Sent to Washington Feb 2, 1967 - evidently prepared just before sending Extra copy (incomplete lacking pages 29 thru 40)

REPORT OF PROGRESS

In an Investigation of

"The Fate of Inhaled Particulate Lead Compounds"
Supported in part (to the approximate extent of one fourth of the cost)
by the U. S. Public Health Service, National Institutes of Health
as a Project identified as A. P. 00220-01-05

by Robert A. Kehoe, M. D., Principal Investigator,
Professor Emeritus of Occupational Medicine
in the Department of Environmental Health, College of Medicine
University of Cincinnati, Cincinnati, Ohio

INTRODUCTION

In the original application for a grant of funds in support of this project, a brief description was given of a systematic, experimental program which had been in progress for some ~~twenty-five~~ thirty-odd years, with the objective of elucidating the metabolism of lead in man, within the limits of intake (dosage) that are surely compatible with the avoidance of any known or demonstrable toxic response. No full account of the results obtained in the course of this experimental program, such as would include the detailed presentation of all of the hematological, biochemical, clinical and analytical observations believed to be useful in establishing normal baselines and certain threshold values, has been assembled. Many of the data have merely been scanned for significance, and, in their apparent acceptability, laid aside for later detailed presentation. The principal fairly findings, i. e., those related most directly to the main objectives of the investigation, have been published, in somewhat less than extended form, from time to time. In 1960, a series of three lectures were given, in an effort to present the high points of these investigations (up to that time), ~~in a running~~, but fairly well illustrated and documented, account. These were published under the title of "The Metabolism of Lead in Man in Health and Disease," as The Harben Lectures, 1960, of the Royal

Institute of Public Health and Hygiene, London, England, in the J. Roy. Inst. Public Health. 24:81; 101-129; 177, 1961. Other papers (prior publications of the Kettering Laboratory), concerned with this series of investigations, were the following: (a) Experimental Studies on the Ingestion of Lead Compounds. J. Industr. Hyg. and Toxicol. 22:381, 1940. (b) Experimental Studies on Lead Absorption and Excretion, and their Relation to the Diagnosis and Treatment of Lead Poisoning. J. Industr. Hyg. & Toxicol. 25:71, 1943. (c) Exposure to Lead. Occup. Med. 3:156, 1947. (d) Experimental Studies on the Inhalation of Lead by Human Subjects. Pure Appl. Chem. 3:129, 1961. The first of these publications (The Harben Lectures) is supplied herewith as a means of summarizing the experimental background of the work of the past twenty-five years.

The physiological, toxicological, and clinical literature relative to lead and lead poisoning, which has appeared during the past decade, has been voluminous, and could scarcely even be referred to, much less abstracted and summarized, within a short period of time or in a report or publication of less than monumental proportions. (The bibliography of pertinent contributions during 1965 alone, abstracted, translated (when necessary), and assembled by the staff of the Kettering Laboratory, runs to 236 single-spaced, typewritten pages.) No other prolonged experimental observations of the type of "balance experiments" on human subjects have been described, however, so that there are no strictly pertinent references to be made than those (a - d) given above.

THE EXPERIMENTAL METHOD

Experimental Subjects, Type and Qualifications

The subjects are recruited from among members of the staff of the Laboratory, or from applicants for positions in the service staff of the Laboratory (including positions as subjects), or from graduate students in the University of Cincinnati whose academic work can be fitted advantageously into the regimen of a specific experimental program.

Each subject is interviewed initially by the principal investigator in an effort to ensure mutually satisfactory personal and working relationships, the probable reliability of the subject in following instructions, and his complete understanding of the purposes and procedures of the experimental program, of the theoretical hazards of his undertaking, of the background of experience and knowledge whereby he is to be maintained in complete safety and well-being, and of the necessity of his continuing in the role of experimental subject throughout the estimated (and actual) duration of the experiment. If the applicant is a graduate student, the approval of the Department of the University in which he is carrying out his academic work is obtained, and every effort is made to avoid any compromise in the requirements of the Department and the University as to time and scholastic performance. All persons employed as subjects are considered from the viewpoint of their continuation in the service of the Laboratory, following the completion of their stints as experimental subjects, should they so desire. Involved in this consideration is the interest and capability of the subject in work which will provide him with suitable employment throughout and after the termination of the experimental program.

Each required and apparently suitable subject is accepted tentatively by the principal investigator, on condition that he shall pass a comprehensive medical examination, augmented by a battery of clinical and biochemical tests in search

of functional deviations of a disqualifying type. If he is found to be acceptable, he is instructed to return to the examining physician, or to the nurse, or to the appropriate technician, as the case may be, at certain intervals required by the experimental regimen, and also to report to the nurse and physician whenever, for any reason, he feels any need for medical attention or advice. (He is also assured of access to the principal investigator as he may feel the need to do so.) From the medical viewpoint, all ailments, including dental, upper respiratory and gastroenteric problems, are to be reported promptly and dealt with appropriately, at no expense to the subject except under obviously irrelevant and agreed upon circumstances.

Each subject is then advised by the principal investigator of the requirements with respect to clothing, changing of clothing and bathing and of the financial provisions for the absorption by the Laboratory of all expense incident to the experimental regimen as specified. He is then instructed, in detail, in the procedures involved in obtaining and delivering suitable specimens of food, beverages (including water), urine and feces. These instructions are reinforced by the advice and supervision of the various persons in the staff of the Laboratory with whom the subject comes in contact in the course of the day's work.

After all preparation has been made, the subject enters upon a preliminary experimental regimen which requires his careful attention to the collection and labeling of all of his urine in 12-hour specimens, all individual fecal evacuations (labeled as to date and hour), and as precise duplicates as can be obtained, by visual and ordinary household methods of measurement (i.e., pouring, spooning, ladling or forking equivalent portions into duplicate articles of tableware, as plates, bowls, cups or tumblers), of all food and beverages consumed during each meal, and at all other times. The procedure involves discarding, from the duplicates, all portions of all items that are not consumed, as bones, fat,

cartilage and fibrous parts of animal tissues, as well as the inedible shells, hulls, peelings, cores and seeds of vegetation. Certain foods are to be avoided, such as the flesh of game animals that have been shot, while apples, for example, are to be peeled entirely, or if eaten without peeling, are to be selected in pairs and halved, and so separated into homologous pairs for eating and sampling. Gum chewing and smoking are also to be avoided, excepting, as for the latter, at such times and under such experimental conditions as may be arranged and agreed upon. The subject maintains a diary in which are listed all unusual circumstances in his life (such as changes in abode, eating places, accidents involving specimens, etc.), and all of the items eaten at each meal. This is furnished to the principal investigator for his private information and for use in cross-checking if and when unusual analytical results are turned up. (From time to time, as desired by one or another of the investigators, certain individual items of food or beverages are collected separately for analysis, especially when somewhat unusual items occur in the dietary or when unusual analytical results are obtained. A number of investigations of this type have provided useful information.)

Clinical and Experimental Observations

During the preliminary observations, the subject reports daily to the designated technical assistant for the collection of a specimen of urine (of recorded volume, to be included in that of the specific day) for quantitative determination of porphyrins and of delta-amino levulinic acid, and for the preparation of films of blood for semi-quantitative estimation of the frequency of occurrence of reticulocytes and of erythrocytes containing basophilic granules ("stippled cells"). (These observations continue throughout the experiment.)

Weekly observations are made and recorded by the responsible clinician as to weight, temperature, pulse, blood pressure, instrumentally measured manual

grip bilaterally, circulatory response to "step exercise," and occular fundoscopic appearances. To these are added any other items of interest or concern to the clinician. Clinical urinalysis (measured volume of urine included in day's output) is performed, and any "follow-up" test that may be indicated, thereby, is carried out. Meanwhile, the well-being of the subject is looked into by the clinician, without creating any pattern of inquiry through leading questions. The hematological status of the subject is investigated further, weekly, by enumeration of the erythrocytes, leucocytes and types of leucocytes and by determination of the hemoglobin, in the peripheral blood. All results, except the personal part of the clinical record (which is dealt with confidentially by the clinician), including all analytical findings, are recorded by the appropriate observer and forwarded to a central record-keeper, by whom they are made available for scanning, charting and statistical handling by designated personnel. These records, after being dealt with as required, are transmitted to the principal investigator for preservation.

The determinations of lead in food, feces, urine and blood (or any other material desired by the investigators) are carried out by methods of preparation and analysis which have been unchanged in their essentials since the initiation of these experiments in 1939, thus insuring valid comparisons of contemporary with earlier findings. Parallel results have been obtained, during certain periods, (or regularly in dealing with certain materials), through the utilization of other analytical methods based on different principles (as a spectrographic method developed in the Laboratory, as well as a technique of atomic absorption adopted for use more recently).

The specimens of blood and urine are analysed promptly and reported in such manner as to be used to reveal the current status of a subject, with respect to his absorption and excretion of lead, with but little lag in time. Other samples,

excepting as there is some special need for information, are fitted into the analytical schedule according to a priority established by the head of the analytical staff, as modified, from time to time, at the request of the principal investigator.

After the preliminary (control) observations have been carried out over a period of time sufficient to disclose the general pattern of the behavior of the subject, including his response to seasonal influences (variations in ambient temperature and, possibly, in the type and intensity of solar radiation, or in other, presently speculative, environmental factors) that are to be expected, the experimental exposure to lead is initiated according to plan. /The details of the design and operation of the chambers have been described elsewhere (1)./

A special procedure, concerned with the inhalation of air-borne particulate lead, has been that of ascertaining, at weekly intervals, the extent of the retention of lead in the respiratory system during the experimental exposure, by determining the lead content of a suitable volume of the expired air (2). /The content of lead in the air of the chamber is determined once or twice (dependent upon the duration of the exposure) during each period of exposure./ On the basis of measurements of the respiratory volume per minute of the individual subject, periodically, under a variety of conditions, approximations can be arrived at as to the quantities of lead made available, experimentally, for absorption. (The precision of these observations leaves something to be desired, in view of the variability of the results obtained from subject to subject and from the same subject from time to time. Moreover, until recently, the extent of the clearance

(1) The Harben Lectures. Lecture II. pp. 41 and 42 of the bound reprint of the lectures.

(2) Ibid. p. 43.

of the lead from the respiratory tree, through mechanisms that operate after the deposition of lead has occurred, has been highly speculative. This matter is still subject to experimental validation, but certain theoretical considerations and observations have provided a basis for assigning numbers to both clearance and absorption (1). With these numbers at hand, one can give quantitative expression to the intake and output of lead into and out of the respiratory system, and to the absorption of lead therefrom, in our experiments, with a degree of confidence in their approximate accuracy.)

(1) Developed by the Task Group on Lung Dynamics for Committee II, of the International Commission for Radiation Protection. Health Physics (Pergamon Press). Vol. 12, No. 2, pp. 173-208, 1966.

EXPERIMENTAL RATIONALE

As indicated in the original application for a grant from the Public Health Service in partial support of this work, the experiment under way at that time, and projected for continuation with such supplementary support, involved 2 men in parallel observations, in which the factor of time (duration of exposure per unit of time over an extensive period of time) was to be the sole significant variable.

The rationale of this experiment lay in certain previously observed facts, as follows: - When experimental subjects had been subjected to the oral administration, daily, of a standard dosage of lead in solution along with their meals, over periods of months and years, thus providing for an elevated, slightly variable but continuous rate of absorption from the alimentary tract during every successive period of 24 hours, the rate of the excretion of lead in the urine, the concentration of lead in the blood, and the rate of accumulation of lead in the body, with some seasonal (and other) variability, continued at substantially uniform yearly rates, without any sign of tapering off during the entire period of the administration of lead, in one instance, for 4.5 years. (Experimental Studies on the Ingestion of Lead Compounds. J. Industr. Hyg. & Toxicol. 22:381, 1940.) When, however, other experimental subjects were confined in respiratory chambers within which they inhaled lead dispersed in air (under standardized and controlled conditions) for 7.5 hours per day on 5 days per week (in simulation of the usual occupational regimen), i.e. intermittently, the rate of the excretion of lead in the urine, and the concentration of lead in the blood increased steadily to a nearly constant level (in about 15 weeks), after which it continued essentially unchanged for as long as the experimental conditions remained constant. (Experimental Studies on

Inhalation of Lead by Human Subjects. Pure Appl. Chem. 3:129, 1961.)

It is obvious, therefore, that continuous exposure to and absorption of lead, above a certain mean level (at which day-to-day variability in intake does not counteract the continuity of continuous absorption), results in progressive accumulation of lead in the body. It may be presumed, reasonably, and in the lack of evidence to the contrary, that this state of affairs persists during the lifetime of the individual, and that this will lead, at a sufficiently high rate of intake, to the accumulation of a dangerous "body burden" of lead. Under the usual environmental conditions in this country, with respect to the absorption of lead by persons in the general population, no such accumulation, or only a negligible degree of accumulation, seems to occur during a lifetime. This phenomenon can be accounted for, on the basis of the relative insignificance of the quantity of absorbed lead that is retained in the body from day to day - a quantity too small to be detected in our "balance experiments," or in the analytical findings at necropsy, in the face of the variability of the terminal findings. Barth (1), Tompsett (2), and Schroeder (3) have submitted evidence, which, in their judgment, was to the contrary, but none of these investigators limited the sources of human specimens for analysis to those definitely identified

---

(1) Barth, E. Untersuchungen über den Bleigehalt der menschlichen Knochen. Virchows Arch. f. path. Anat. 281:146, 1931.

(2) Tompsett, S. L. The Determination and Distribution of Lead in Human Tissues and Excreta. Analyst 81:330, 1956.

(3) Schroeder, H. A., et al. Abnormal Trace Metals in Man: Lead. J. Chronic Diseases 14:408, 1961.

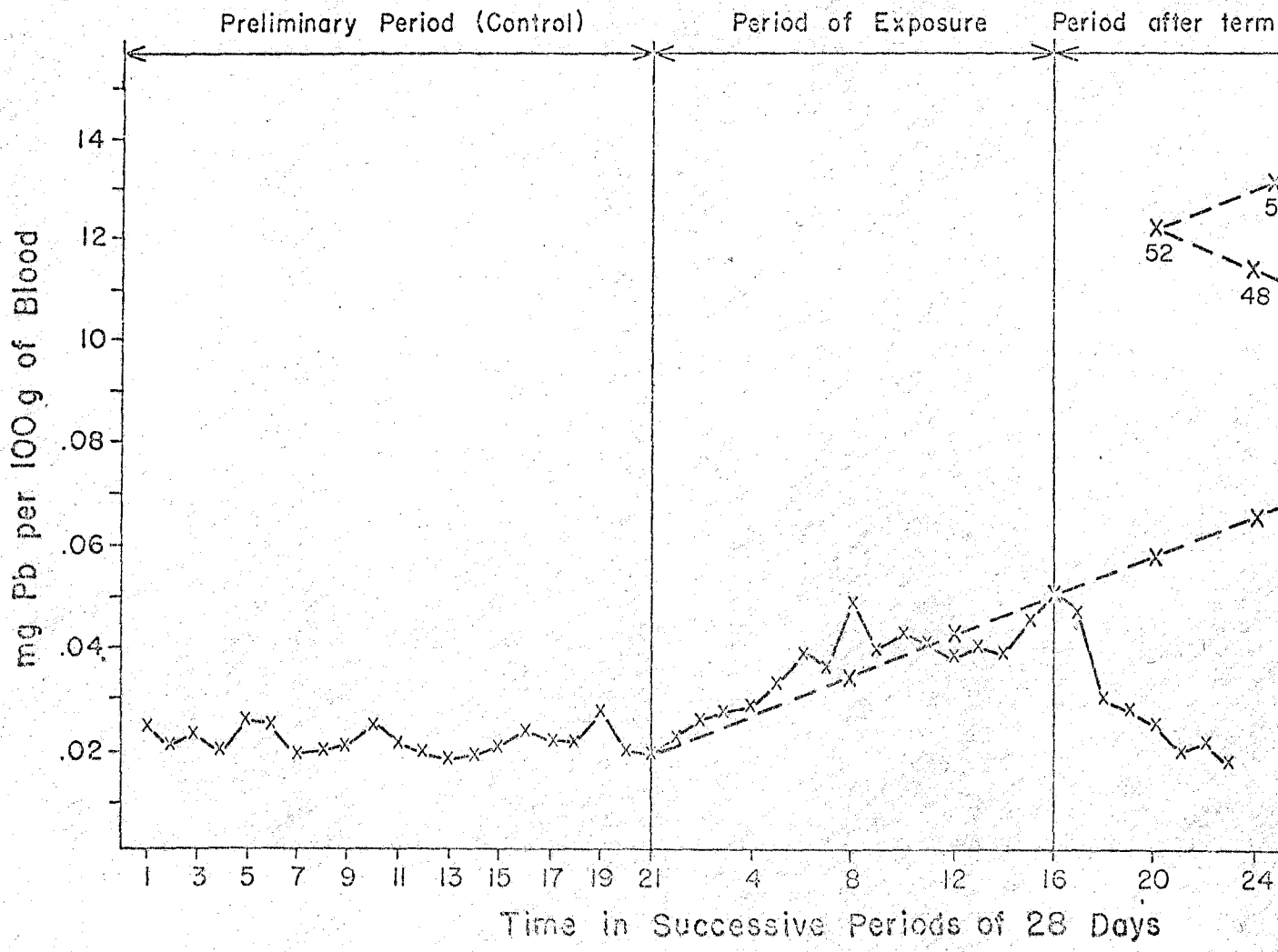
as lacking in any influence other than the current general environmental exposure to lead. This point is crucial. An additional feature of the environmental exposure to lead contributes further to the paucity of evidence indicative of the progressive accumulation of lead in the bodies of persons in the general population with advancing age. This factor is the variability in the rate of intake and in the consequent variability in the rate of absorption of lead, from person to person, in the population, because of the variable selection and utilization of food, qualitatively and quantitatively, by individuals. Bodily size, occupation, climate, habits of life and of upbringing, social and economic status, and a variety of other factors influence the manner of eating and the quantity and variety of food consumed. These factors, in turn, determine the quantities of lead taken into the alimentary tract. Moreover, at different periods in a single lifetime, from infancy through childhood and adolescence, adulthood and old age, the quantity and variety of the food (and its content of lead) consumed by an individual undergo considerable variation, with the result, akin but not equivalent to that of temporal intermittency of exposure, that there are times when lead is being lost from the body, and others when the reverse is true. A seasonal factor (or factors) promotes loss of lead from the body in the summer months in the temperate zone. The balance experiments on "normal," healthy, young subjects have also demonstrated once and for all time, if there has been any very serious doubt about the matter, that the lead which finds its way into the human skeleton is actively involved in the metabolism of the body. The osseous metabolism, as lead is involved therein, is slow, as

KE

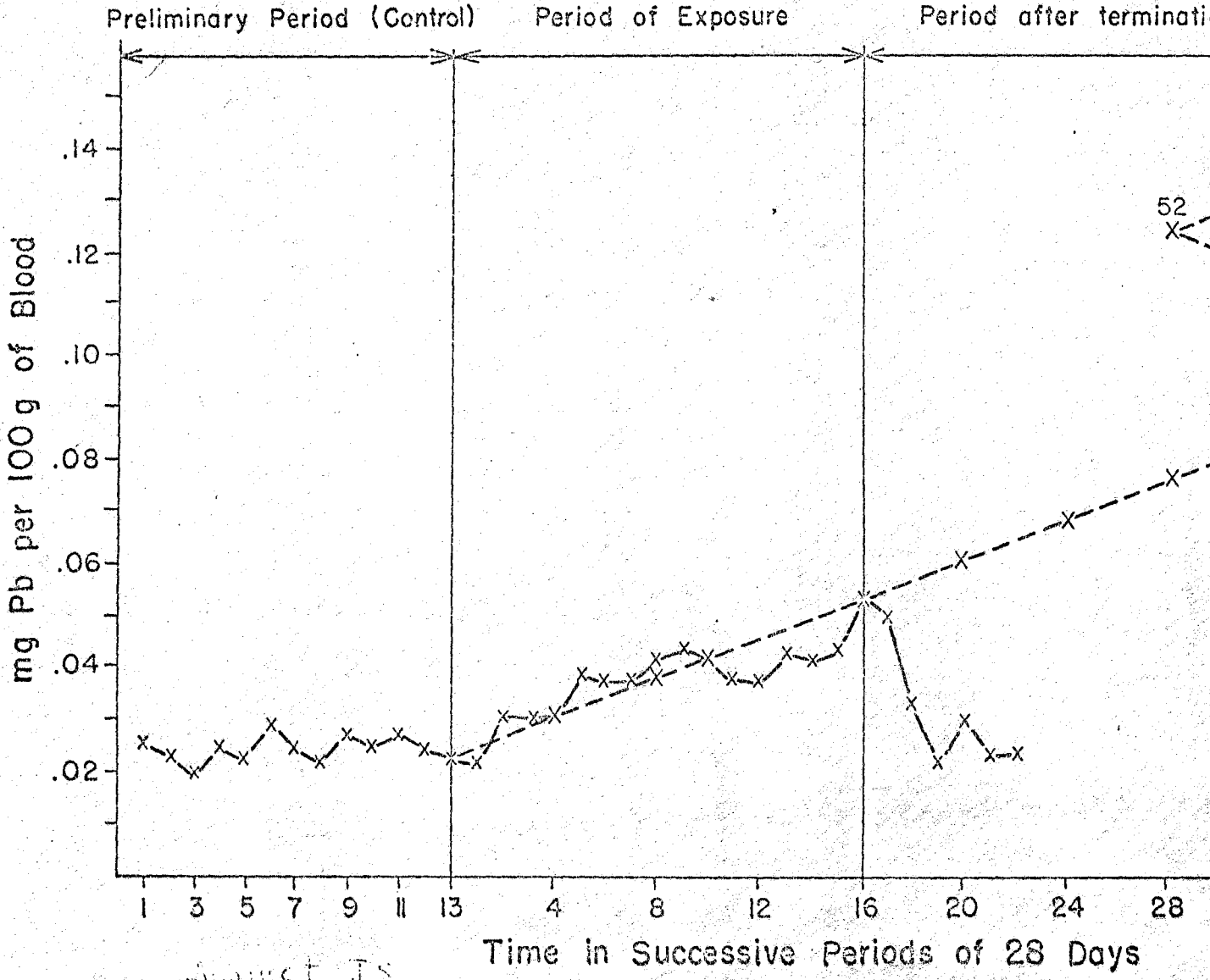
compared to that of other tissues of the body, but it responds promptly, although in lesser degree, to conditions that disturb the metabolic equilibrium, such as increase and decrease in the rate of absorption of lead into the body, or the increased loss of lead from the "soft tissues" of the body following chelation therapy. (The metabolism of lead is not influenced appreciably, under the ordinary conditions of life, or within the limits of cautious experimentation, so far as present evidence is concerned, by that of calcium or phosphorous or of any other mineral element, but is, rather, strikingly stabile and predictable in its behavior in the body, as has been demonstrated clearly by these experiments. Moreover, the uniformity in the responses of a series of subjects to a variety of experimental conditions, has been little short of remarkable.)

Because of the facts recited above, it seemed that the demonstration of the maximum concentration of a reproducible dispersion of absorbable lead in the air that could be inhaled continuously, with complete safety, by normal healthy human subjects, would require direct observation of the metabolic effects of continuous exposure at levels above those now prevalent in the community. Since such direct experimental observation was not feasible, except, perhaps, through the expenditure of inordinate sums of money for the actual creation of conditions of housing, subsistence and prolonged maintenance of an essentially normal conduct of life by the experimental subjects, an alternative approach was thought to be that of establishing the gradient of the response of human subjects to incremental increases in the duration of intermittent but quantitatively constant exposure, from which to extrapolate to a near

X——X Average of mean values of findings obtained by dithizone and spectrographic



X——X Average of mean values of findings obtained by dithizone and spectogr



Subject J.S.
Fig. 2

approximation to the metabolic effects of continuous exposure. The use of the expression, "approximation" arises from the likelihood that, as the experimental intermittency approaches continuity, the shortening periods of freedon from exposure and the lengthening periods of exposure will operate against the achievement of a balance between the intake and output of lead, so that the curve indicative of the progress of events will assume a trend bending upward from the straight line indicated in Fig's. 1 and 2, to which detailed attention will be drawn later. From the strictly practical point of view, this deviation should have little importance, especially when the concentration of lead in the respired air is low.

EXPERIMENTS CARRIED OUT SINCE 1962

Preliminary Experiment

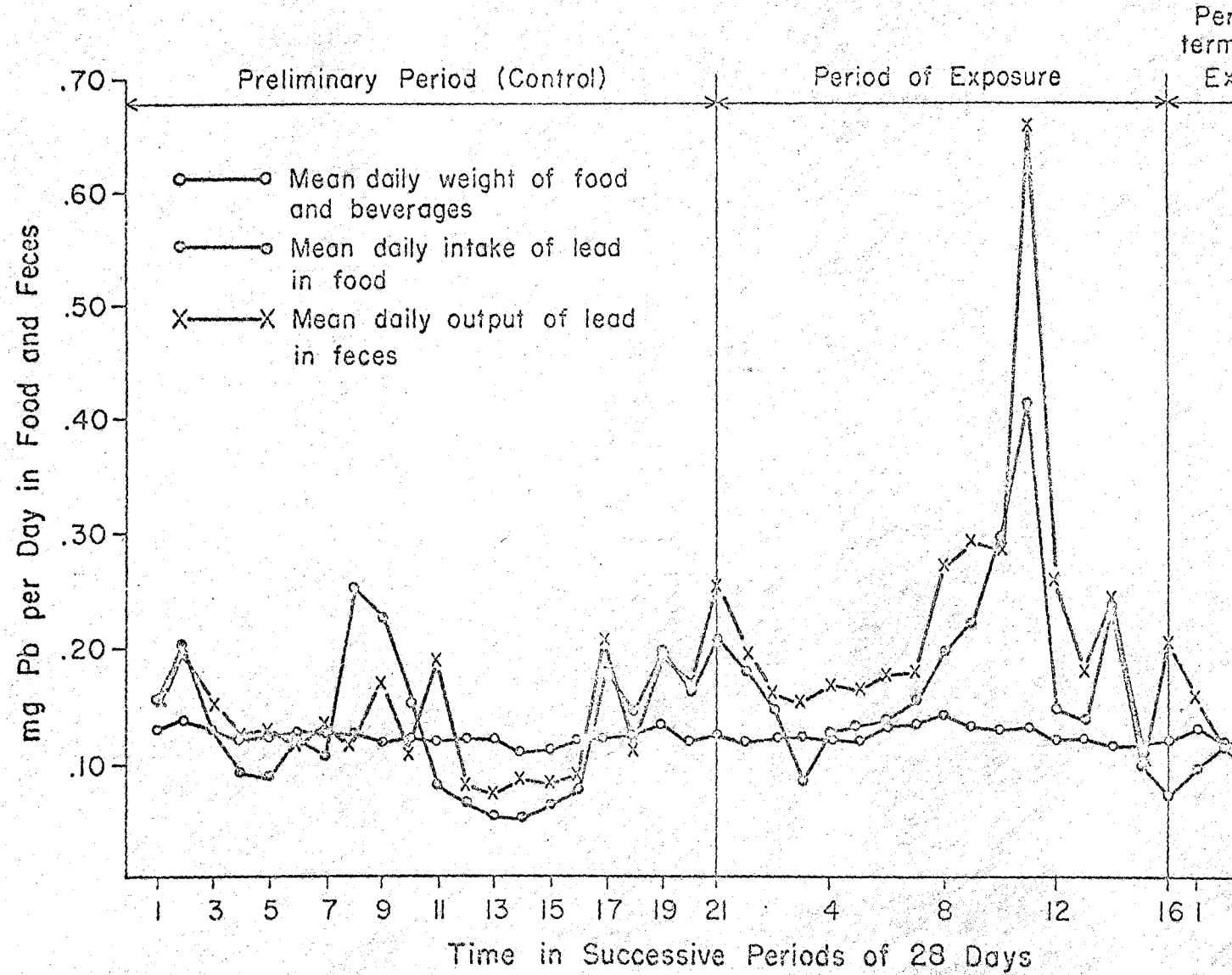
The feasibility of the experimental approach described above was tested in an experiment involving detailed observations (including all of the procedures described in prior balance experiments) of two human subjects over the period of 144 and 178 weeks, respectively. (The period of observation of one of the subjects, prior to the initiation of experimental exposure to lead, was unusually prolonged so as to enable the other, secured later, to undergo his initial period of experimental exposure to lead on the same date as the first, and so to continue parallel procedures.)

Subjects [redacted] and [redacted] were engaged in daily preliminary (control) observations, (determinations of their daily intake of lead in food and beverages, and their output of lead in the feces and urine, in association with the usual battery of hematological, clinical, microscopic, and physical examinations),

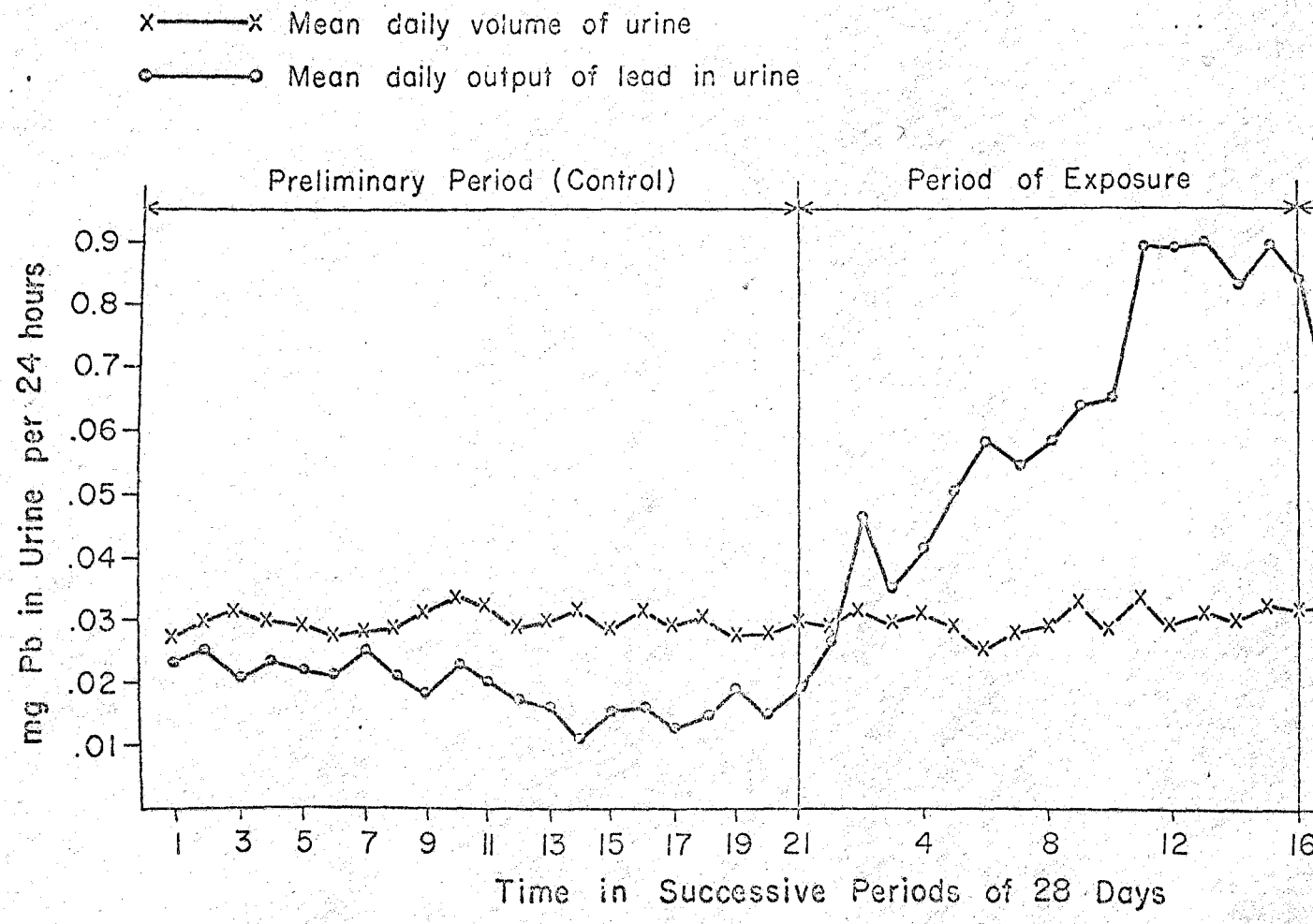
over periods of 56 and 86 weeks, respectively.

They entered two correspondingly designed respiratory chambers into which air containing 150 micrograms of lead per cubic meter, as the sesquioxide, dispersed in particles ranging up to 0.18 micron in diameter, and having the mean diameter of 0.05 micron, was being introduced and evenly distributed, and from which it was being evacuated, stripped (by electrostatic precipitation) of its lead, and discharged into a stack. The design of the experiment called for exposure to these conditions on the following schedule: 16 weeks for 10.5 hours per week; 16 weeks for 21 hours per week; and so on, 16 weeks at a time, for 31.5 and 42 hours per week. This schedule did not result in carrying the severity of the exposure beyond the level which has been established, by both occupational experience and physiological experimentation, as being well within the limits of safety.

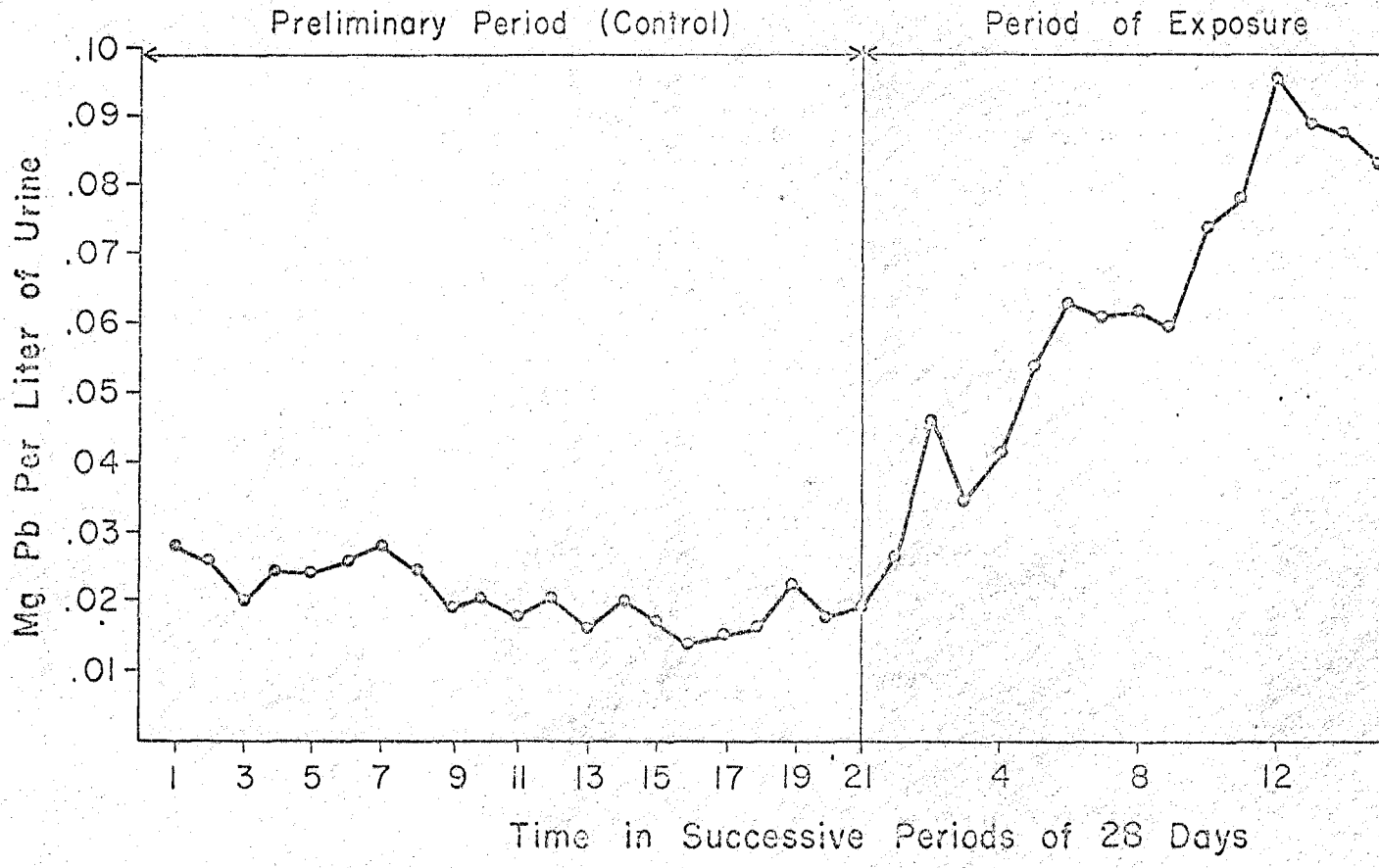
During each successive period of 16 weeks, as has been the case in every other experiment involving intermittent exposure to lead in simulation of occupational experience (i.e. usually 37.5 hours per week), the level of the urinary output of lead per day, and the concentration of lead in the urine of the subjects, as well as the concentration of lead in their blood, rose up to a certain point and then continued at essentially constant levels, so long as the conditions of exposure remained unchanged; each incremental increase in the weekly duration of the exposure at least resulted in a further graduated increase in the levels of the lead in the urine and blood; a line connecting the points representing the final levels (or the leveled out plateaux) of the concentration of lead in the blood reached in the successive periods of 16 weeks became a straight line of uniform upward slope. The results of the extension



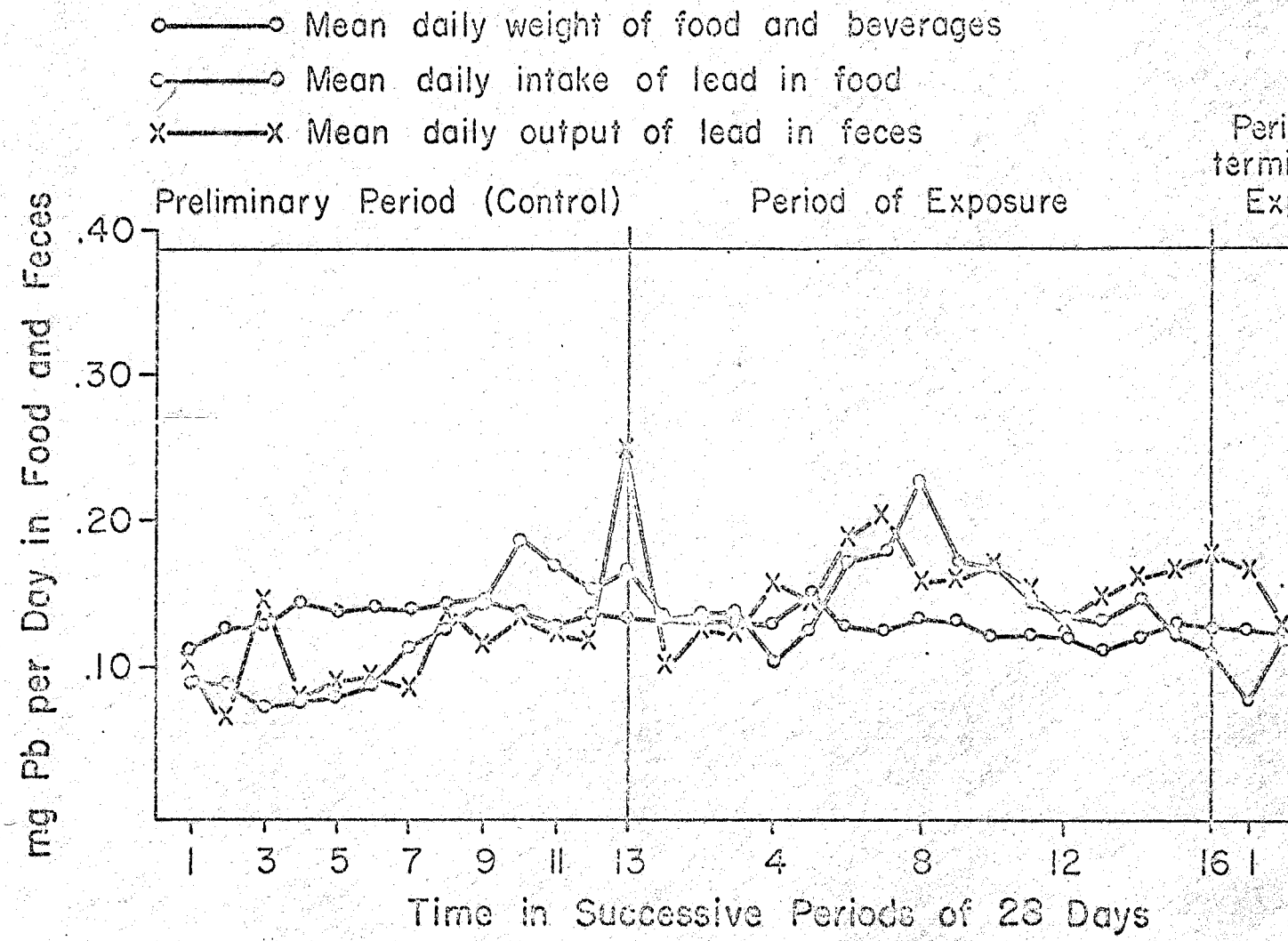
Subject 10.
Fig 2



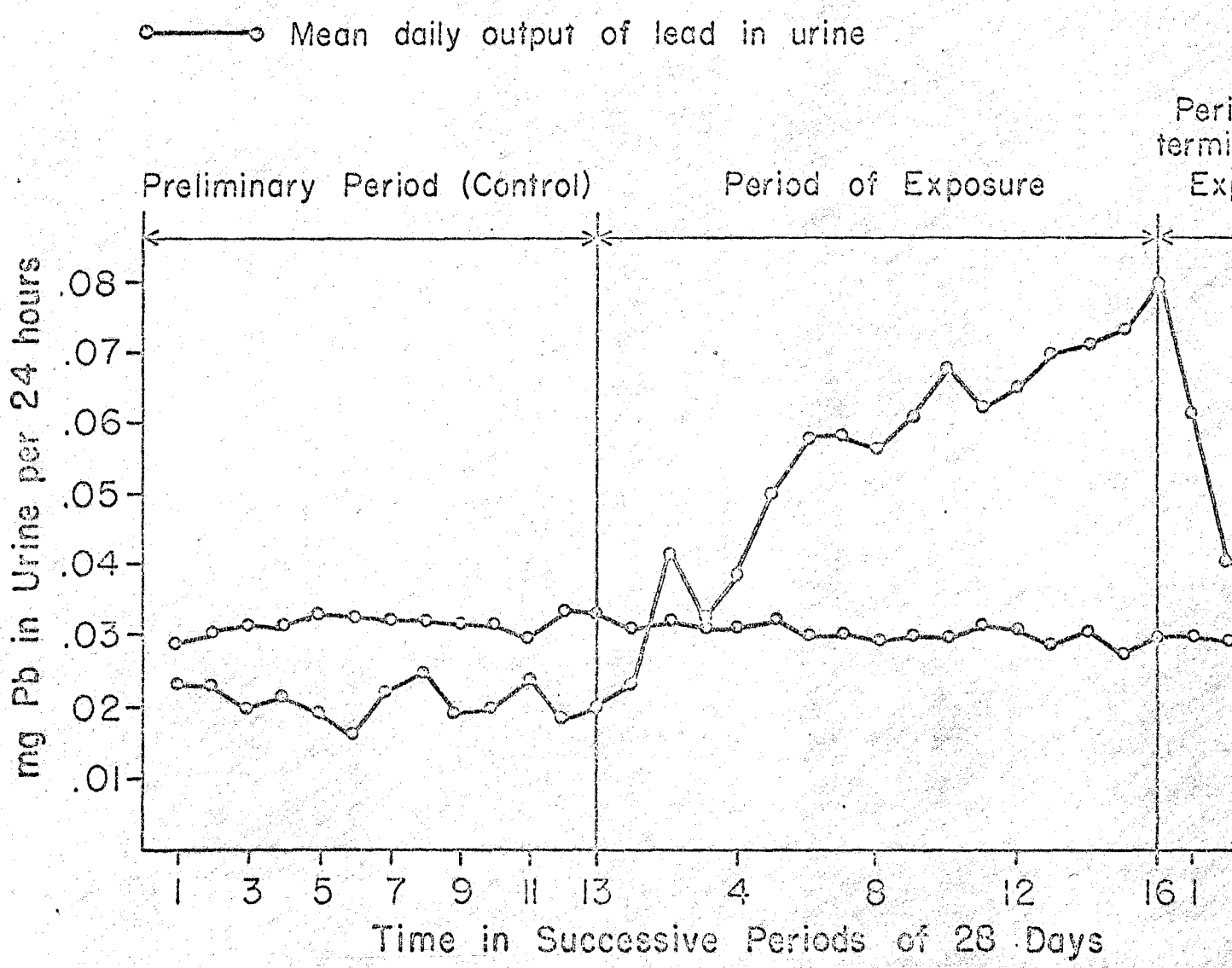
Subject A.D
Exp. 4


Preliminary Period (Control)
Period of Exposure
Mg Pb Per Liter of Urine
.10
.09
.08
.07
.06
.05
.04
.03
.02
.01
1 3 5 7 9 11 13 15 17 19 21 4 8 12
Time in Successive Periods of 28 Days

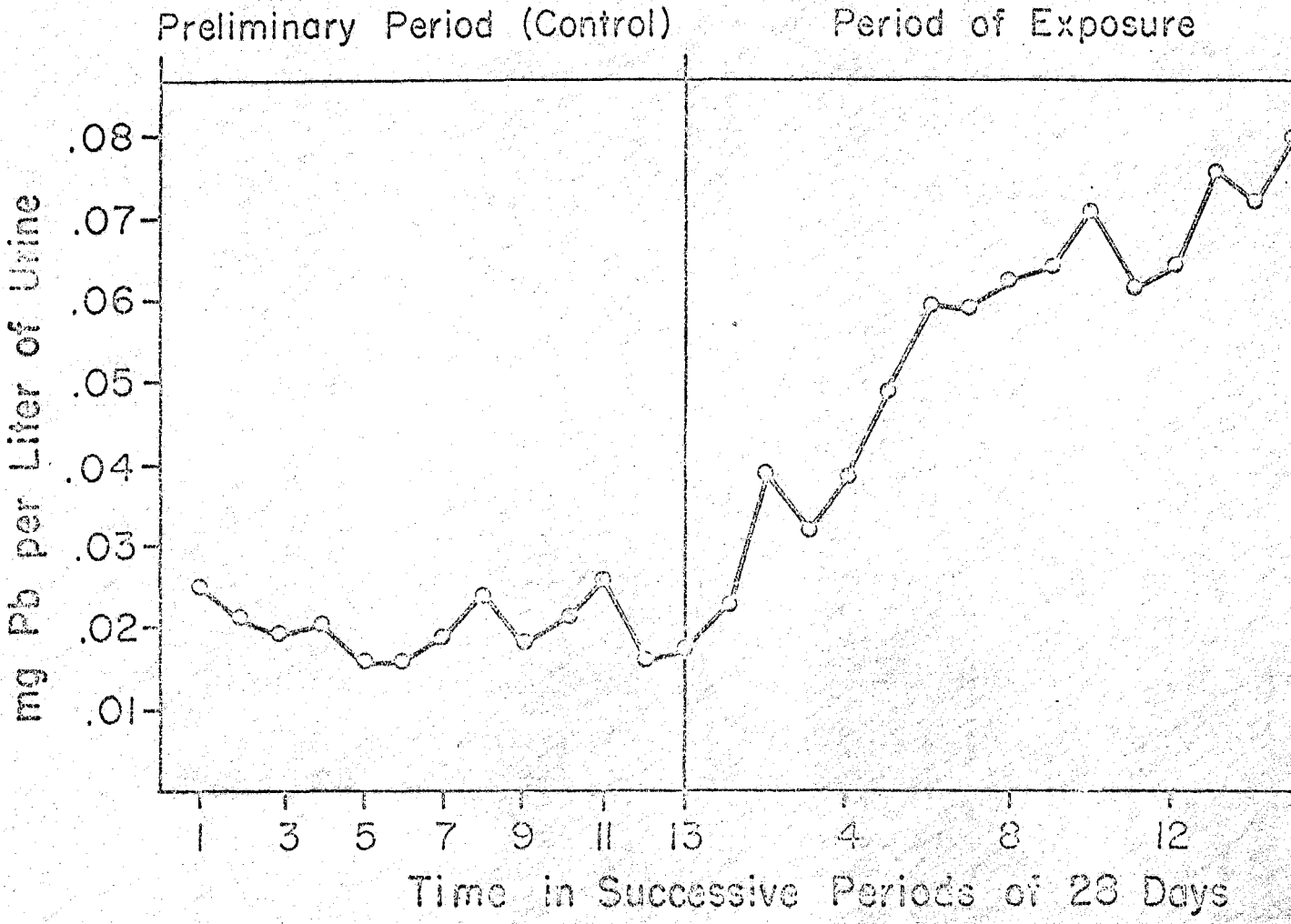
Subject AD


Mean daily weight of food and beverages
Mean daily intake of lead in food
Mean daily output of lead in feces
Preliminary Period (Control)
Period of Exposure
Peri
termi
Ex
mg Pb per Day in Food and Feces
.40
.30
.20
.10
1 3 5 7 9 11 13 4 8 12 16 1
Time in Successive Periods of 28 Days

Subject J.S



Subject J.S.
Fig. 7


Preliminary Period (Control)
Period of Exposure
mg Pb per Liter of Urine
.08
.07
.06
.05
.04
.03
.02
.01
1
3
5
7
9
11
13
4
8
12
Time in Successive Periods of 28 Days

of this line, at the same slope, to a point that corresponded, in the case of each subject, to continuous exposure (i. e. 168 hours per week) is shown in Figure 1, (Subject [redacted]), and in Figure 2, (Subject [redacted]). Other analytical data of this experiment have been grouped and charted in Figures 3, 4, and 5 (Subject [redacted]), and in Figures 6, 7 and 8 (Subject [redacted]). The general features of these charts are similar to those observed in previous experiments and commented on elsewhere. It may be useful, however, to call attention to certain of their characteristics. First and foremost is the similarity of the two sets of charts. We have learned to expect that the behavior of human subjects will be much the same under the same experimental conditions, but it is none the less impressive to note that two individuals have reacted so nearly identically when, in the Laboratory, their environments were as nearly identical as they could be made, while differing elsewhere according to the customs of two different households, and the habits of life of two individuals, one of whom ([redacted], Fig. 1), a craftsman, was married and "settled down," the other, ([redacted], Fig. 2) was unmarried, a night-school student, and looking toward a technical career. The concentration of lead in the blood of Subject [redacted], at the start, was slightly lower than that of Subject [redacted] during the initial (control) period (0. 022* vs. 0. 024*mg. per 100 grams), still slightly lower by about the same margin, at the end of the total period of experimental exposure, and still in the same relationship as the result of the extrapolation, the slope of the curves, as plotted, being remarkably similar.

It is instructive to compare the end results of the intermittent respiratory

* These differences are negligible in the practical sense. They are remarked upon only because they have resulted from calculations from data that were

exposure to air containing 150 micrograms of lead, with those that emerge, from the extrapolative process, as an expression of continuous respiratory exposure. Thus, the concentration of lead in the blood of the two subjects was 0.045* and 0.053* milligrams per 100 grams, for subjects [redacted] and [redacted] respectively, after 16 weeks of exposure for 42 hours per week (comparable roughly to the industrial week), while it reached the derived levels of 0.145* and 0.148*, respectively, at some point in time (certainly less than 3 years), after the subjects had been subjected, continuously, to the inhalation of air containing the same concentration of lead. /The evidence furnished by observation of the effects of occupational exposure to a well maintained level of lead concentration in the industrial atmosphere justifies the conclusion that the concentration of lead in the blood of workmen increases for a time, until it reaches a level which undergoes little or no further increase over the period of many years. Under conditions in which the concentration in the blood of every workmen remains below 0.08 mg. per 100 grams (that is, in view of the analytical deviation of ± 0.01, not in excess of 0.07 mg. per 100 grams), no case of lead poisoning will occur, in our experience. The concentration of approximately 0.15 milligram of lead per 100 grams of blood, on the other hand, is unduly high, and while it does not follow that every individual whose blood contains such a concentration of lead has or will have lead poisoning, some become ill promptly and others later, while some, unaccountably, remain well/.

* These differences are negligible in the practical sense. They are remarked upon only because they have resulted from calculations from data that were limited to the second decimal.