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BIOLOGICAL SIGNIFICANCE OF SOME METALS AS AIR POLLUTANTS

Progress report

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CONTENTS

I DETERMINATION OF GLUCOSE-PHOSPHATE DEHYDROGENASE (G-6PD)

The deficiency of glucose-6-phosphate dehydrogenase (G-6PD) activity can be measured quantitatively and qualitatively. Quantitative methods are based on reduced triphosphopyridine nucleotide (TPNH) and reduced dichloro-indophenol (DCIP) light absorption measurements, while the basis for qualitative tests is the reaction of the produced TPNH either with some dyes, such as brilliant cresyl blue, dichloro-indophenol, or methylene blue, or with some basic components of erythrocytes, such as oxidized glutathione and methemoglobin. In addition, there are also tests involving production of new blood pigments or new morphologic products, as well as the test employing the TPNH fluorescence.

Quantitative methods have been developed by several authors (I-1-5). They differ in the composition of the reaction mixture, temperature, and the concentration of hydrogen ions. One group of methods is based on direct determination of the TPNH formation (I-1, -2, -3, -5) using spectrophotometry in the ultraviolet region (340 nm). The method by Ells and Kirkman (I-4) is based on the determination of enzymatic activity by means of dichloro-indophenol using spectrophotometry in the visible region (620 nm).

Qualitative tests are reported in the literature more frequently than quantitative ones. In dye tests (I-6-11) the enzymatic activity is the function of the dye disintegration rate. The glutathione stability test (I-12) shows the average concentration of reduced gluthatione (GSH) in erythrocytes with a reduced G-6PD activity to be lower

than in erythrocytes with a normal enzymatic content. In the methemoglobin reduction test (I-12-16) the reduction is followed of the artificially produced methemoglobin by TPNH methemoglobin reductase in the presence of an electron carrier (methylene blue, Nile blue sulphate). The main point in methods based on the production of new blood pigments (I-17, -18) is that G-6PD deficient erythrocytes are oxidized with ascorbate more quickly than normal ones; this oxidation produces a new blood pigment which in its colour differs from the colour of the normal blood, and this allows a visual following of the reaction. Tests with the production of new morphologic products by means of (3-(4,5-dimethylthiazolil-2)-2,5-diphenyltetrazolium bromide (MTT) (I-19) and also fluorescent tests (I-20, -21) are of a more recent date.

Four methods have been investigated: two qualitative - the method of Brewer et al. (I-13) and that of Fairbanks and Lampe (I-9) - and two quantitative - the method of Zinkham et al. (I-3) and that of Ells and Kirkman (I-4). The methods have been developed and are being checked. However, as persons with a congenital G-6PD deficiency are encountered very rarely, a final assessment of these methods will be possible only after employing them in a larger number of subjects, which is believed to be done by the end of 1969.

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II ALKALINE PHOSPHATASE ACTIVITY IN BLOOD SERUM

For the estimation of the activity of alkaline phosphatase the method by King and Armstrong was selected (II-1) which is based on the measurement of hydrolysis of disodium phenyl phosphate by the enzyme at 37°C yielding equivalent amounts of phenol and phosphate. After the precipitation of proteins with the Folin-Ciocalteu's reagent the liberated phenol is determined by spectrophotometric measurement of the optical density (620 nm) of the blue colour formed on addition of sodium carbonate. King-Armstrong's unit of alkaline phosphatase activity is defined as the amount of enzyme which liberates 1 mg of phenol from 0.005 M disodium phenyl phosphate within 15 minutes at 37°C in a carbonate-bicarbonate buffer pH 10.

The method was calibrated with series of pure phenol solutions of different concentrations. The regression equation, obtained by the method of least squares, reads as follows:

mgs of phenol = 34.97 x optical density

Milligrams of phenol are equal to the number of K.A. units.

In Table II-1 the results are presented of the estimation of alkaline phosphatase activity in 32 "normal" serum samples. The results obtained are within the normal range proposed in the literature (II-1).

Table II-1

Number of sample	Alkaline phosphatase activity (K.A. units)	Number of sample	Alkaline phosphatase activity (K.A. units)
1	3.74	17	4.76
2	4.89	18	4.48
3	3.95	19	4.41
4	3.53	20	6.08
5	3.25	21	5.24
6	6.78	22	6.12
7	2.34	23	6.54
8	4.06	24	7.27
9	6.05	25	5.35
10	7.90	26	6.26
11	5.17	27	6.85
12	4.30	28	5.73
13	5.91	29	6.22
14	6.29	30	5.14
15	2.76	31	7.34
16	3.74	32	6.08

Amelung et al. (II-2) indicated that the activities of most frequently used enzymes in clinical biochemistry are sufficiently stable at room temperature only up to 6 hours following the collection of blood samples. As the majority of blood samples to be analysed in the course of the present project are to be collected far from the laboratory and will have to be transported to the laboratory and analysed with a delay, the stability of the enzyme at various temperatures and through various storage periods is being studied.

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III ACTIVITY OF GLUTAMIC-PYRUVIC TRANSAMINASE AND GLUTAMIC-OXALACETIC TRANSAMINASE IN BLOOD SERUM

The simplified colorimetric method described by Reitman and Frankel (III-1) was chosen for the assay of serum glutamic-pyruvic transaminase (SGPT) and serum glutamic-oxalacetic transaminase (SGOT). The estimation of SGPT activity is based on the liberation of pyruvate from a substrate containing α-keto-glutaric acid and dl-aspartic acid. The formed pyruvate is subsequently determined colorimetrically in the form of pyruvate hydrazone. The SGOT activity is estimated by determining the change of substrate containing α-keto-glutaric acid and dl-alanine yielding oxalacetate which is subsequently partially transformed into pyruvate. The hydrazones of the mixture of oxalacetate and pyruvate are determined colorimetrically.

In Fig.III-1 the obtained calibration curves for the estimation of SGPT and SGOT are presented.

In table III-1 and III-2 the results are presented of the estimation of SGPT and SGOT resp., in 32 "normal" human serum samples. The results are within the normal range as cited in the literatures (III-2).

STANDARD CURVES FOR ESTIMATION OF TRANSAMINASES

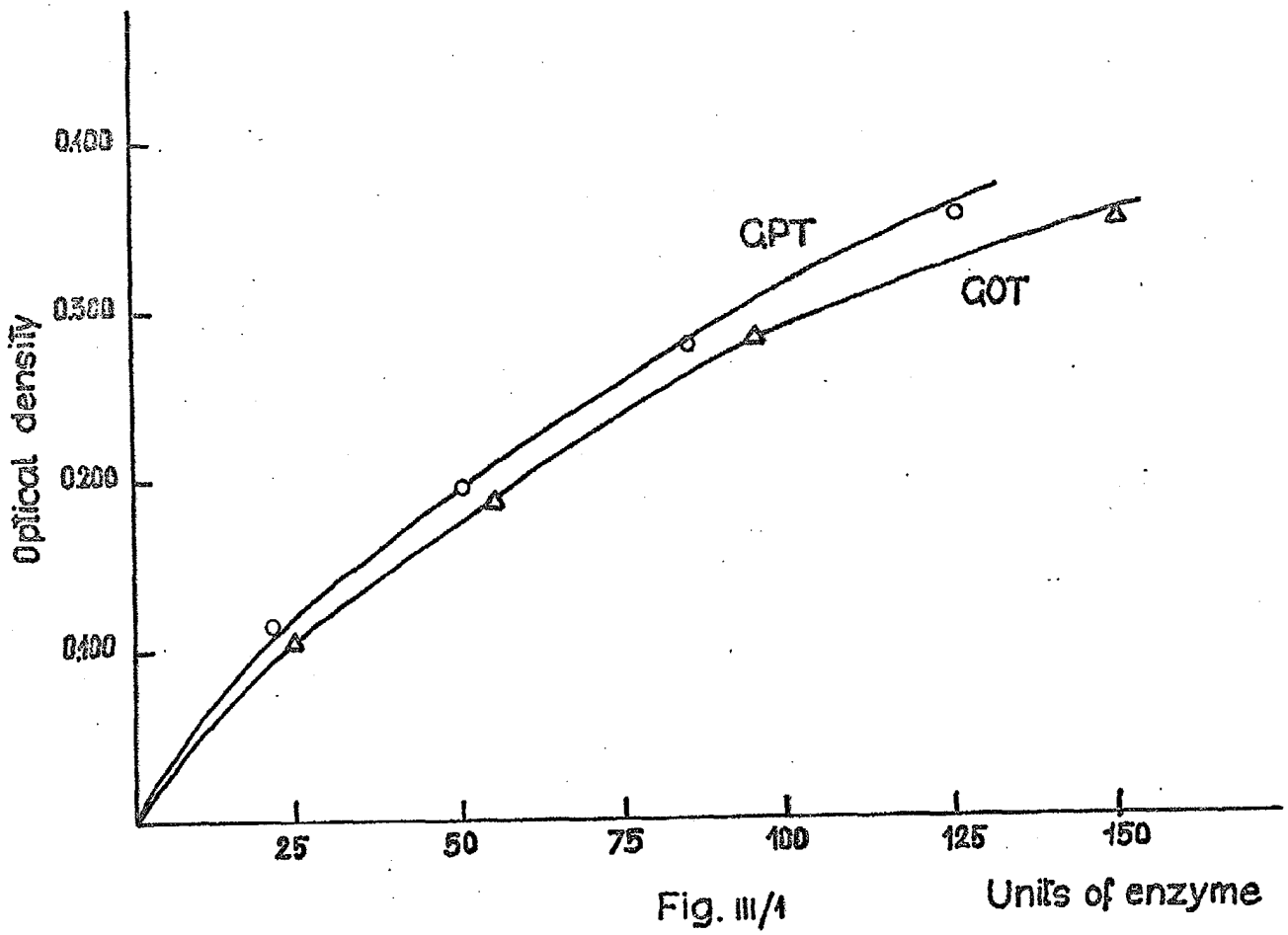

Fig. III/1

Table III-1

Number of samples	SGPT activity (Karmen units)	Number of sample	SGPT activity (Karmen units)
1	9.0	17	5.0
2	8.0	18	9.5
3	2.5	19	5.0
4	2.0	20	8.5
5	3.0	21	3.0
6	6.0	22	5.5
7	2.0	23	4.0
8	2.5	24	5.0
9	3.5	25	5.5
10	3.0	26	13.5
11	2.0	27	4.5
12	2.5	28	spoiled
13	1.5	29	3.0
14	8.0	30	3.0
15	6.0	31	1.5
16	1.0	32	3.5

Amelung et al. (III-3) have emphasised the greater instability of transaminases than that of alkaline phosphatase. For the same reason as mentioned under "alkaline phosphatase" the stability of SGPT and SGOT through various storage periods at various temperatures is being studied.

Table III-2

Number of samples	SGOT activity (Karmen units)	Number of sample	SGOT activity (Karmen units)
1	11.5	17	7.0
2	11.5	18	8.0
3	10.0	19	8.0
4	8.0	20	12.5
5	9.0	21	11.0
6	9.5	22	9.5
7	8.0	23	11.0
8	10.0	24	7.0
9	8.5	25	13.5
10	9.0	26	12.0
11	9.0	27	12.0
12	8.0	28	11.5
13	7.0	29	11.0
14	10.0	30	9.5
15	11.0	31	8.5
16	8.5	32	12.0

References

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IV DETERMINATION OF BLOOD GLUTATHIONE

Methods for the glutathione determination take advantage of two glutathione properties: (1) that it activates the glyoxalase enzyme which transforms methylglyoxal in to lactic acid, and (2) that it contains sulfhydril groups which are very reactive. In our investigations we decided in favour of the methods based on the oxidation of sulfhydril groups, of which photometric methods either with nitroprusside (IV-1, -2) or the Ellman (5,5-ditiobis-2-nitrobenzoic acid) (DTNB) reagent (IV-3) are best known. Methods with nitroprusside are not suitable for routine investigations securing no colour and reagent stability and being extremely sensitive to temperature. There are no such shortcomings with the Beutler method with DTNB (IV-3) developed on the basis of Ellman's determination of sulfhydril groups (IV-4), and for this reason this method has been selected as the most suitable.

The maximum absorption for reduced DTNB in the visible part of the spectrum has proved to be at 412 nm (Fig.IV-1). The experimental points for various concentrations of glutathione in water and the same standard added to 0.2 ml blood are shown in Fig.IV-2.

The results of the analysis of glutathione in blood and water are represented with a single regression line since the difference in the slope and in the variance of the two individual regression lines were not statistically significant (see the table).

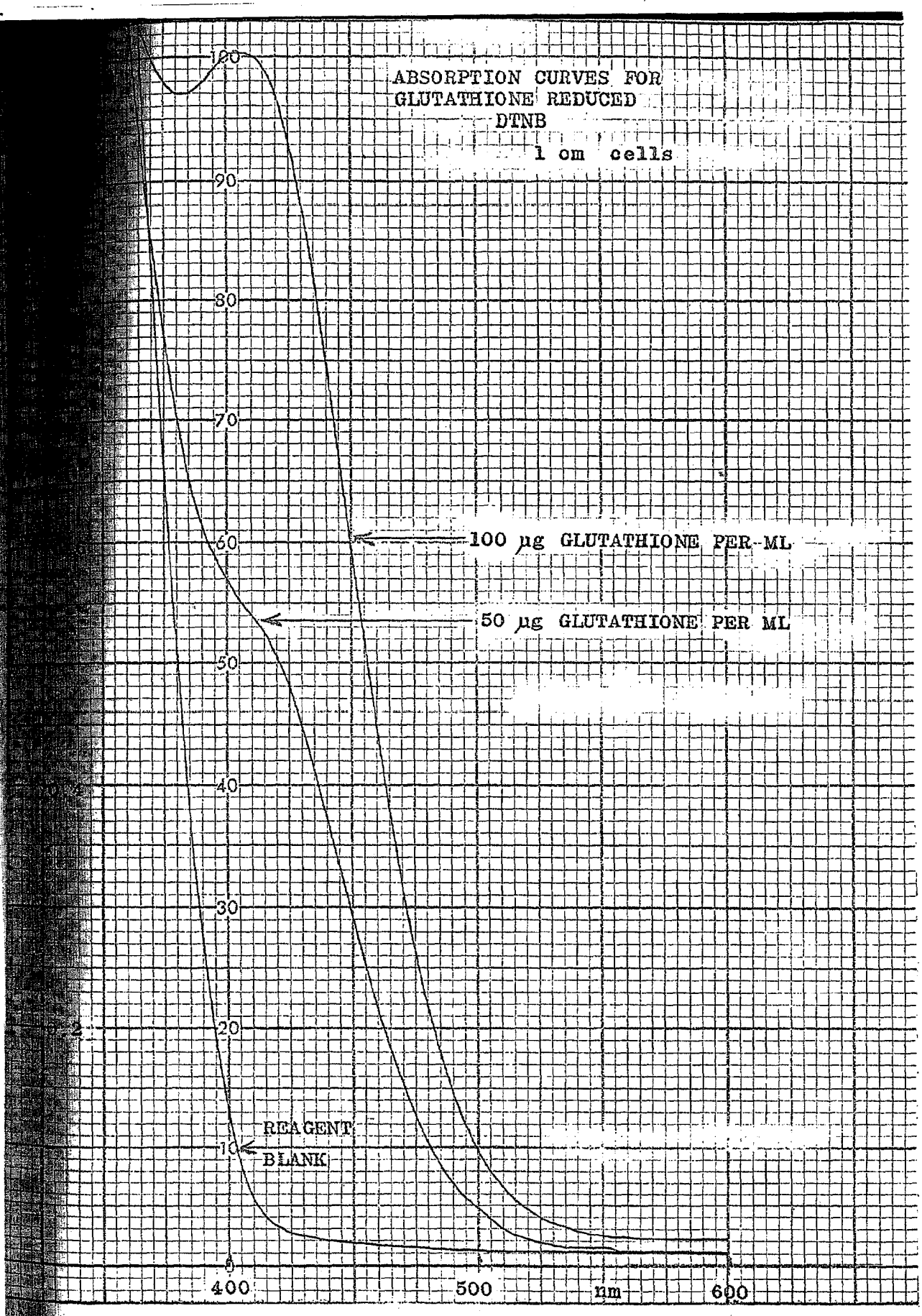

Fig. IV-1

CALIBRATION CURVE FOR THE DETERMINATION OF GLUTATHIONE

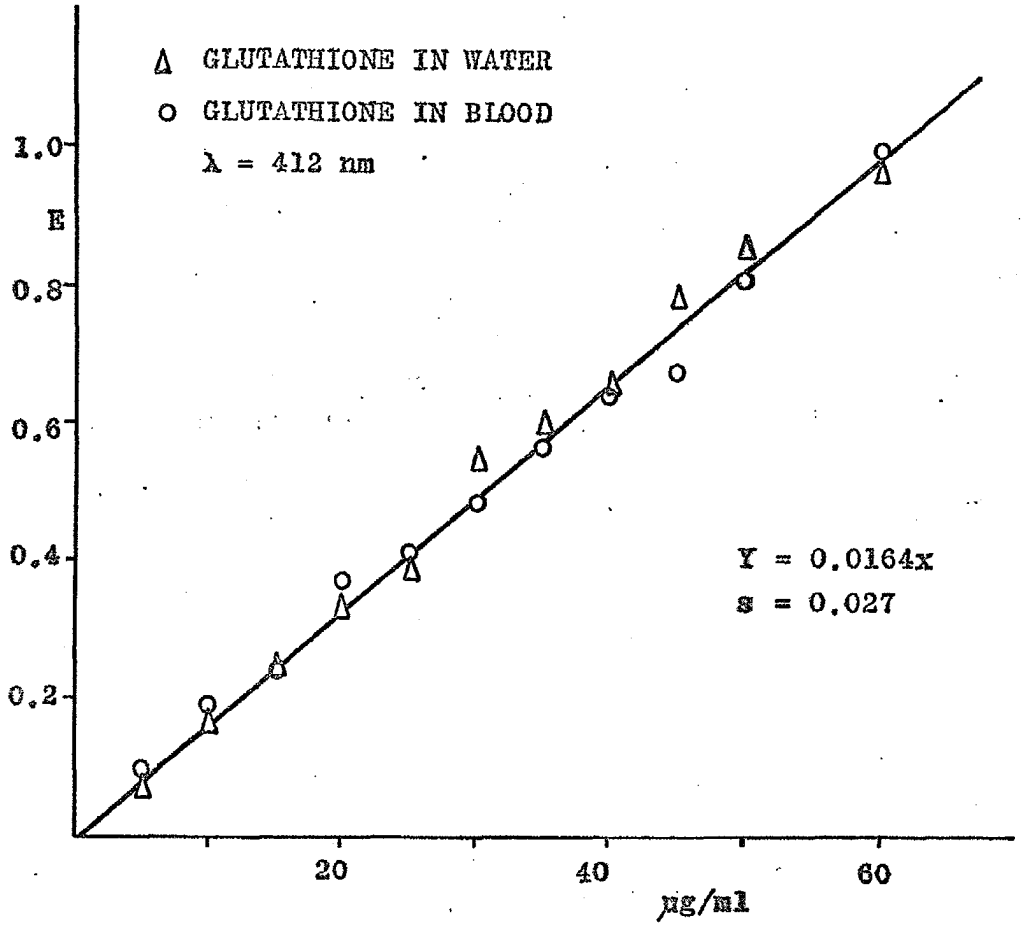

Fig. IV-2

	blood	water	
b	0.01614	0.1665	
s^2	0.733	0.651	F = 1.12
s	0.0271	0.0255	P > 0.05
s_b	0.000236	0.000222	
Δb	0.000513		
t	1.6		
$t_{0.05(\nu=20)}$	2.086		

The method has been checked on 10 blood samples of healthy subjects. The glutathione concentrations ranged from 65 to 79 mg per 100 ml of blood. Further investigations on the normal glutathione concentration are in progress.

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V SPECTROPHOTOMETRICAL DETERMINATION OF HEMOGLOBIN (Hb)

To determine hemoglobin (Hb) spectrophotometrically, the cyanmethemoglobin method has been used. Its principle is that by means of an oxidant (potassium ferricyanide) and cyanide the blood hemoglobin is transformed into cyanmethemoglobin (hemiglobincyanide, HiCN) which has a wide absorption band with the maximum absorption at 540 nm. By measuring the intensity of the coloured complex and taking into account the dilution value, it is possible, using the standard with the known Hb concentration, to determine Hb in the sample examined.

The International Committee for Standardization in Haematology (ICSH) (V-1) has strictly defined conditions of the analysis and recommended, as a universal standard, the solution containing 52.2 mg HiCN in 100 ml and corresponding to the concentration of 14.36 g Hb. Such standards are produced in Europe by the Institut voor de Volksgezondheid, Utrecht, the Netherlands, and our preliminary investigations have been conducted by means of such a standard which contained 59.0 mg HiCN and was equivalent to 14.8 g Hb.

The extinction coefficient at the wave length of 540 nm was found to be $\mathcal{E} = 44.6$ and the factor for further calculations was determined as $f = 36.7$. Both values proved to be in agreement with the characteristics of the standards determined by the Rijks Institut.

Analysing the stability of the cyanmethemoglobin complex it has been evidenced that in the interval of 0.5 to 24 hours, at $4^{\circ}C$, the colour of hemiglobincyanide

practically remains unchanged. Several blood samples were analysed simultaneously, and the reproducibility of the results proved satisfactory.

Investigations of normal values in a larger population are in progress.

Reference

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VI DETERMINATION OF DELTA-AMINOLEVULINIC ACID (ALA) IN URINE

According to literature data, the method of Mauzerall-Granick (VI-1) is used most frequently for the determination of delta-aminolevulinic acid (ALA) in cases of lead exposure and lead intoxication. The method is very sensitive but time-consuming and therefore not always suitable for routine investigations in large populations. The method employing alkaline picrate after Schuster (VI-2), though quick, is not suitable either, because it cannot separate porfobilinogen which interferes with the reaction of ALA with picric acid. Porfobilinogen also interefers in the reaction with p-dimethyl-amino-benzaldehid reagent (DMAB) but the method of Mauzerall-Granick separates it by means of the anion-exchange resin Dovex-2. However, according to some authors (VI-3, -4, -5), porfobilinogen is rarely excreted in increased quantities in lead poisoning. On the basis of these observations Williams and Few (VI-6) have concluded that the porfobilinogen determination is superfluous if ALA is used as a screening test in workers exposed to lead. They have modified the Mauzerall-Granick method by neglecting the porfobilinogen determination and also changing the conditions of ALA elution and ALA condensation with acetil-acetone. The method was tested, against the original method, in a group of exposed workers and the results proved very good (the correlation coefficient r = 0.99). Grabecki et al. (VI-7), neglected both the porfobilinogen and the urea removal shortening in this way the procedure time considerably. By a combined method of Mehani (VI-8) ALA is separated from porfobilinogen by the Mauzerall-Granick

method and further analysed by the Schuster method. In this way, in the author's opinion, the advantages of both methods are preserved: there is no interference of porfobilinogen with the reaction with picric acid and the speed of the procedure is considerably increased.

From the methods described the method of Mauzerall-Granick (VI-1) and that of Mehani (VI-8) have been investigated so far.

The modification after Williams and Few (VI-6) of the Mauzerall-Granick method, has been adopted, that is the first 3 ml 0,5N NA acetate passed through the Dowex-50 column during the ALA elution are not discarded because this eluate has proved to contain some ALA as well. The elution is conducted by first adding 4,5 ml and then 5,0 ml 0,5N NA acetate, and then the combined eluates collected in one container.

Measurements of the absorption spectra in the 700-400 nm range for the ALA and DMAB product have shown that for the Beckmann DB-G spectrophotometer the maximum absorption is at 555 nm (Fig.VI-1).

The standardization of the method has been carried out with the known concentrations of ALA x HCl in water and the same concentrations added to the urine. For the 0-100 µg/ml range, and the optical path of 0,5 cm, the curve has proved to follow the Beer-Lambert law (Fig.VI-2).

The Mehani method (VI-8) has been slightly modified. The saturated picric acid solution, originally suggested by the author, has been replaced by 0,3M solution. The optimal amount of picric acid of this concentration for the reaction with ALA is 0,3 ml. As regards the amount and

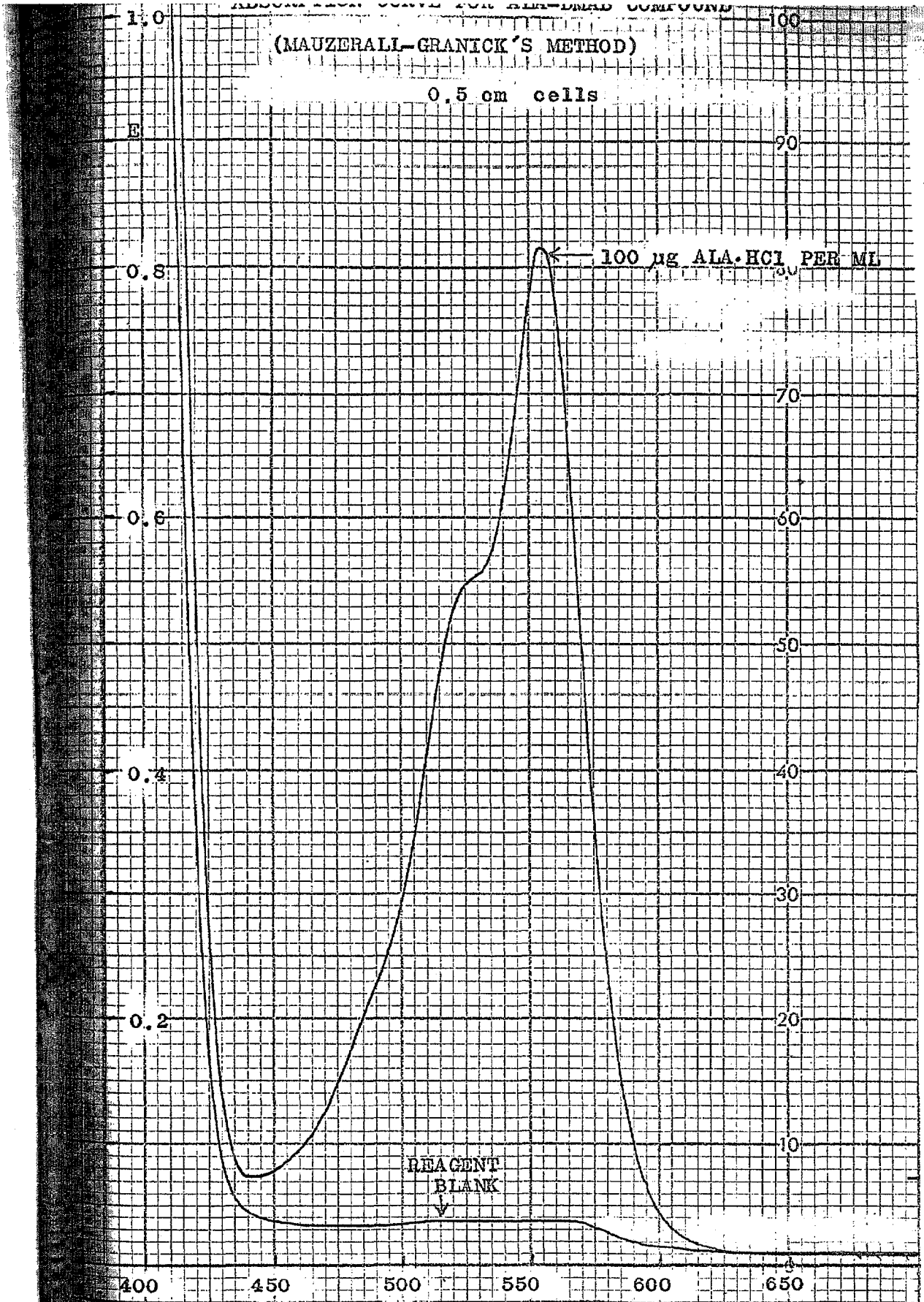

Fig.VI-1

CALIBRATION CURVES FOR DETERMINATION OF ALA

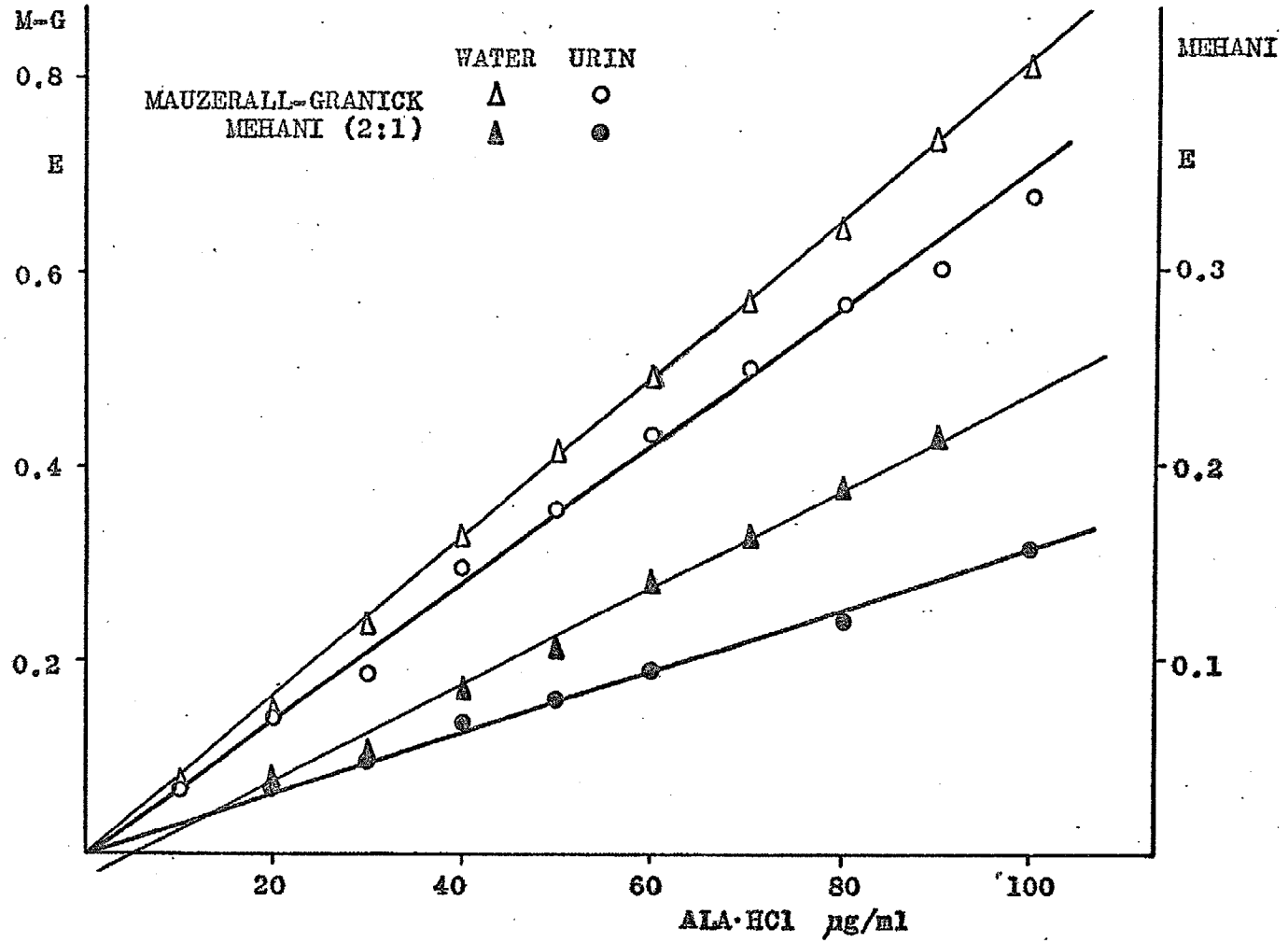

Fig.VI -2

concentration of sodium hydroxide and hydrochloric acid no changes have been introduced except that the shaking time is strictly defined, amounting 5 minutes after addition of sodium hydroxide and 5 minutes after addition of hydrochloric acid.

Measurements of the absorption spectrum for the 700-250 nm wave lenght range have shown that for the absorption measurement of the ALA-Na piorate product the wave lenght range in the vicinity of 500 nm is far more suitable than that in the vicinity of 450 nm (Fig.VI-3) as suggested by the author. Thus, on the basis of a series of analyses, the wave lenght of 495 nm has been selected as the most suitable.

The method is standardized with the known concentrations of ALA x HCl in water and the same concentrations in the urine. The standard curves at the 495 nm wave lenght and 0.5 cm optical path are shown along with the standard curves for the Mauzerall-Granick method (Fig.VI-1). These curves, too, follow the Beer-Lambert law for the 0-100 µg/ml concentration.

The effect of creatinine has been analysed separately. In addition to reacting with ALA, alkaline picrate also reacts with creatinine (VI-9) normally contained in the urine, while the added hydrochloric acid is supposed to remove this interference. By using the known creatinine concentration (100 µg/ml) it has been proved that under the conditions described, the effect of creatinine is completely eliminated.

By summing up the results obtained in the application of Mauzerall-Granick and Mehani methods, it may be concluded

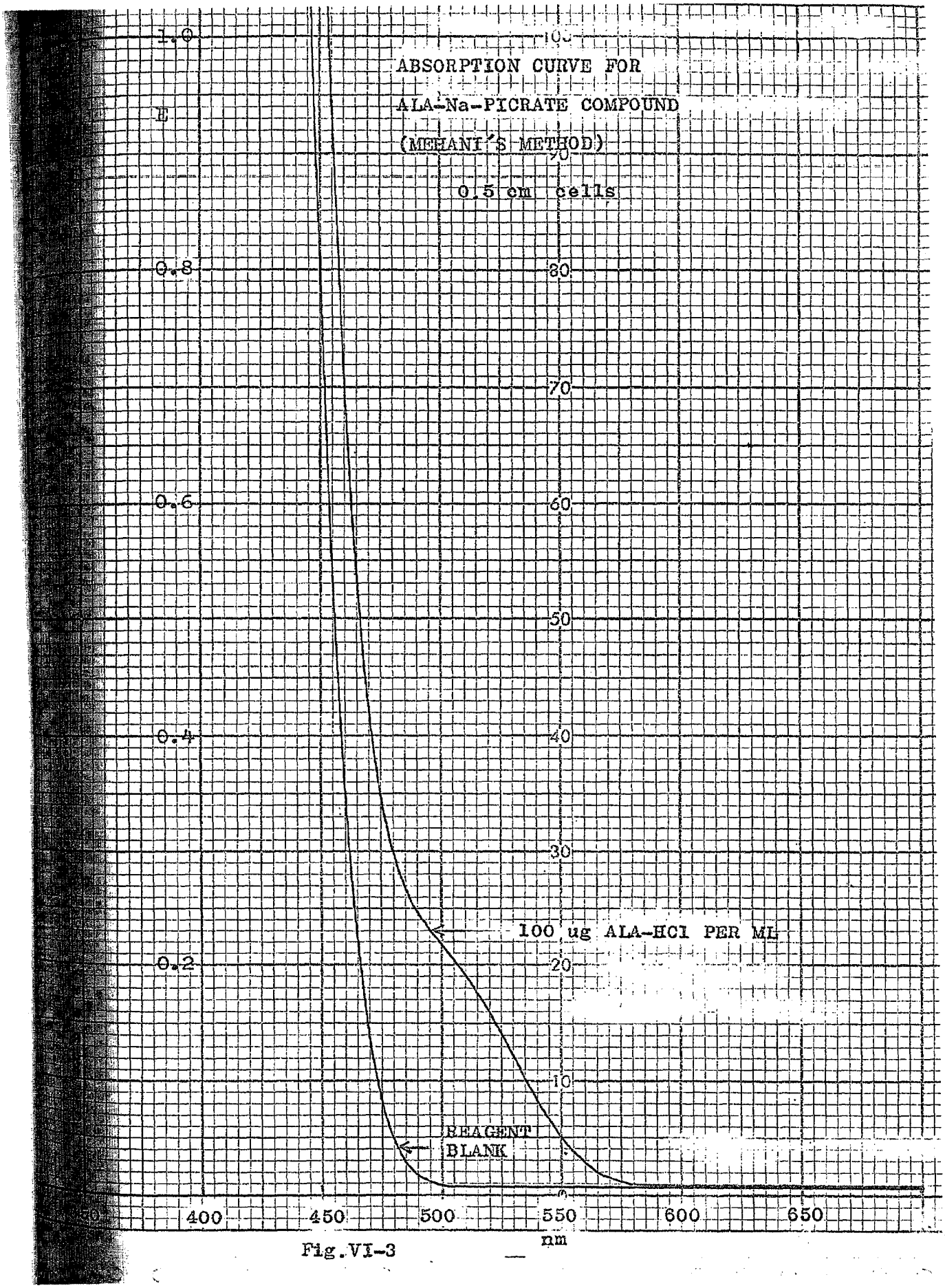

Fig.VI-3

that both methods can be applied for the determination of ALA concentrations in the urine. However, as regards sensitivity, the Mauzerall-Granick method appears preferable. Further investigations should clarify if the modification after Williams-Few, i.e. omission of porfobilinogen removal, could be used, because it would simplify and speed, up the procedure and make it more suitable for the examination of a larger number of subjects.

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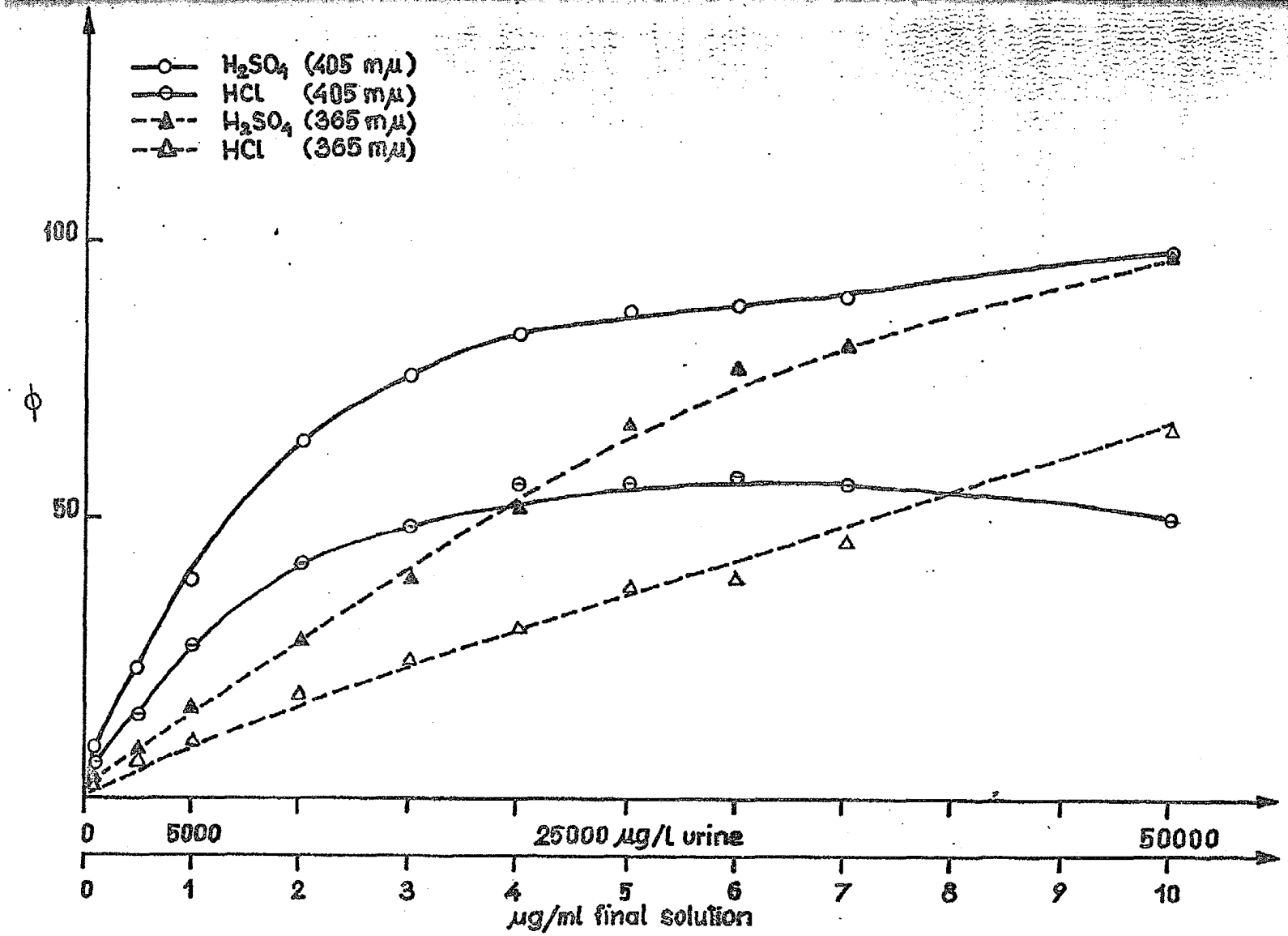
VII DETERMINATION OF COPROPORPHYRIN IN URINE

A modification of the fluorimetric method proposed by Schwartz et al. (VII-1) has been used in our laboratories for the determination of total urinary coproporphyrins for a number of years. In the original method the urine sample is acidified with buffered acetic acid, the coproporphyrin is extracted with ethyl acetate, the extract washed with sodium acetate, the coproporphyrin precursors oxidized with iodine, and the coproporphyrin is finally extracted by several successive portions of hydrochlorid acid. The fluorescence intensity of the last extract is measured and the coproporphyrin concentration read from a calibration curve.

We have shown that chlorine ions in the last extract exhibit a quenching effect on the fluorescence of coproporphyrin lowering the sensitivity of the method and that this can be obviated by subtituting sulphuric for hydrochloric acid (VII-2). The quenching effect is of practical significance only at very high coproporphyrin concentrations, as can be seen from Fig.VII-1 where two fluorimetric calibration curves with coproporphyrin in 1.5 N hydrochloric acid and two in 10% sulphuric acid are presented. They show the dependance of relative fluorescence intensity upon the coproporphyrin concentration. Two curves were obtained using a home-made fluorimeter with the exciting radiation of mainly 365 nm, and two curves using a Farrand fluorimeter with the exciting radiation of mainly 405 nm.

In addition to the afore mentioned quenching effect of chlorine ions on the coproporphyrin fluorescence, the main reason for which we have switched over to the



COPROPORPHYRIN CONCENTRATION

Fig. VII/1

sulphuric acid final extraction in the urine analysis for coproporphyrin is the very strong corrosive effect of hydrogen chloride which evaporates from the 1.5 N hydrochloric acid in the fluorimeter in spite of covered cuvettes.

In the present project urinary analysis of coproporphyrin I and III isomers is planned to be performed in addition to the determination of total urinary coproporphyrin in a part of subjects under investigation. We have chosen the thin-layer chromatographic method described by J. Jensen (VII-3), and P.Koskelo and I.Toivonen (VII-4) for the analysis of coproporphyrin isomers. In this method the extraction of coproporphyrin is performed with ether instead of ethyl acetate and the final extraction with hydrochloric acid. Besides, 60 - 80 ml of urine is needed for the isomer analysis instead of 5 ml only needed for the determination of total coproporphyrin. As we want to use the same urine sample for both the determination of total urinary coproporphyrin and the analysis of isomers, and in order to avoid totally separate analyses for the two purposes, we are trying to modify the methods. Our experiments conducted so far have shown that the substitution of ether for ethyl acetate lowers the sensitivity of the method for the determination of total coproporphyrin. Whether this effect is due to the positive influence of ethyl acetate on the fluorescence intensity of coproporphyrin or to reduced extractibility of coproporphyrin by ether, is not yet clarified. The experiments are being made to check whether sulphuric acid used for the final extraction in our method would interfere with the isomer analysis.

Eriksen has suggested (VII-4) that the oxidation of coproporphyrin precursors with iodine might change the isomer distribution. We have therefore checked the role of iodine in the coproporphyrin analysis and found that iodine, if applied as suggested in the original procedure of Schwartz et al. (VII-1), lowers the results of total coproporphyrin determination. The iodine concentration in the reaction solution ought to be reduced or iodine possibly ommitted if further experiments show that the precursors are quantitatively transformed into coproporphyrin on standing without addition of an oxidant (which assumption is based on some experimental evidence produced in preliminary testing), and particularly if they show at the same time that the oxidant does change the distribution of isomers.

If the isomer analysis is not to be included into the project, the analysis of total urinary coproporphyrin will be made by our present modification of the method proposed by Schwartz et al. further modified as to the oxidation of precursors.

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VIII DETERMINATION OF MERCURY IN BLOOD

The photometric method proposed by Jacobs and co-workers was chosen for the determination of mercury in blood (VIII-1, -2). The method consists of an incomplete digestion of the biological sample, subsequent extraction of mercury with dithizone, decomposition of the dithizone complex by heating to liberate the mercury in the form of vapour, and final determination of mercury by ultraviolet photometry in gaseous phase.

The combustion-ultraviolet photometric equipment consists of a heating chamber connected to a U-tube containing non-absorbent cotton connected in turn to a cold trap, a rotameter, an additional cold trap, a modified Beckman mercury vapour meter Model 23, and a vacuum pump. The mercury vapour meter is modified by replacing the grille with a cylindrical optical cell with removable silica end windows and inlet and outlet ports for the air stream drawn by the vacuum pump.

The heating chamber consists of a Bunsen burner mounted in a Nichrome wire cage in opposite sides of which two openings are out for a Pyrex ignition tube. The ignition tube passes through a wire cylinder fitted between two openings of the heating chamber.

The calibration curve for the estimation of mercury from the readings on the Beckman mercury meter is presented in Fig.VIII-1. The sensitivity of the method obtained was 3×10^{-9} grams of mercury per milliliter of blood.

In table VIII-1 the results are presented of mercury determination in 32 blood samples taken from subjects with no known exposure to mercury.

STANDARD CURVE FOR MERCURY ESTIMATION

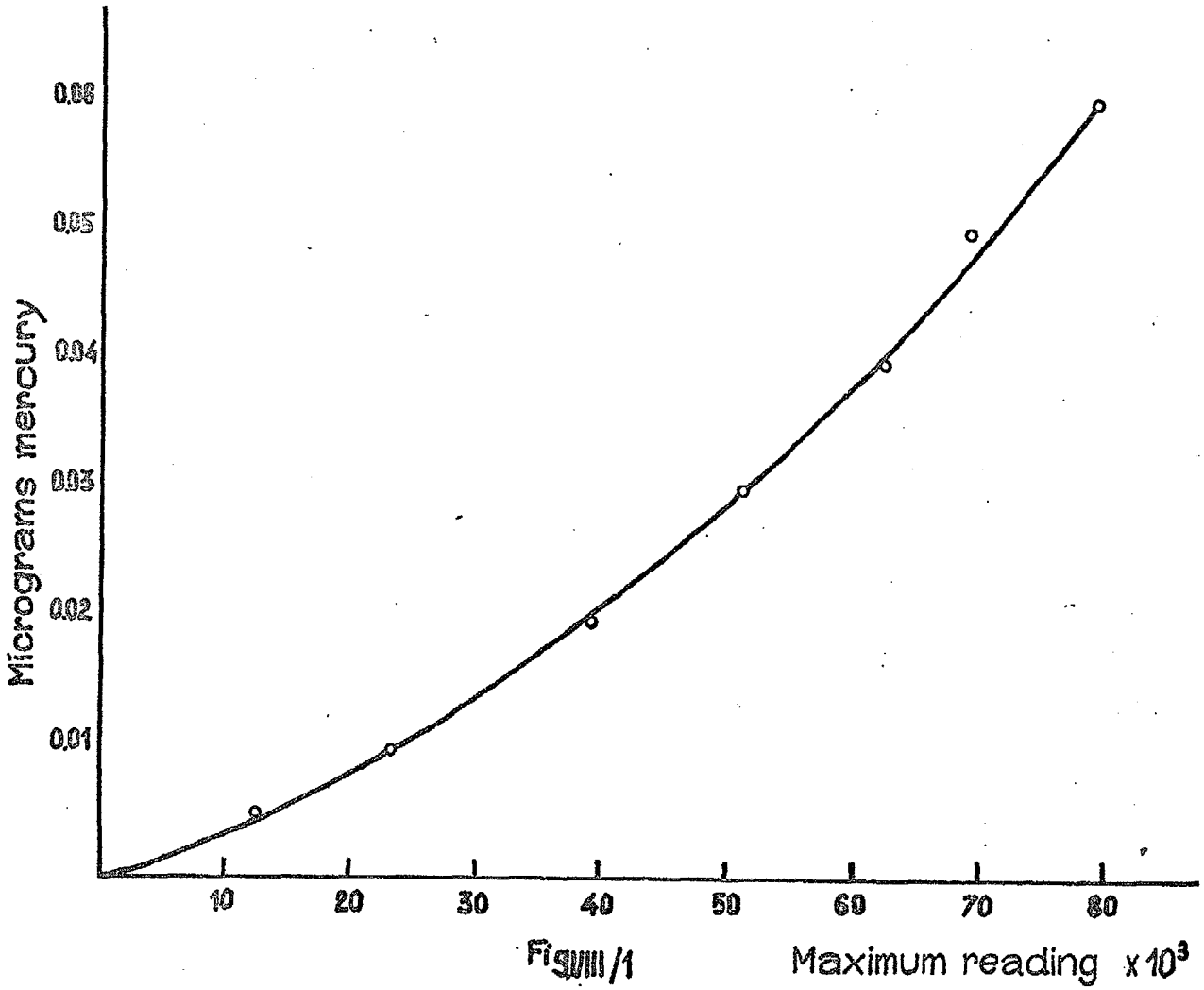

Fig VIII/1

Table VIII-1

Number of sample	Mercury µg/100 ml	Number of sample	Mercury µg/100 ml
1	0.60	17	< 0.30
2	0.30	18	1.65
3	< 0.30	19	0.90
4	< 0.30	20	1.65
5	< 0.30	21	1.80
6	< 0.30	22	2.70
7	0.30	23	6.00
8	< 0.30	24	0.90
9	0.30	25	2.40
10	< 0.30	26	5.10
11	0.60	27	3.60
12	0.75	28	3.78
13	2.10	29	5.40
14	0.60	30	5.10
15	0.90	31	6.00
16	1.20	32	6.00

As can be seen from the table, mercury content of 7 samples (about 22%) was below the analytical sensitivity of the method (0.3 µg per 100 ml).

In order to avoid "zero" results (or rather results indicating the concentrations below 0.3 µg/100 ml) in analyses of "normal" human blood samples two steps have been taken:

I. An attempt has been made to avoid the measurement of the ultraviolet radiation absorption by mercury vapour in a dynamic system i.e. in the current of air, as is

being proposed in the original method. The optical cell proposed to be used in the mercury ultraviolet photometer has been made tight so that vacuum can be created inside the cell. By means of this vacuum the total quantity of mercury is drawn into the cell and the absorbance of mercury is measured in the statical system.

The results obtained thus far do not yet permit a definite conclusion as to the applicability of the modification.

2. The method recently suggested by Magos and Cernik (VIII-3) for rapid estimation of mercury in undigested biological samples is being introduced and checked. The method consists of liberating mercury from sulfhydryl bonds by means of stannous chloride in the presence of cystein, and subsequent measurement of mercury vapour in the same ultraviolet photometer. The checking of the method will be completed within two months.

References

VIII-1 Jacobs,M.B. et al.: Am.Ind.Hyg.Assoc.J., 21 (1960) 475.

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IX DETERMINATION OF LEAD IN AIR

A ring-oven method (IX-1) for the determination of lead as chromate, developed earlier (IX-2), was applied for the analysis of low volume samples of air-borne particulates and verified against atomic absorption spectrophotometric (AAS) determination of lead in parallel high volume (HV) samples. Two wet ashing procedures for the preparation of HV samples for analysis were also compared. An attempt was made to compare just the two analytical methods by analysing the aliquots of the same HV samples by both methods, thus eliminating the influence of sampling efficiency on the results. The relative change in the flow rate during the sampling period was recorded for both sampling sistems and compared. The retention of lead in the low volume sampling system was also verified.

Methods

1. Sampling techniques

1.1 High volume air samples

24-hour samples from about 2000 m^3 of air were collected on 8 x 10 " MSA 1106-B flashed fired glass fiber filters by means of a General Metal Work HV sampler.

1.2 Low volume air samples ("smoke" samples)

24-hour samples from about 2 m^3 of air were collected on 5.5 cm Munktell's No 00 filters, situated in a standard British smoke sampling filter holder of a 1/2" sampling surface by means of a Dymax II A diaphragm pump (IX-3).

The samplers were situated side by side on a roof of a low building.

2. Preparation of samples for analysis

2.1 High volume samples

At first the samples were ashed in a muffle furnace at 500 to 550°C according to the recommonded standard procedure (IX-4). As this procedure proved to cause high losses of lead, it was abandoned and replaced by the "California" acid digestion procedure (IX-5). According to this procedure, a mixture of nitric, sulphuric, and perohloric acid, and deionized water in the ratio 3.79 : 0.5 : 1 : 4.79 was used for digestion. Since we did not use membrane filters, the procedure had to be modified in that the solution had to be separated from the glass fiber filter pulp by filtration through a Whatman No 42 filter paper. As this last step may represent a new possibility of lead loss, another approach to the wet "ashing" of samples was also tried and that is the Soxhlet extraction of samples or aliquots with the redistilled nitric acid, and the two procedures were compared.

2.2 Smoke samples

No special preparation of smoke samples for analysis was necessary. Dissolution, separation, and analysis were performed directly on the filter paper used for sampling.

3. Analysis of air samples

3.1 Analysis of HV samples

3.1.1 Atomic absorption spectrophotometry

The sample extracts (or aliquots) were evaporated to dryness and redissolved in 1% EDTA solution(pH 8) in order to complex lead and thus achieve better atomization. A UNICAM SP 90 instrument was used for the analysis. The concentration of lead was read from the calibration curve obtained with standard lead solutions containing 5 to 50 µg of lead per ml of 1% EDTA. The optimal conditions for measurement were found to be : lamp current 6 mA, wave length 2170 A, slit 0.2 mm, flame height 0.8 cm, and acetylene flow 1.0 l/min.

3.1.2 The ring-oven method

Two 1-cm-diameter disks were cut out of HV samples on glass fiber filters and each disk was fixed face-down with three micro-drops of "UHU" glue (IX-6) and analysed by the chromate method using the ring-oven technique.

3.2 Analysis of smoke samples

Smoke samples were analysed by the chromate method using the ring-oven technique.

AGREEMENT BETWEEN TWO INDEPENDENTLY PROCESSED ALIQUOTS ANALYSED BY ATOMIC ABSORPTION PROCEDURE

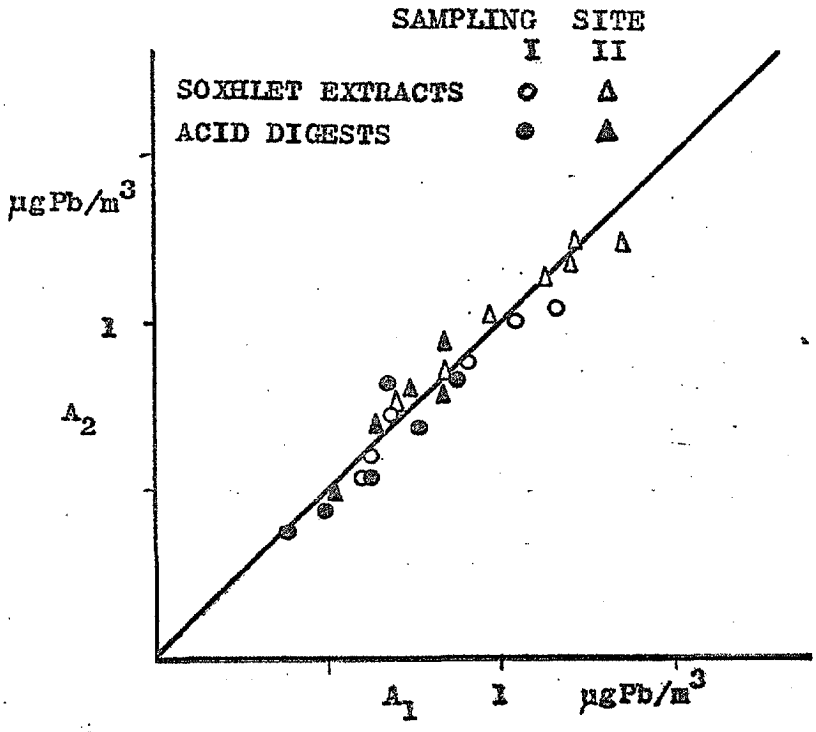

Fig. IX-1

EFFECT OF DIGESTION PROCEDURE ON LEAD RECOVERY
AD=ACID DIGESTION, SOX=SOXHLET EXTRACTION

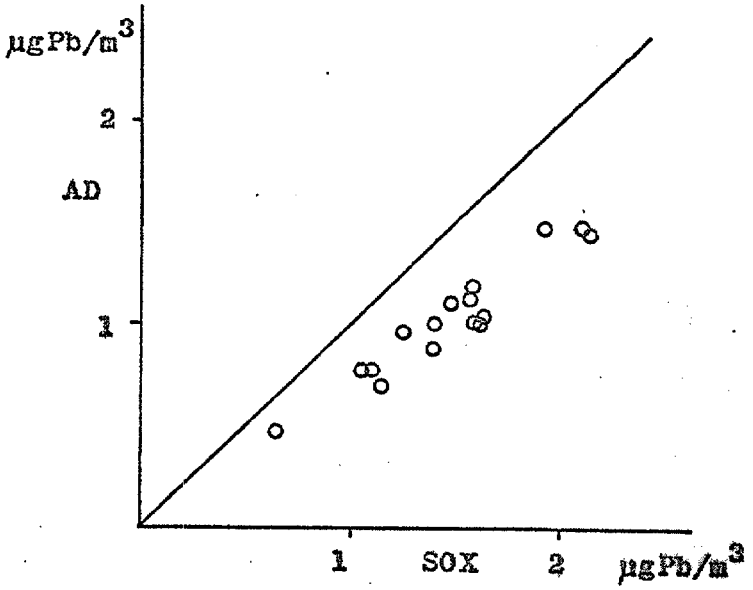

Fig. IX-2

EFFECT OF CONCENTRATION ON RESULTS OBTAINED BY
ATOMIC ABSORPTION SPECTROPHOTOMETRY

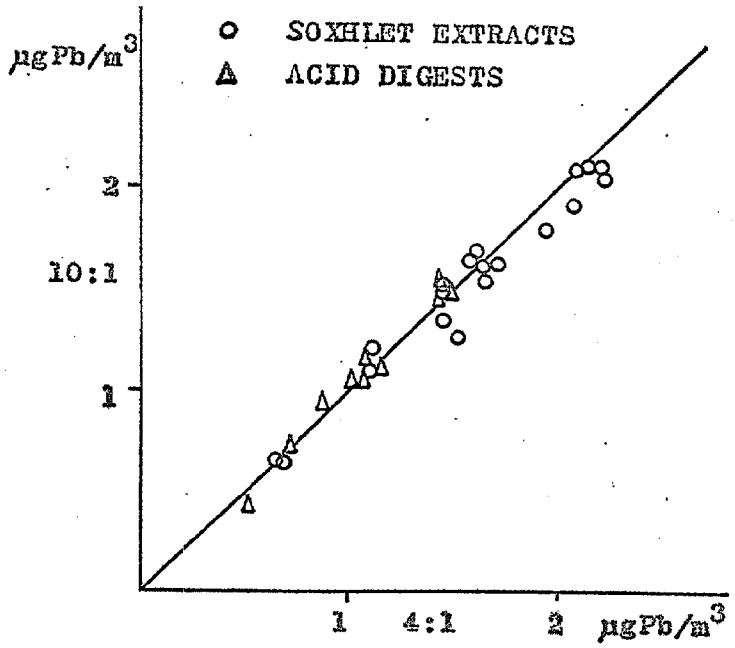

Fig. IX-3

RING-OVEN ANALYSIS
AGREEMENT BETWEEN TWO ALIQUOTS OF A HV SAMPLE

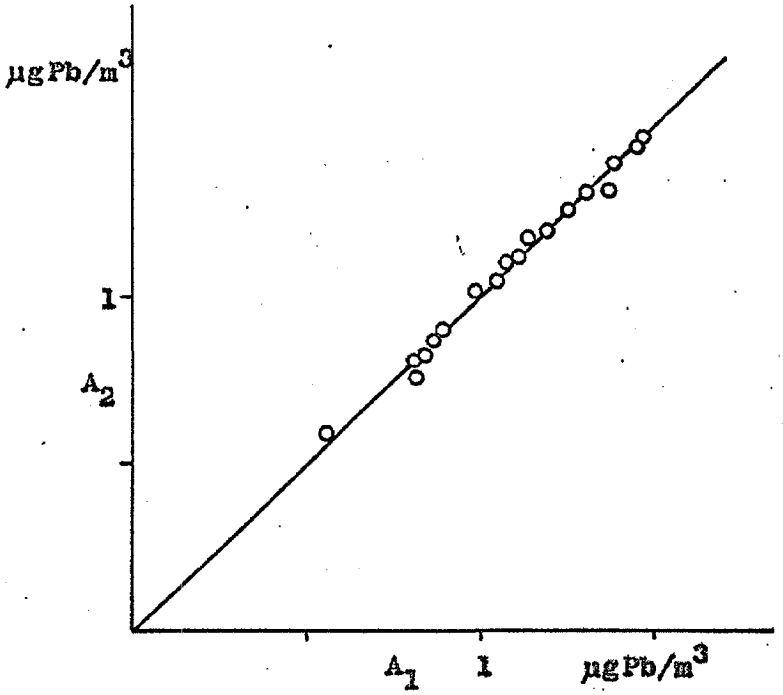

Fig. IX-4

RING-OVEN ANALYSIS
AGREEMENT BETWEEN TWO PARALLEL SMOKE SAMPLES

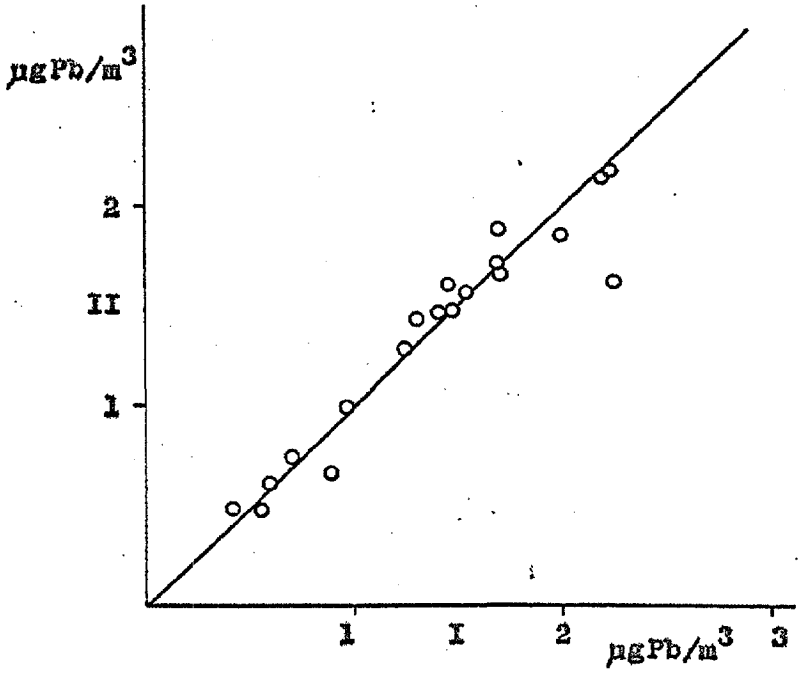

Fig. IX-5

Results and discussion

Digestion procedures

Two pairs of 8 x 10 cm aliquots of the HV samples were processed by both the Soxhlet extraction and the acid digestion and analysed using AAS. There was a very good agreement between the two independently processed aliquots (Fig.IX-1)[x], which shows that all the mentioned procedures yield reproducible results. But, by comparing the results obtained by the Soxhlet extraction and the acid digestion it was shown that the latter was yielding systematically lower results (Fig.IX-2). A blank, run with every three samples, did not indicate any contamination of the Soxhlet extracts. The reason for the difference in the results obtained by the two digestion procedures may be the loss of lead on filter and glass surfaces in the course of the mixed acid digestion procedure.

Analytical methods

In order to check the effect of concentration on the atomizing efficiency of the flame in the AAS analysis, two aliquots of different volumes (50 and 20 ml) were evaporated and redissolved in the same volume of 1% EDTA solution (5 ml), thus yielding final solutions of different concentrations. No indication of salt concentration influencing the signal was observed (Fig.IX-3).

The two HV sample aliquots analysed by the ring-oven method were in excellent agreement (Fig.IX-4), and the two independently collected parallel smoke samples analysed by the same method gave also very well matched results (Fig.IX-5), showing that the ring-oven method yields reproducible results.

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[x] The straight lines on the diagrams represent a theoretical agreement.

RING-OVEN VERSUS ATOMIC ABSORPTION SPECTROPHOTOMETRY
(SOXHLET EXTRACTS)
ALIQUOTS OF THE SAME HV SAMPLES

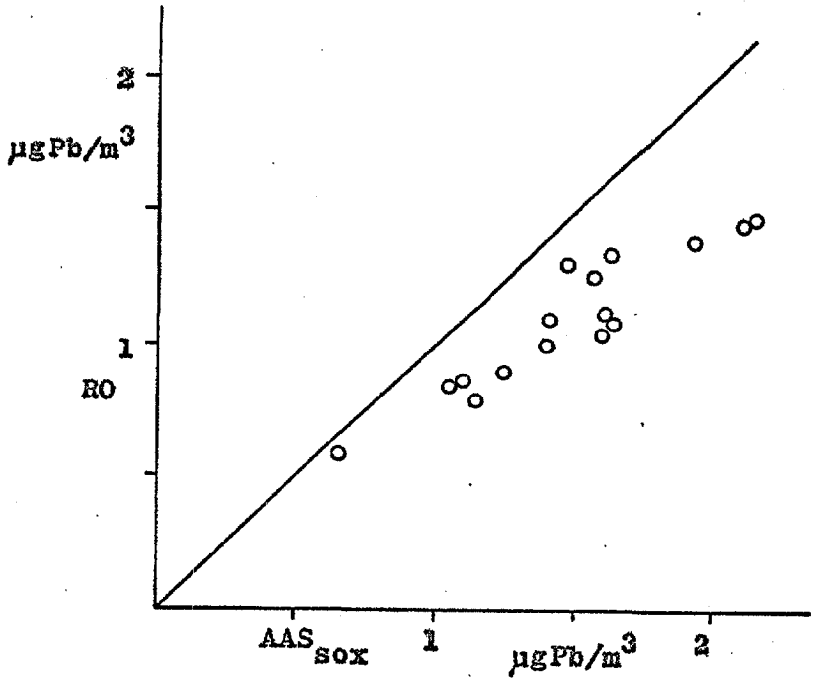

Fig. IX-6

RING-OVEN VERSUS ATOMIC ABSORPTION SPECTROPHOTOMETRY
(ACID DIGESTS)
ALIQUOTS OF THE SAME HV SAMPLES

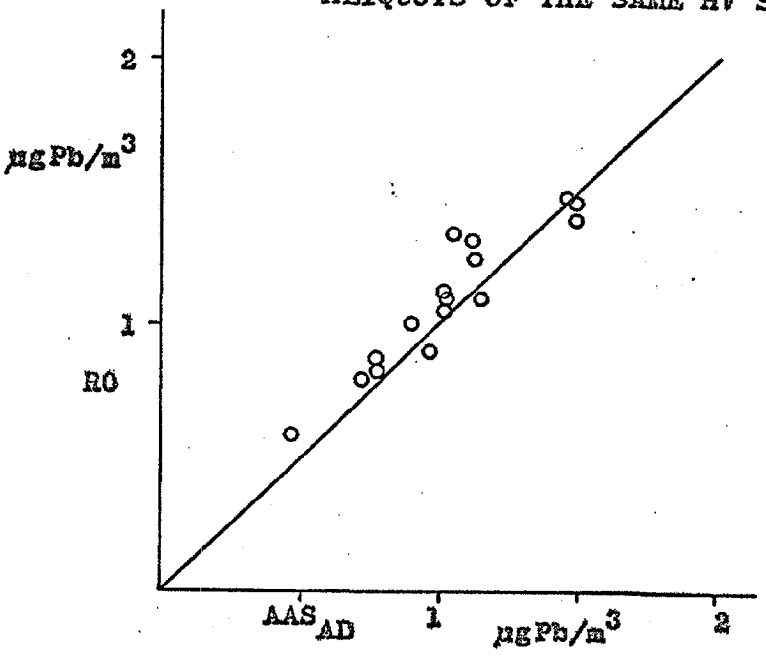

Fig. IX-7

CORRELATION BETWEEN THE RESULTS OBTAINED BY RING-OVEN ANALYSIS OF SMOKE SAMPLES AND ATOMIC ABSORPTION SPECTROPHOTOMETRY OF HIGH VOLUME SAMPLES

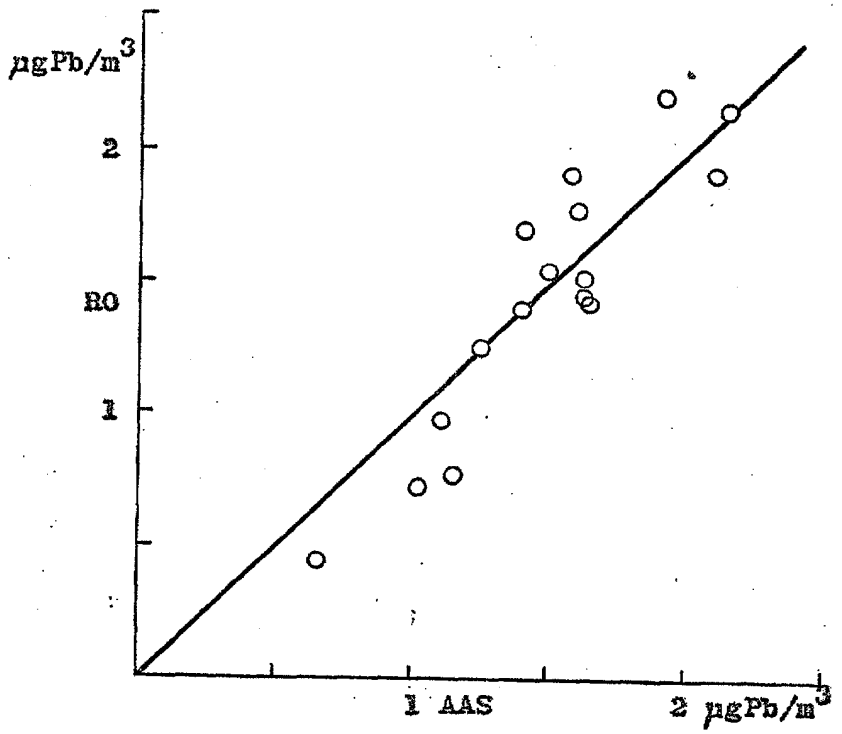

Fig. IX-8

DAY TO DAY FLUCTUATIONS OF LEAD IN AIR OBTAINED BY DIFFERENT PROCEDURES

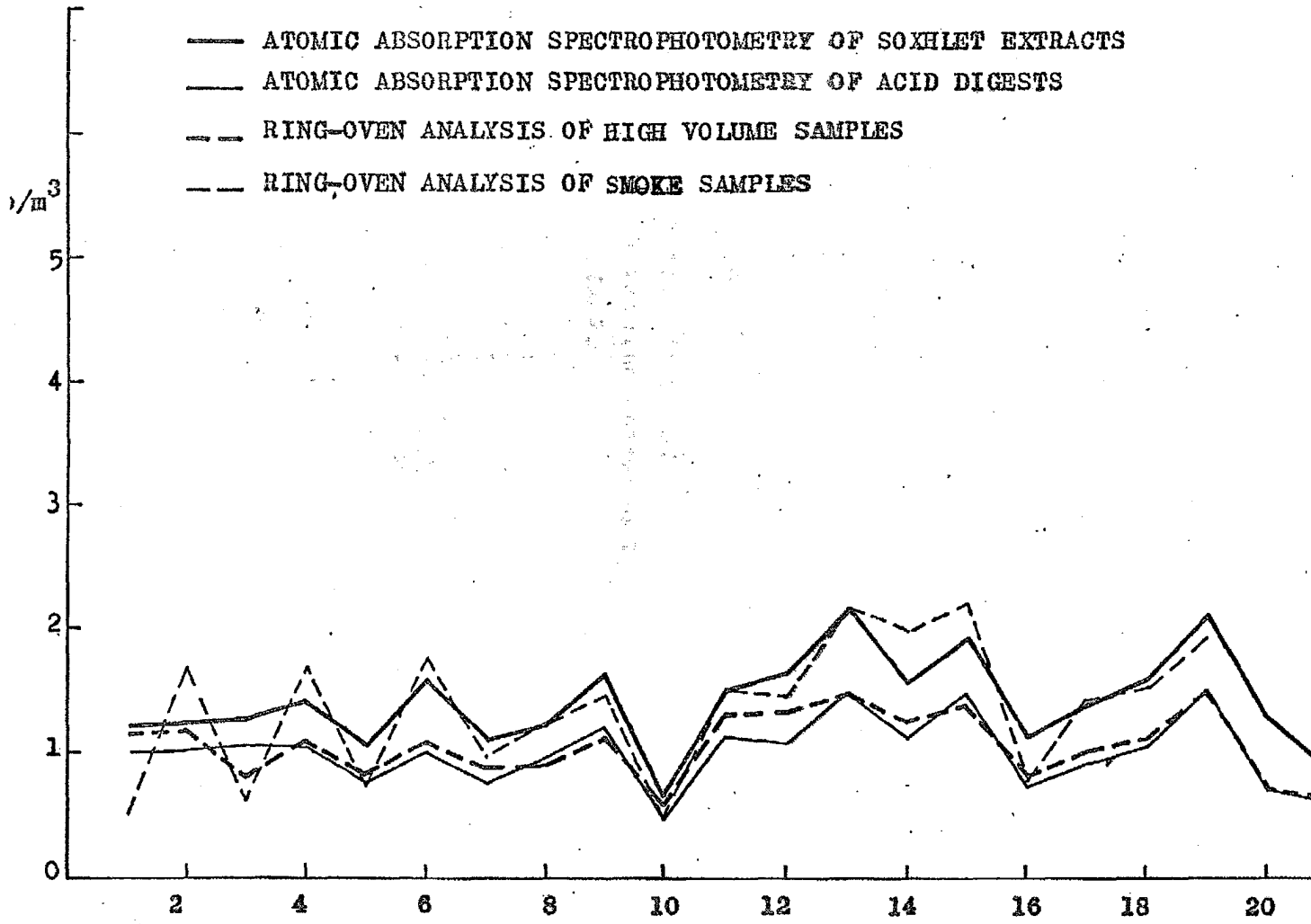

Fig. IX-9

The lead concentration obtained by the ring-oven analysis of the HV samples was always lower than the concentration obtained by the AAS analysis of Soxhlet extracts (Fig.IX-6), but it was in fair agreement with the concentration obtained by the AAS analysis of acid digests (Fig.IX-7). The lower results obtained by the ring-oven analysis of the HV samples are most probably caused by a poor diffusion through glass fiber filter web.

Total procedures

The correlation between the two total procedures, that is between the ring-oven analysis of smoke samples and the AAS analysis of the Soxhlet extracts of HV samples (Fig.IX-8) was very favourable ($r = 0,93$). If the influence of different kinds of filter media and of a different flow rate on sampling efficiency is taken into account (IX-7), as well as a different way of recording air volume and the fact that each of the two analytical methods measures a different quality of lead, a better correlation may hardly be expected.

Day to day variations of the lead concentration according to the results obtained by different procedures are shown in Fig.IX-9.

Reliability of sampling

The sampling efficiency, although being a very critical part of air pollution measurement, is very often neglected. In order to put sampling efficiency under control, the sampling systems were regularly calibrated and checked for leakage. The air flow was measured at the beginning and at the end of each sampling period. A set

of 15 consecutive measurements showing a relative decrease in the flow rate after a 24-hour sampling period is shown in Table IX-1.

Table IX-1

HV sample			Smoke samples			
			I		II	
Total weight of sample mg	Total volume of air m^3	Relative decrease in air flow %	Total volume of air m^3	Relative decrease in air flow %	Total volume of air m^3	Relative decrease in air flow %
147	2043	97.8	3.28	98.8	2.05	98.6
162	1790	92.8	2.87	93.8	1.82	99.4
320	1957	92.1	1.76	80.0	1.93	81.4
292	1920	94.0	2.04	95.9	2.23	98.0
389	1907	90.0	1.77	83.2	1.88	79.2
298	1965	92.0	1.87	89.0	2.00	86.1
283	1964	94.0	1.78	86.1	1.87	83.2
240	1950	86.0	1.87	87.1	2.03	86.2
305	1903	95.8	1.79	89.0	1.91	82.0
329	1870	95.0	1.90	90.0	2.08	88.0
169	1906	92.0	1.83	91.6	1.93	88.0
191	1998	96.0	1.90	88.7	2.04	82.4
245	1964	92.0	1.82	88.1	1.87	76.8
175	1976	98.0	1.78	88.4	1.88	80.0
288	1890	88.0	1.71	85.7	1.85	81.5

The results show that both the low- and the high-volume samplers change the flow rate during the sampling period. Changes in the flow rate were more frequently higher for smoke samples, which means that all the fractions of the day were not equally represented in the 24-hour average of the two samples collected in a different way. The difference was, however, in most cases not too large, although it may have contributed to the scattering of the results obtained by the two procedures.

The efficiency of Munktell's No 00 filters for lead retention was checked by putting a glass fiber filter behind. The experiment was run for 27 days with the same glass fiber filter, while four Munktell's filters were exchanged. The results are shown in Table IX-2.

Table IX-2

		Volume of air m^3	Total amount of lead µg	% of total
I Munktell's No 00	1.	11.3	4.0	21.9
	2.	10.9	4.0	21.9
	3.	10.6	3.5	19.1
	4.	10.8	5.0	27.3
Munktell's 1 - 4.		43.6	16.5	90.1
II Glass fiber filter		43.6	1.8	9.9
Munktell's + glass fiber		43.6	18.3	100.0

The retention efficiency of Munktell's No 00 filter is about 90%. The results reported earlier were not corrected in accordance with this finding.

In further investigations a number of parallel HV and smoke samples are planned to be collected at various sites differing in the quantity and composition of air-borne particulate matter, and analysed by both the AAS and the ring-oven method, for the final evaluation of the latter. Should the ring-oven method prove to be reliable under all conditions, this would allow the determination of lead in air within a large scale survey, since the method is fast and simple, fairly accurate and reproducible, and requireing inexpensive equipment for sampling and analysis.

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X DETERMINATION OF LEAD IN BLOOD

In 1952 a "mono-colour" dithizone method for determination of lead in blood was developed in our laboratory (X-1). The special characteristic of the method was the elimination of iron with cupferron prior to dithizone extraction. The method was used for the determination of normal values of lead in blood in the population of Zagreb and some rural districts, and for the determination of lead in blood of occupationally exposed subjects, and showed to be very reliable.

For each determination two parallel analyses, requiring 5 ml of blood each, have to be run, which means that only for lead determination 10 ml of blood would be necessary from each subject. In the frame of this investigation an attempt was made to modify the method in such a way that smaller volumes of blood could be used for the analysis.

In the modified procedure one or two ml portions of venous blood are put into a Kjeldahl flask and digested with several 3 to 5 ml portions of double distilled nitric acid allowing each portion to evaporate to dryness before the next one is added. After the content of the flask becomes clear, the digestion is continued with two or three portions of hydrogen peroxide. The dry residue is dissolved in double distilled water, transferred into a separating funnel, the iron is precipitated with cupferron (2% water solution) and the iron cupferrate is extracted with carbon tetrachloride. Aqueous layer is again evaporated to dryness with addition of nitric acid in order to destroy the excess of cupferron. The residue is boiled with 10 ml hydroxylamine hydrochloride solution (20 g in 100 ml), 10 ml of

citrate solution are added (20 g per 100 ml), a few drops of ammonia to bring the pH to 10.5, and then 15 ml of a pH 10.5 buffer (2 g of citric acid, 4 g of potassium cyanide, 2 ml of double distilled ammonia and double distilled water up to 100 ml). Lead is extracted with one 5 ml portion followed by a few two ml portions of dithizone solution (0.001% in carbon tetrchloride). The organic phase is collected in another separating funnel and shaken with 40 ml of potassium acetate buffer (pH 4.5 - 5) in order to destroy lead dithizonate and bring lead back into the aqueous phase. The so isolated lead is again extracted with the dithizone solution after the pH of the aqueous phase has been set to 10.5. The lead dithizonate extracts are combined, separated from water by centrifugation, made up to 10 ml with carbon tetrachloride and the absorbance of the solution is measured against blank at 520 mm in 1 cm cells with a Beckman Model DU spectrophotometer.

The procedure has been applied to one and two ml aliquots of a blood sample with addition of various amounts of a lead standard. The results obtained with one ml aliquots were very scattered, but those obtained with two ml aliquots were in favourable agreement with the expected values. In the first part of our field investigations the new modification of the procedure will be verified against the original method and if reliable results are obtained, it will be used in the further investigation.

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