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Note

Determination of physiological levels of Cr(III) in urine by gas chromatography

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The relative importance of chromium is generally associated with its toxicity. Its presence in the environment is a threat to human health, yet, a trace amount of chromium in human blood is essential for normal glucose metabolism. While the determination of chromium in the ppm range is well established, the analysis at the low ppb** level presents serious difficulties, as is obvious from disconcerting disagreement on trace chromium determinations[1]. This is true whether inter- or intralaboratory comparisons are made: Different analytical techniques yield disparate results. Methods employed to determine chromium in biological fluids were described[2] and include neutron activation analysis (NAA)[3,4], atomic absorption (AA)[5–7], coulometry[8], polarography[9], and gas chromatography (GC)[10]. In GC, the chromium is converted to a volatile chelate, usually a fluorinated acetylacetonate. The detection of the chemical species is conveniently done by electron capture[11–15], flame photometry[16,17], or microwave plasma emission[18].

The work presented here was initiated by participation in an interlaboratory comparison of trace determinations of chromium, an inquest decided necessary in a workshop on chromium[19]. The international participants of the workshop acknowledged that before 1970, most of the published values for chromium in blood serum ranged from 20–50 ppb or more. Today the accepted values are typically, but not always, below 5 ppb. While the principal problem in the determination of biological chromium appears to be contamination, some recent interlaboratory comparison studies, as well as an independent observation[20], suggest the loss of volatile chromium. An improved GC method has been developed and compared with instrumental methods.

EXPERIMENTAL

The study started with the investigation of two promising detectors: (a) the flame photometric detector (FPD) and (b) the electron capture detector (ECD). The GC parameters were chosen so as to elute the *cis*- and *trans*-$Cr(tfa)_3$ as a single peak.

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** Throughout this article, the American billion (10^9) is meant.

A series of standard solutions was made from $Cr(tfa)_3$ (Pierce, Rockford, Ill., U.S.A.) in benzene and stored in a refrigerator. Another set of solutions was made *in situ* by reacting varying amounts of $CrCl_3 \cdot 6H_2O$ with the hexane solution of distilled trifluoroacetylacetone (Htfa) under identical conditions as the analysis of the urine samples described below. A ^{63}Ni-ECD equipped with a linearizer (Tracor) was evaluated with the two sets of standards.

Glassware

The reaction tubes (Pasteur pipettes sealed at one end) were boiled in aqua regia for a minimum of 4 h (in a ventilated hood). The vials in which the excess Htfa was reacted with NaOH were boiled in concentrated HCl for at least 4 h. All the volumetric flasks used to prepare the standard solutions were soaked in concentrated HCl overnight. Pipettes for aliquot measurements were acid washed. All glassware was rinsed with distilled deionized water.

Analysis of the urine samples

The "freeze-dried urine standards" distributed to five U.S. and three European laboratories were obtained from the National Bureau of Standards through the National Institute of Occupational Safety and Health. The samples were reconstituted with 1 ml of distilled deionized water. Chromium was present as Cr(III).

The urine samples were buffered with sodium acetate–acetic acid to pH 5.8–6.2. Fifty microliters were measured into the reaction tube using an Eppendorf pipette. A 0.5-ml portion of doubly distilled Htfa (1:100 in hexane) was added. The reaction tube was cooled in ice, then quickly sealed with a flame. Groups of five tubes were wrapped in aluminum foil. After shaking for 15 sec, they were laid on their sides inside a GC oven at 175° for 30 min. The reaction mixtures were allowed to cool to room temperature, then centrifuged for 5 min for better hexane/water separation. Each tube was broken and the hexane phase from it was pipetted into a 5-ml vial containing 0.5 ml of 1.0 *N* NaOH. solution The vials were covered with PTFE-lined caps and shaken vigorously on a mechanical shaker for 2 min to remove the excess Htfa from the hexane phase. The vials were placed in a freezer after a 5-min centrifugation. Once the aqueous layer was frozen, the hexane phase containing the $Cr(tfa)_3$ was decanted into another vial. Reagent blanks were run along with the samples. Aliquots were injected in the gas chromatograph.

RESULTS AND DISCUSSION

The ECD has a detectable amount of 0.1 pg chromium injected as the $Cr(tfa)_3$ chelate. A linear range up to 10 ng was obtained by the use of a linearizer. Fig. 1 shows (a) the chromatograms of 0.1 pg Cr in a standard $Cr(tfa)_3$ solution, (b) 0.4 pg Cr from a $CrCl_3 \cdot 6H_2O$ solution (complexed as $Cr(tfa)_3$) and, (c) a complexed urine sample. The results of the urine analyses show no interfering peaks even at the 3 ppb level samples. The calibration curves in Fig. 2 demonstrate that the yield of $Cr(tfa)_3$ from the buffered $CrCl_3 \cdot 6H_2O$ solution is close to theoretical. The recovery of $CrCl_3 \cdot 6H_2O$ from urine is found to be better than 88%. (It should be noted in this context that a $Cr(tfa)_3$ sample obtained earlier from a different company gave chromatographic responses four times lower. Consequently, preliminary analyses of

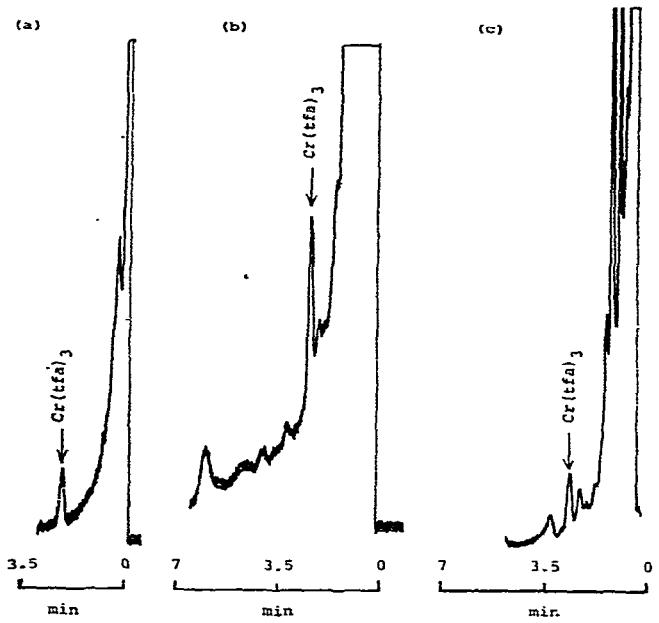

Fig. 1. (a) 0.1 pg Cr from $Cr(tfa)_3$ standard; (b) 0.4 pg Cr as $Cr(tfa)_3$ from $CrCl_3 \cdot 6H_2O$; (c) $Cr(tfa)_3$ from urine. ECD linearizer attenuation ×1 for (a) and (b) and 2 × for (c); column, 6 ft. × 2 mm I.D., 3% OV-17 on Gas-Chrom Q, 100–120 mesh; carrier gas (10% methane–argon) flow-rate, 6.0 ml/min; column temperature, 140°; ECD temperature, 315°.

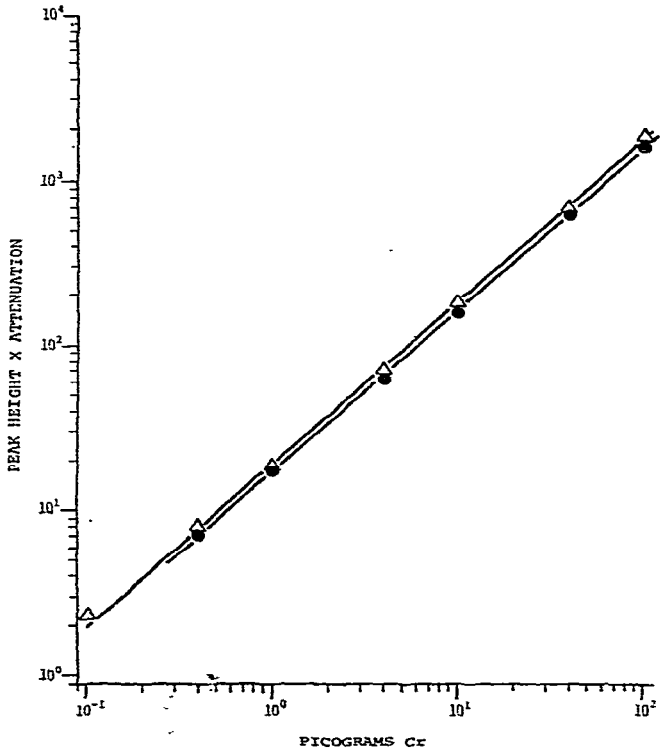

Fig. 2. Calibration curves of $Cr(tfa)_3$ standards and $CrCl_3 \cdot 6H_2O$ complexed as $Cr(tfa)_3$, using ECD linearizer. GC conditions, as in Fig. 1. △, $Cr(tfa)_3$; ●, $CrCl_3 \cdot 6H_2O$ + 3 Htfa.

distilled deionized water spiked with $CrCl_3 \cdot 6H_2O$ showed recoveries much higher than 100%. Further reaction of this $Cr(tfa)_3$ with Htfa improved the response although still not to the appropriate level.)

The GC measurements of chromium in normal and elevated urines are shown in Table I together with the results obtained by different methods and laboratories. Except for three laboratories, the chromium in normal urine is about 3 ppb. The present results, if compared to previously reported values, seem to satisfy the problem of defining the normal level of chromium in biological materials. It appears, therefore, that the normal chromium level in biological materials is, in fact, significantly lower than previously believed. The problem of contamination and/or loss of Cr, whether in sampling, analysis, or both, is evident.

TABLE I

INTERLABORATORY COMPARISON OF CHROMIUM LEVEL IN URINE
NAA = Neutron activation analysis; AA = atomic absorption.

Lab. No.	*Technique*	*No. of replicates*	*Range*	*Mean and standard deviation*
Normal urine				
—	GC–EC	3	2.3 – 3.0	2.7 ± 0.36
1	NAA-Separation	10	2.3 – 3.9	2.9 ± 0.44
1	AA-Furnace	3	2.53– 2.75	2.66 ± 0.12
2	NAA-Separation	3	2.34– 3.42	2.88 ± 0.54
3	AA-Furnace	4	7.40–11.0	9.01 ± 1.68
4	AA-Furnace	4	2.5 – 3.6	3.15 ± 0.47
5	AA-Furnace	5	2.41– 2.78	2.60 ± 0.14
6	AA-Furnace	8	6.5 – 8.7	7.2 ± 0.8
6	AA-Furnace	3	6.0 – 7.4	6.73 ± 0.70
7	NAA-Instrumental	2	10.5 –11.5	10.9
Elevated urine				
—	GC–ECD	4	38.4 –43.0	41.4 ± 2.20
1	NAA-Separation	8	46.0 –55.2	49.5 ± 3.9
1	Flame emission	3	41.0 –51.4	47.0 ± 5.4
1	AA-Furnace (1000°)	3	51 –54	51.7 ± 2.08
1	AA-Furnace (700°)	3	60 –61	60.5 ± 0.58

In the GC determination of chromium (and some other metals), a β-diketone derivative is formed. This technique is by now almost classical. Troubles, however, persist at the picogram range, especially when biological materials are analyzed. Without proper precautions, the magnitude of the reagent blanks can make the analysis impossible. In the present work, the following measures proved necessary: The NaOH extraction mixture must be vigorously shaken to remove traces of Htfa in the hexane solution. Htfa from the supplier must be redistilled to obtain a clean chromatogram. Syringes with metal parts are not recommended for measuring aliquots; rather, Eppendorf pipettes or other non-metallic sampling devices should be used. Samples must be buffered to about pH 6.0 to obtain highest recovery. In flame-sealing the reaction tube, a large area of melted glass consistently gave slightly higher Cr values. This could be due to the exposure of newly created, non-acid washed surface to the reaction mixture, although this was not investigated thoroughly.

Use of quartz reaction tubes is no better, presumably because the high temperature needed to make the seal caused the loss of sample.

If the precautions outlined above are followed, GC of $Cr(tfa)_3$ can be used to determine not only elevated levels arising from occupational exposure, but also the extremely low, normal levels of Cr in human urine.

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