations during the period of lead administration, and that 110 mg. of lead were retained in the body of the subject during the 1477 day period.

Subject E. B.

Subject E. B., who ingested 2.0 mg. of lead daily in addition to the lead in his normal diet, was studied for 646 consecutive days. Observations on this experimental period have also been reported in some detail in previous articles (1, 3). Again, it should be noted that there was an irregular but progressive increase in the urinary and blood lead concentrations during the period of lead administration, and that 118 mg. of lead were retained in the body of the subject during the 646 day period.

Subject I. F.

Subject I. F. tood 3.0 mg. of lead daily in addition to the lead in his freely chosen diet for 113 consecutive days. The mean daily urinary lead concentration increased rapidly

and regularly from a pre-experimental level of 0.024 mg. per liter to 0.077 mg. per liter in the final 29 days of the experimental period, while the mean daily urinary lead output increased from 0.023 mg. to 0.083 mg. During this period of increased lead ingestion, the blood lead concentration increased from 0.023 mg. to 0.083 mg. During this period of increased lead ingestion, the blood lead concentration increased from 0.031 mg. per 100 grams of blood to 0.051 mg. per 100 grams of blood. Figure 5 shows how the lead content of the urine and blood varied during and after the period of increased lead ingestion. These findings had been expected after a study of the results of the ingestion of lesser amounts of lead by Subjects M. R. and E. B. (1, 3). The curves shown in Figure 5 are similar in character to those of the other subjects, but are much steeper, representing an increased rate of retention of lead in the body.

Of the 362.12 mg. of lead ingested by I. F. during the period of increased lead ingestion, 306.91 mg. (85%) were eliminated from the body, 299.54 mg. (83%) in the feces, and 7.47 mg. (2%) in the urine, leaving 55.21 mg. (15%) retained in the body. This compares with a 6% retention in Subject M. R. (1), and an 8% retention in Subject E. B. (3) on a lead dosage of 1.0 mg. and 2.0 mg. respectively (Table 8). The mean daily alimentary lead intake of Subject I. F. during the period of increased lead ingestion

was 3.243 mg., and lead was retained in the body at the rate of about 15 mg. each 28-day period. This compares with a retention of 5 mg. per 28-day period by E. B. in association with a mean daily intake of 2.31 mg., and a retention rate of 2.5 mg. per 28-day period on the part of M. R., whose mean daily intake was 1.34 mg. (3).

Subject S. W.

Subject S. W. took 0.3 mg. of lead daily in addition to the lead in his chosen diet. It had previously been estimated, for the purpose of designing the experiment, that an oral intake of about 0.5 or 0.6 mg. of lead per day would probably serve to counteract the imbalance between the intake and output of lead as previously observed in this and other subjects while under study during periods of normal or incidental lead intake (3).

The subject was studied in the usual manner, while taking this dose of lead, for 15 periods of 28 days each. At the end of that time, the mean daily urinary lead output, the mean daily urinary lead concentration, and the average blood lead concentration were within accepted normal limits, the lead in the urine having increased intermittently to but a slight degree, that in the blood imperceptibly. On the other hand, lead retention had occured at the rate of about 1.0 mg. per 28-day period to reach the total of 13.34 mg. The mean daily alimentary lead intake during these 15 lunar months was

was 0.504 mg. It is now apparent that the amount of soluble lead which can be ingested daily over a long period of time without causing lead accumulation in the human body is small, approaching indeed the amount in the normal adult diet. In fact, in view of all of the limitations of, and opportunities for, errors in these methods of studying lead metabolism at low levels of alimentary intake, it seems possible that there may be some almost imperceptible and perhaps intermittent accumulation of lead in the bodies of normal healthy persons whose dietary intake of lead varies within present normal or incidental limits. Some variations occur, no doubt, by reason of geographic or localized factors, and also because of variations in quantity and qualitative composition of the diet of individuals, in association with variations in appetite, effort, temperature and the like.

Lead Excretion Following Discontinuance of Abnormal Lead Intake

Subject I. F.

Following discontinuance of the experimental ingestion lead by this subject, the amount of lead in the feces was at a normal level in three days and remained so thereafter for the remaining period of the observations (168 days). The mean daily urinary lead output, the mean daily urinary concentration and the average lead concentration in the blood all reached normal levels by the second 28-day period, as shown in Figure 5, but there still remained 47 mg. of lead which had

been absorbed into the body during the period of increased lead intake. Table 9 clearly shows that the excess lead excreted by I. F. in the 168 days following the discontinuance of oral lead administration was present largely in the feces, a fact which had been shown before but which was believed due to sampling errors (3). Disregarding the first 28-day period after the discontinuance of oral lead administration, during which there was a very large negative accumulation due to the large amounts of lead passed in the feces on each of the first three days, the subject was losing lead from his body at the rate of 3.0 mg. per 28-day period. Even assuming that this rate would continue, and it subsequently will be shown that it would not, eighteen periods of 28 days each would be required to rid the body of the subject of the 55 mg. of lead retained during a period of oral administration of only four lunar months.

Subject E. B.

Tables 9 and 10 and Figure 8 show the loss of lead from the body of Subject E. B. following discontinuance of the abnormally high oral lead intake. It was previously suspected that the apparent loss of retained lead in the feces over the first 280 days after discontinuance of oral lead administration might be due, at least in part, to sampling errors, and that the retained lead had been lost largely through the urinary system instead (3). After 908 days, however, we find that the

excess lead excreted in the urine is still less than that apparently excreted by the gastro-enteric tract. Subject I. F. showed a similar but more pronounced pattern, but the period of observation was only 168 days. Since the effect of sampling errors would appear to have been excluded to a large extent in the case of E. B. by the long period of the observations, it is evident that there must be some actual excretion of lead into the alimentary tract and loss from the body in that manner. The extent of this loss seems to be the greater the larger the amounts of lead available for excretion within comparable periods of time.

Further study of these 908 days of observation of Subject E. B. shows that the lead content of the feces reached mean normal levels in five days, but that the amount of lead in the urine was greater than normal for 23 periods of 28 days each. Over these 23 periods, the urinary lead excretion gradually returned to normal, the amount of lead in the urine being greater in the initial than in the latter part of this period. Similarly, the amount of accumulated lead decreased rapidly during the first few lunar months, but the amount lost each month became gradually less as time progressed, until after the 23 such periods, the loss of retained lead was going on at approximately the same rate as the loss of lead from the normal subjects, I. F. and S. W., while on normal diets. For the last nine months of observation, E. B. appeared

to be in normal lead balance as determined by the studies on I. F. and S. W., although at the end of this time, 41.87 mg. of lead had still not been recovered. At the normal rate of lead excretion, it would have required an additional three years for the subject to have lost this amount of lead from his body. Unfortunately, this part of the experiment was terminated at this point when the subject was inducted into the Armed Services. Three and one-half years later, this same subject was studied again for a period of twelve weeks while on a normal diet. As was to be expected, he was in a state of normal lead metabolism, as determined by comparison of these results with those obtained in the study of Subject I. F. and Subject S. W. under normal conditions.

SUMMARY

A series of six long-term experiments on the metabolism of lead by four volunteer human adults has been studied. The experimental techniques and procedures required for conducting prolonged metabolic studies on a metal of which the total daily amounts involved are measured in milligrams and fractions of milligrams are given in considerable detail. Previously reported results have been mentioned briefly for the sake of completeness and for comparison with heretofore unpublished results on subjects given soluble lead at different levels of

daily dosage. Previously unreported results have been given in some detail, although the experimental data have been given in greatly contracted form. Certain conclusions regarding lead metabolism at normal or incidental levels of ingestion and at abnormally high levels of ingestion (up to ten times the normal or incidental level) have been reached. Metabolic studies on calcium, phosphorus and aluminum, which were carried on concurrently with the lead metabolism studies, have not been included in this report.

Considerable time was spent in an attempt to discover certain other physiological relationships that might be revealed by the available data with particular reference to the water balance, but no remarkable or clean-cut conclusions were warranted and this phase of the work was not included in this report.

REFERENCES

1. Kehoe, R. A., Cholak, J., Hubbard, D. M., Bambach, K. McNary, R. R., and Story, R. V. : Experimental Studies on the Ingestion of Lead Compounds, J. Ind. Hyg. and Toxicol., 22: 381 (1940).

2. Kehoe, R. A., Thamann, F., and Cholak, J. : On the Normal Absorption and Excretion of Lead, II, J. Ind. Hyg. and Toxicol., 15: 273 (1933).

3. Kehoe, R. A., Cholak, J., Hubbard, D. M., Bambach, K., and McNary, R. R. : Experimental Studies on Lead Absorption and Excretion and their Relation to the Diagnosis and Treatment of Lead Poisoning, J. Ind. Hyg. and Toxicol., 25: 71 (1943).

4. Kehoe, R. A., Thamann, F., and Cholak, J. : Normal Absorption and Excretion of Lead, J. A. M. A., 104: 90 (1935).

5. Kehoe, R. A., Thamann, F., and Cholak, J. : An Appraisal of the Hazards Associated with the Distribution and Use of Gasoline Containing Tetraethyl Lead, II, J. Ind. Hyg. and Toxicol., 18: 42 (1936).

6. Kehoe, R. A. : The Diagnosis of Lead Poisoning in the Light of Recent Information, J. of Med. (Cincinnati) 16: 527 (1935).

7. Kehoe, R. A., Thamann, F., and Cholak, J. : On the Normal Absorption and Excretion of Lead, I, J. Ind. Hyg. and Toxicol., 15: 257 (1933).

8. Cholak, J. : The Quantitative Spectrographic Determination of Lead in Urine, Am. Chem. Soc., 57: 104 (1935).

9. Cholak, J. : The Quantitative Spectrographic Determination of Lead in Biological Material, Ind. Eng. Chem. Anal. Ed., 7: 287 (1935).

10. Cholak, J. and Story, R. V. : Spectrographic Analysis of Biological Material, Ind. Eng. Chem. Anal.Ed., 10: 619 (1938).

11. Cholak, J. and Story, R. V. : Spectrochemical Determination of Trace Metals in Biological Material, J. Optical Soc. of Am., 31: 730 (1941).

12. Hubbard, D. M. : Determination of Lead, Ind. Eng. Chem. Anal. Ed., 9: 493 (1937).

13. Bambach, K. : Determination of Lead by Dithizone, Ind. Eng. Chem. Anal. Ed., 11: 400 (1939).

14. Cholak, J., Hubbard, D. M., McNary, R. R., and Story, R. V. : Determination of Lead in Biological Material, Ind. Eng. Chem. Anal. Ed., 9: 488 (1937).

15. Cholak, J. and Story, R. V. : Determination of Lead in Biological Material, Ind. Eng. Chem. Anal. Ed., 13: 583 (1941).

16. Kehoe, R. A., Cholak, J., and Story, R. V. : A Spectrochemical Study of the Normal Ranges of Concentration of Certain Trace Metals in Biological Material, J. Nutrition, 19: 579 (1940).

17. Largent, E. J., Ind. Eng. Chem. Anal. Ed., 13: 93 (1941).

18. Quantitative Clinical Chemistry, Peters and van Slyke, Vol. II (Methods), Chap. XXV, Williams and Wilkins Co., 1932.

19. McCrudden, F. H. : J. Biol. Chem., 7: 83 (1909-1910).

20. Ibid., 10: 187 (1911-1912).

21. Methods of Analysis, Assoc. of Official Agricultural Chemists, 5th Ed., 1940, p. 127, Method XII-10.

22. Ibid., p. 22, Method II-10, 11, 12.

TABLE 1

The Daily Occurence of Lead in the Food and Feces of Normal Human Subjects while on Normal Diets

Subject I. F.			Subject S. W.		
Lead in mg.	Frequencies of Occurence of Amts. of Lead Indicated		Lead in mg.	Frequencies of Occurence of Amts. of Lead Indicated	
	In Food	In Feces		In Food	In Feces
0.00-0.19	200	207	0.00-0.19	210	174
0.20-0.39	203	179	0.20-0.39	121	129
0.40-0.59	32	46	0.40-0.59	11	31
0.60-0.79	8	9	0.60-0.79	7	12
0.80-over	6	8	0.80-over	12	15
Total	449	449	Total	361	361
Mean	0.240	0.247	Mean	0.217	0.259
P. E. $\pm$	0.005	$\pm$0.005	P. E.	$\pm$0.006	$\pm$0.007
St. Dev. $\pm$	0.156	$\pm$0.171	St. Dev.	$\pm$0.181	$\pm$0.203
Co. V.	65.00%	69.23%	Co. V.	83.41%	78.38%

TABLE 2

The Daily Urinary Volume, Lead Content and Urinary Lead Concentration of a Normal Subject, S. W., while on a Normal Diet

Volume in ml.	Lead Content in mg.						Concentration mg./liter					
	0.000 0.019	.020 .039	.040 .059	.060 .079	.080 over	Total	.000 .019	.020 .039	.040 .059	.060 .079	.080 over	Tot
0-399	0	0	0	0	0	0	0	0	0	0	0	0
400	2	6	0	0	0	8	1	6	1	0	0	8
800	14	60	14	1	1	90	2	68	16	4	0	90
1200	2	85	29	8	2	126	5	98	20	2	1	126
1600	0	41	24	16	3	84	3	67	13	1	0	84
2000	0	2	21	11	1	35	2	32	1	0	0	35
2400	0	0	0	1	0	1	0	1	0	0	0	1
2800	0	0	2	1	0	3	0	3	0	0	0	3
TOTAL	18	194	90	38	7	347	13	275	51	7	1	347

Mean and Probable Error

Volume	Lead Content in mg.	Lead Conc. in mg./liter
1472.8 $\pm$ 15.4	0.0397 $\pm$ 0.0006	0.0332 $\pm$ 0.0004

TABLE 3

The Daily Urinary Volume, Lead Content and Urinary Lead Concentration of a Normal Subject, I. F., while on a Normal Diet

Volume in ml.	Lead Content in mg.						Concentration mg./liter					
	0.000 0.019	.020 .039	.040 .059	.060 .079	.080 over	Total	.000 .019	.020 .039	.040 .059	.060 .079	.080 over	Tot
0-399	0	0	0	0	0	0	0	0	0	0	0	0
400	38	40	4	0	0	82	5	52	20	5	0	82
800	49	135	47	9	2	242	21	151	55	12	3	242
1200	6	47	33	8	3	97	8	58	30	1	0	97
1600	0	9	4	8	1	22	1	15	6	0	0	22
2000	0	4	1	0	0	5	4	1	0	0	0	5
2400	0	0	0	0	0	0	0	0	0	0	0	0
2800	0	0	1	0	0	1	1	0	0	0	0	0
TOTAL	93	235	90	25	6	449	40	277	111	18	3	449

Mean and Probable Error

Volume	Lead Content in mg.	Lead Conc. in mg./liter
1070.4 ± 5.4	0.0329 ± 0.0005	0.0325 ± 0.0004

TABLE 4

Retention of Lead in a Normal Subject, I. F., while on a Normal Diet

Successive Periods of 28 Days	Ingested Lead in mg.	Excreted Lead in mg.			Retained Lead in mg.	
		Fecal	Urinary	Total	This Period	Cumulative Total
1	5.00	4.42	1.04	5.46	-0.46	-0.46
2	5.91	5.41	0.92	6.33	-0.42	-0.88
3.	5.42	5.17	0.81	5.98	-0.56	-1.44
4	6.59	5.28	0.59	5.87	+0.72	-0.72
5	7.50	6.43	0.72	7.15	+0.35	-0.37
6	6.78	7.53	1.18	8.71	-1.93	-2.30
7	7.10	6.36	1.10	7.46	-0.36	-2.66
8	6.44	8.64	1.15	9.79	-3.35	-6.01
9	8.88	7.94	1.21	9.15	-0.27	-6.28
10	6.70	7.27	1.11	8.38	-1.68	-7.96
11	5.70	5.44	1.11	6.55	-0.85	-8.81
12	5.80	5.30	0.72	6.02	-0.22	-9.03
13	9.14	9.04	0.73	9.77	-0.63	-9.66
14	10.98	9.13	0.77	9.90	+1.08	-8.58
15	5.08	5.63	0.67	6.30	-1.22	-9.80
16	10.06	10.10	0.74	10.84	-0.78	-10.58
TOTAL	113.08	109.09	14.57	123.66		-10.58

TABLE 5

Retention of Lead in a Normal Subject, S. W., while on a Normal Diet

Successive Periods of 28 Days	Ingested Lead in mg.	Excreted Lead in mg.			Retained This Period	Lead in mg. Cumulative Total
		Fecal	Urinary	Total		
1	6.27	5.35	0.93	6.28	-0.01	-0.01
2	5.70	4.94	1.03	5.97	-0.27	-0.28
3	5.55	4.59	0.99	5.58	-0.03	-0.31
4	8.27	6.85	1.30	8.15	+0.12	-0.19
5	7.14	6.55	1.33	7.88	-0.74	-0.93
6	4.98	5.62	1.22	6.84	-1.86	-2.79
7	5.20	5.82	1.03	6.85	-1.65	-4.44
8	4.53	5.72	0.82	6.54	-2.01	-6.45
9	9.17	6.60	1.20	7.80	+1.37	-5.08
10	5.12	4.68	1.10	5.78	-0.66	-5.74
11	5.32	4.89	1.22	6.11	-0.79	-6.53
12	4.32	4.66	1.23	5.89	-1.57	-8.10
13	5.48	5.13	0.78	5.91	-0.43	-8.53
TOTAL	77.05	71.40	14.18	85.58		-8.53

TABLE 6

The Occurence of Lead in the Blood of Normal Subjects while on Normal Diets

Subject I. F.		Subject S. W.	
Lead Level mg./100 g. Blood	Frequency of Occurence	Lead Level mg./100 g. Blood	Frequency of Occurence
0.010 - 0.019	3	0.010 - 0.019	0
0.020 - 0.029	36	0.020 - 0.029	6
0.030 - 0.039	10	0.030 - 0.039	19
0.040 - 0.049	6	0.040 - 0.049	19
0.050 - 0.059	0	0.050 - 0.059	4
0.060 - 0.069	1	0.060 - 0.069	0
TOTAL	56	TOTAL	48

Mean and P. E.	0.029 ± 0.0009	0.039 ± 0.0007
St. Dev.	± 0.010	± 0.008
Co. V.	29.41%	20.51%

TABLE 7

The Occurence of Lead in the Blood Cells and the Blood Plasma of a Normal Subject, S. W., while on a Normal Diet

Partition Number	Lead in mg./100 g. of Blood	Total Lead in Sample in mg.	Lead in Cells		Lead in Plasma	
			mg.	Percent of Total	mg.	Percent of Total
1	0.036	0.0145	0.0130	89.7	0.0015	10.3
2	0.040	0.0154	0.0140	90.9	0.0014	9.1
3	0.035	0.0142	0.0120	84.5	0.0022	15.5
4	0.060	0.0255	0.0240	94.1	0.0015	5.9
5	0.035	0.0144	0.0135	93.8	0.0009	6.2
6	0.030	0.0122	0.0111	91.0	0.0011	9.0
AVERAGE	0.039	0.0160	0.0146	91.3	0.0014	8.7

TABLE 8

Lead Intake and Output of Normal Subjects
During Periods of Oral Lead Administration

	Subject M. R. 1.0 mg. - 1477 days		Subject E. B. 2.0 mg. - 646 days		Subject I. F. 3.0 mg. - 113 days	
	mg.	Percent of Total Ingested	mg.	Percent of Total Ingested	mg.	Percent of Total Ingested
Lead Ingested in Food and Beverages	1966.60	100	1419.33	100	362.12	100
Lead Excreted in Urine	113.62	6	73.07	5	7.37	2
in Feces	1734.79	88	1236.19	87	299.54	83
Total	1848.41	94	1309.26	92	306.91	85
Lead Retained	118.19	6	110.07	8	55.21	15

TABLE 9

Lead Intake and Output of a Normal Subject, I. F., over a Period of 168 Days after Discontinuance of Oral Lead Administration

Ingested Lead in Food and Beverages		31.89 mg.
Eliminated Lead		
in Urine	6.42 mg.	
in Feces	40.51 mg.	
Total		46.93 mg.
Excess of Elimination		15.04 mg.
Excess Excreted		
in Urine*	0.89 mg.	
by Gastro-enteric route	14.15 mg.	
Excess of Elimination		15.04 mg.

* Arrived at by calculating the normal urinary output for the period on the basis of the mean daily urinary lead output of the control period, and subtracting this amount from the amount of lead actually eliminated in the urine.

TABLE 10

Lead Intake and Output of a Normal Subject, E. B., over a Period of 908 Days after Discontinuance of Oral Lead Administration

Ingested Lead in Food and Beverages		280.03 mg.
Eliminated Lead		
in Urine	54.46 mg.	
in Feces	293.87 mg.	
Total		348.33 mg.
Excess of Elimination		68.30 mg.
Excess Excreted		
in Urine*	20.86 mg.	
by Gastro-enteric route	47.44 mg.	
Excess of Elimination		68.30 mg.

* Arrived at by calculating the normal urinary output for the period on the basis of the mean daily urinary lead output of the control period, and subtracting this amount from the amount of lead actually eliminated in the urine.

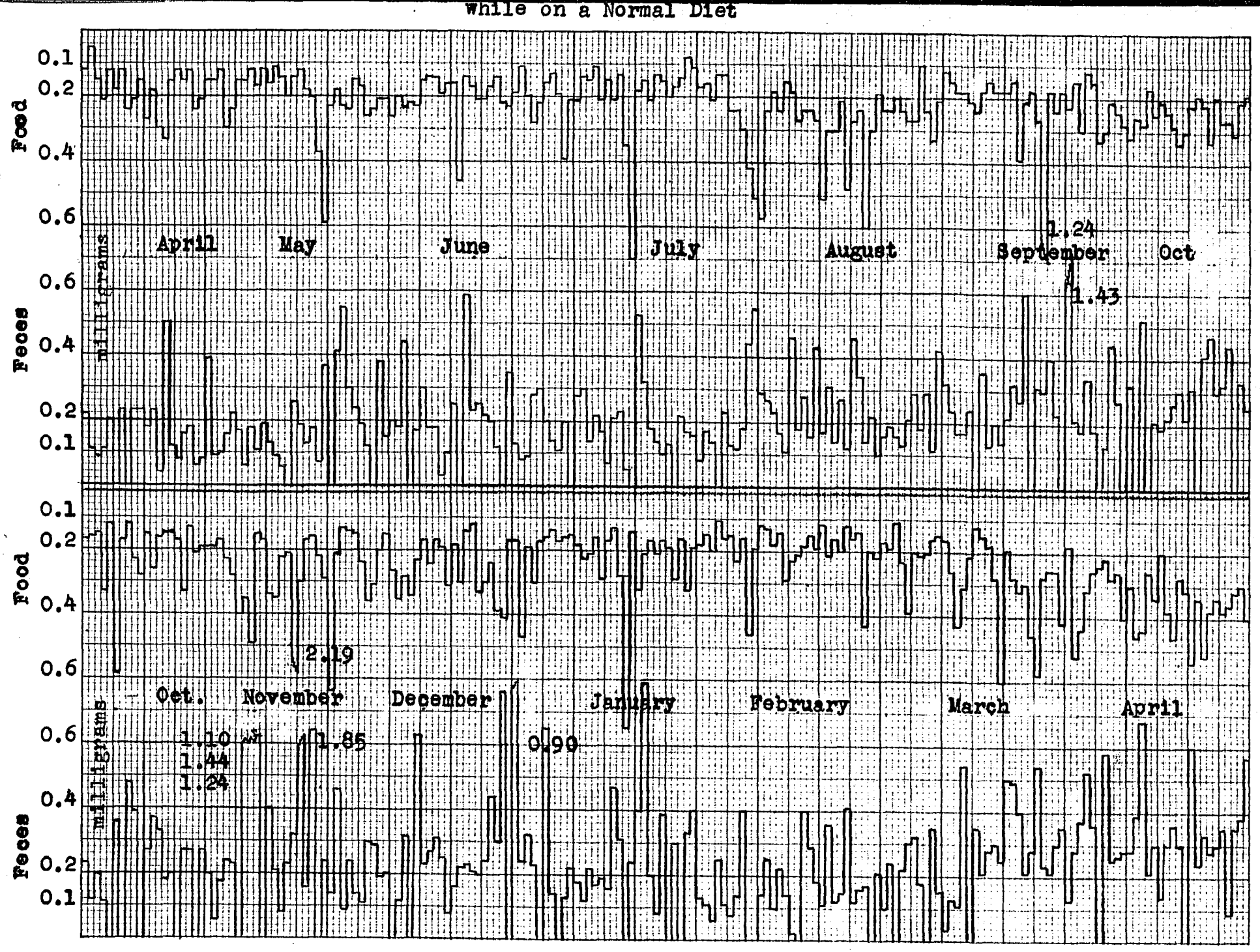

Figure 1

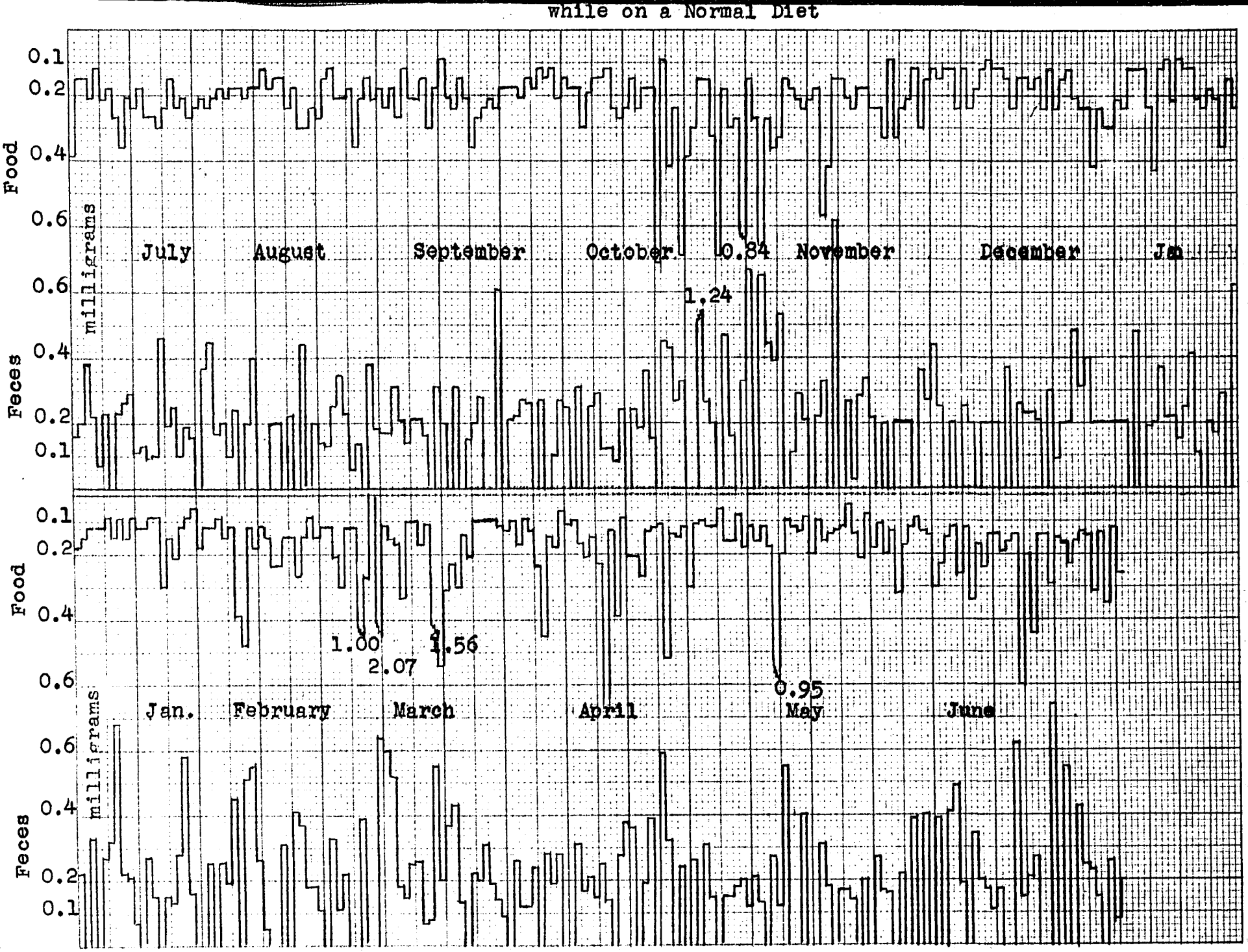

Figure 2

while on a Normal Diet

Figure 3

while on a Normal Diet

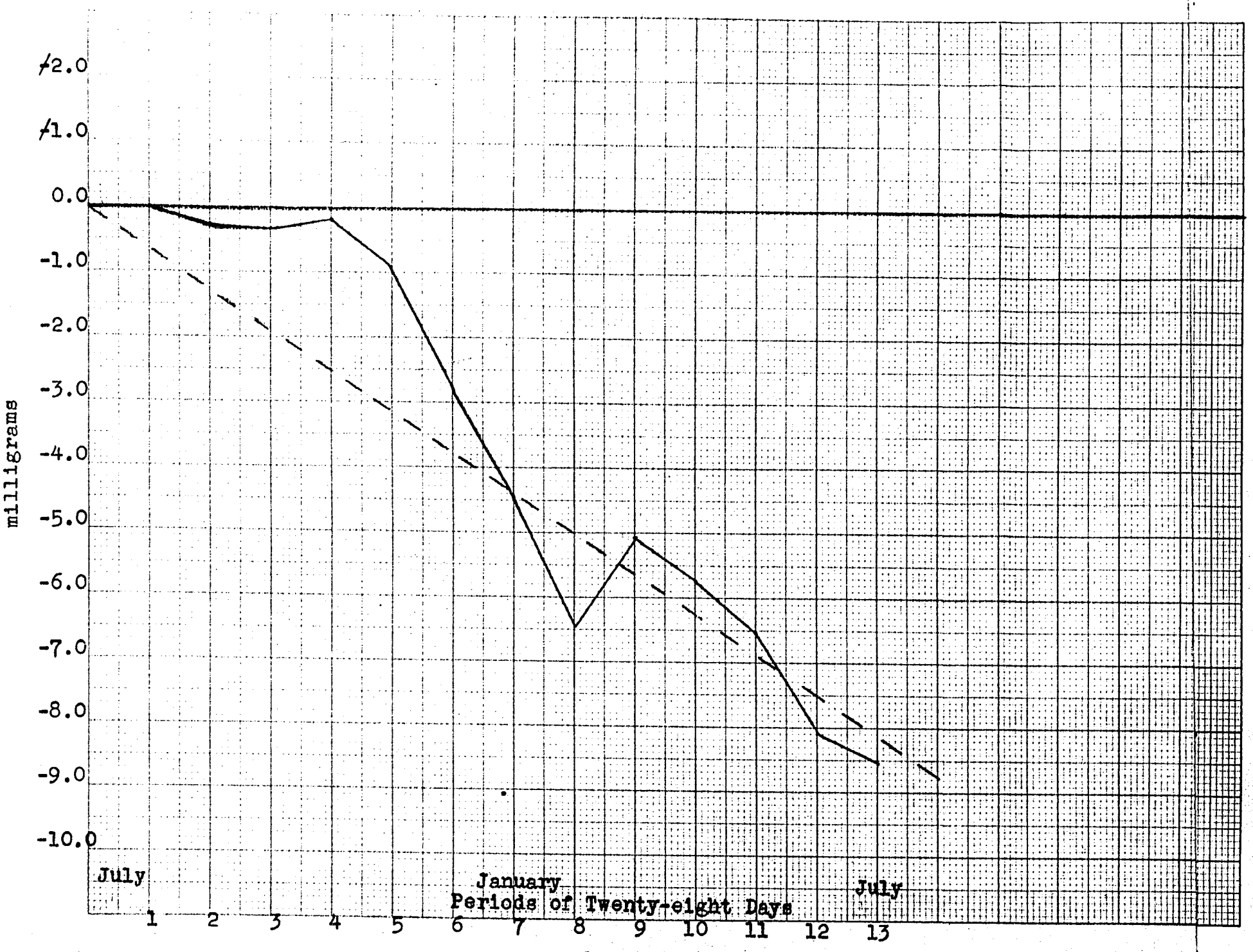

Figure 4

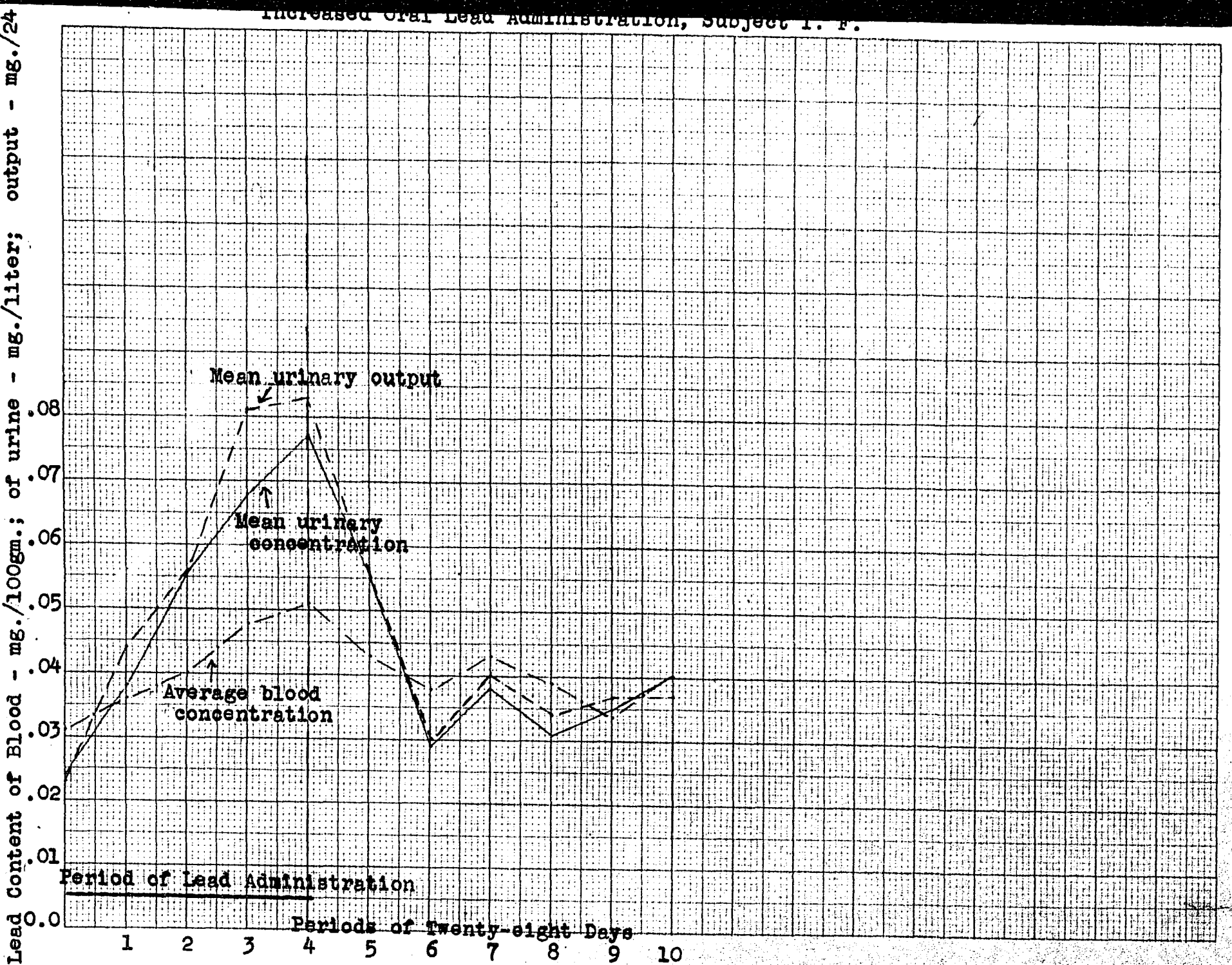

Figure 5

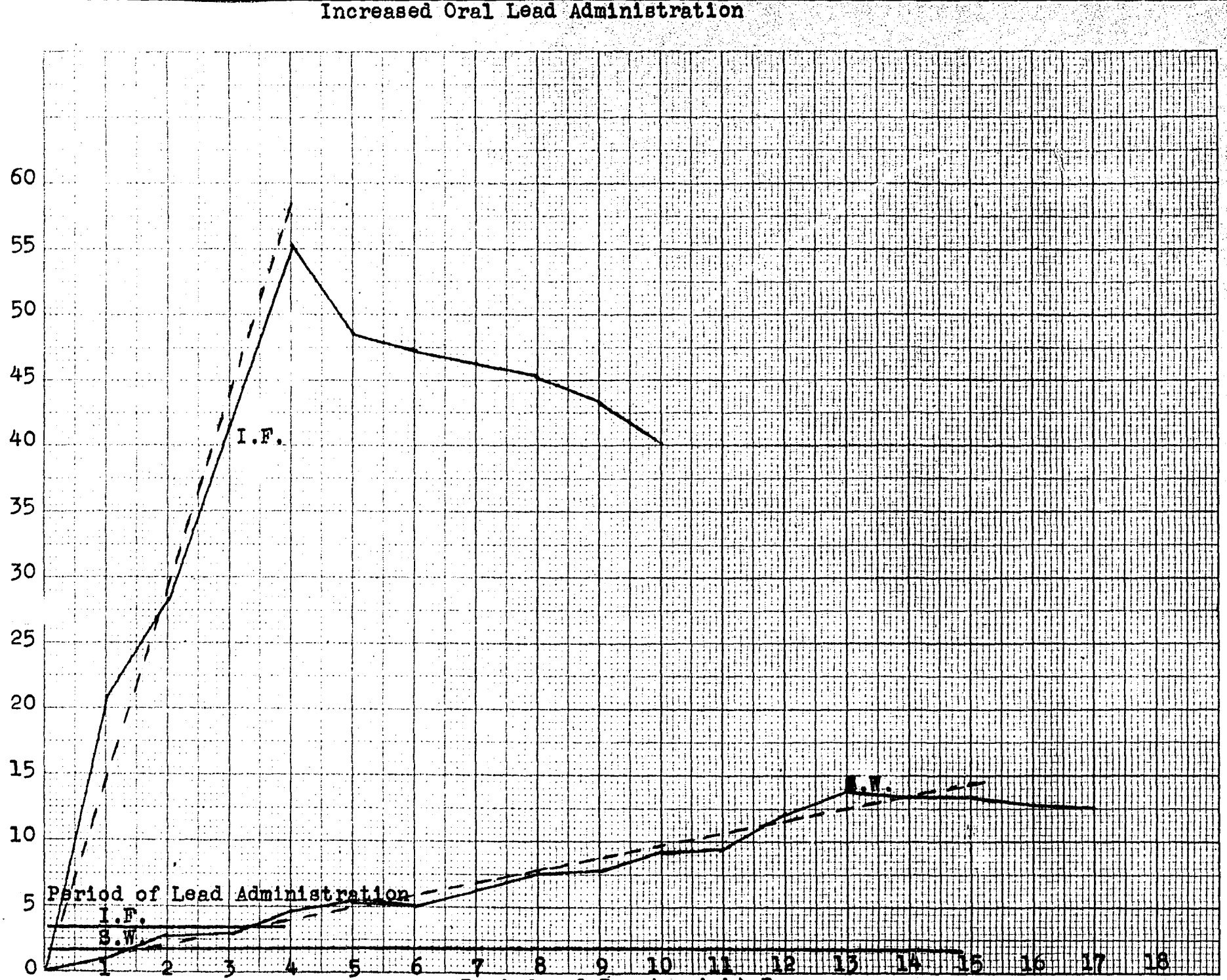

Figure 6

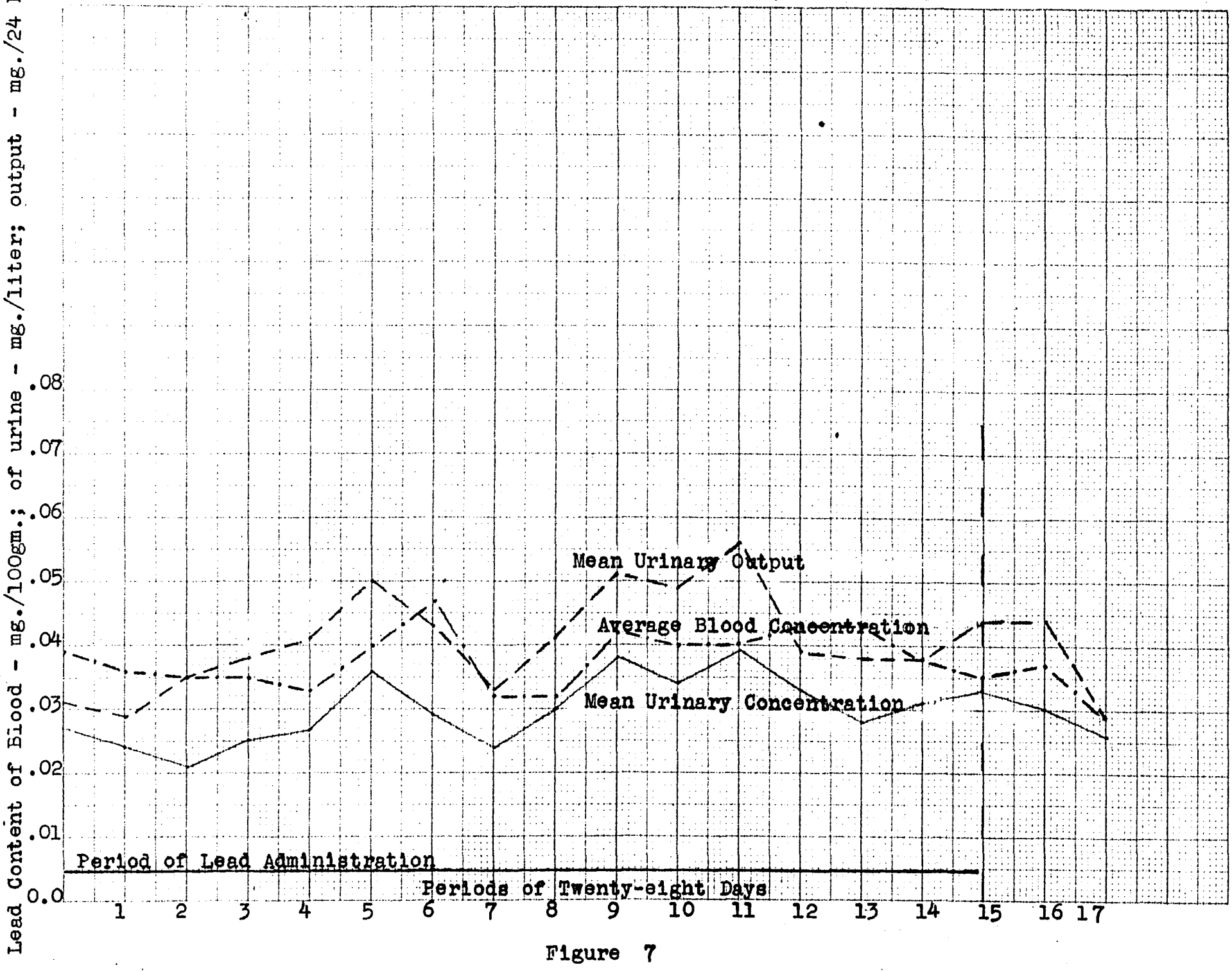

Figure 7

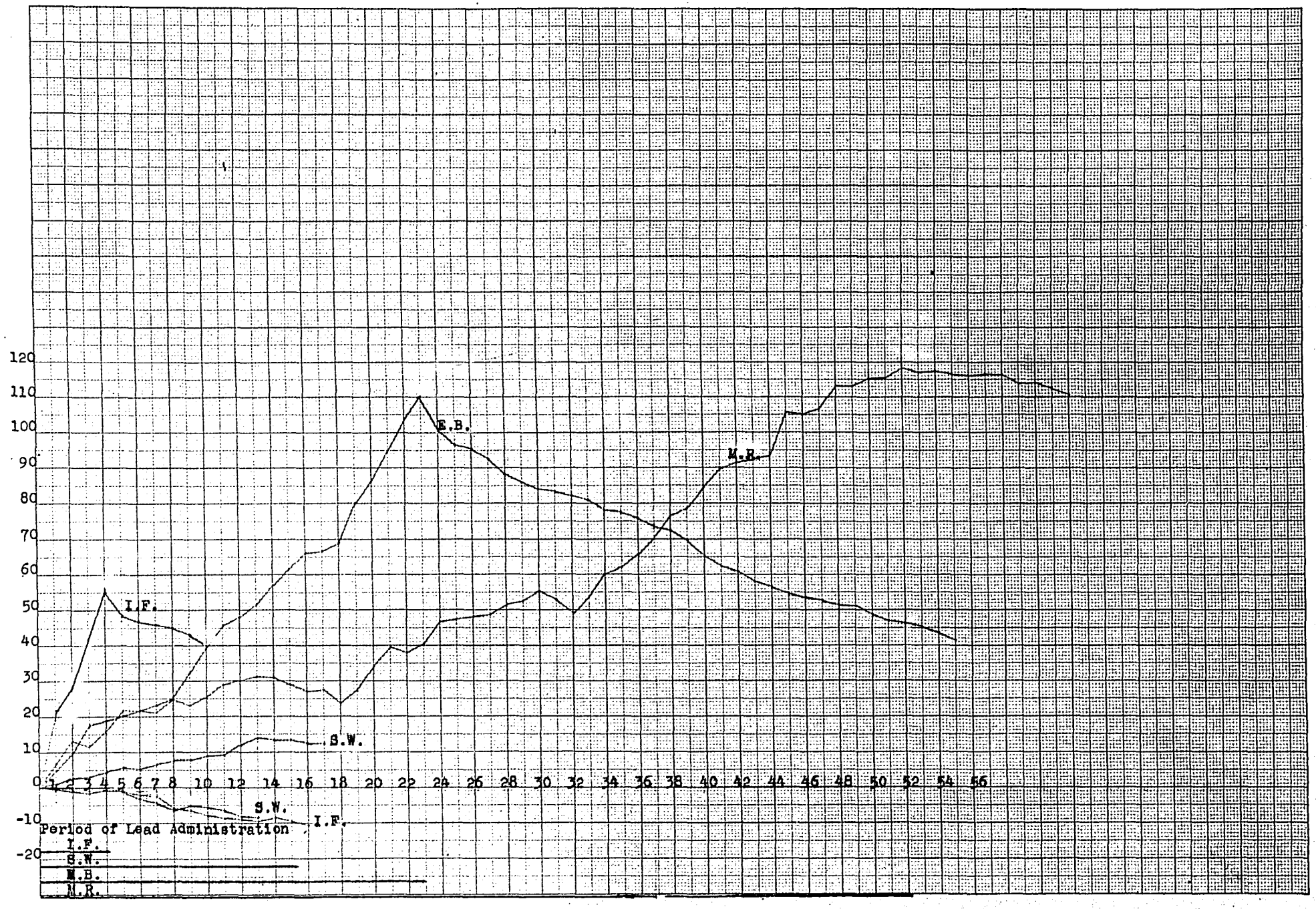

Figure 8