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Specific determination of benzene in urine using dynamic headspace and mass-selective detection

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Abstract

A method for the determination of benzene in urine was developed, based on dynamic headspace and preconcentration of the analyte on a solid sorbent. The subsequent analysis by thermal desorption of the sorbent, capillary gas chromatography and mass-selective detection ascertained a low limit of detection (6.5 ng/l) and a highly specific determination. The limit of detection is an order of magnitude lower than that reported earlier and allows reliable quantitation of occupational exposure and of most environmental exposures. Samples could be stored frozen for at least a month without significant loss. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Dynamic headspace; Benzene

1. Introduction

Benzene is myelotoxic and causes leukemia in humans. As a constituent of petrol, it is probably the most widely spread compound of those classified as group I carcinogens by the International Agency for Research on Cancer [1]. Among the highest occupationally exposed workers are those involved in the production, distribution and handling of petrol [2]. Moreover, most people in the industrialised world are exposed daily to benzene from vaporised petrol and exhaust gases. Tobacco smoke is also an important source of benzene exposure. Thus, there is a need for reliable and sensitive methods for the assessment of both occupational and environmental exposure.

Biological monitoring has the advantage of reflecting the total uptake in the body, regardless of administration route. This is important since dermal absorption may contribute considerably to the total exposure [3]. Biological monitoring based on analysis of urine samples has the advantage of using a non-invasive sampling procedure. Benzene is metabolised in a complex way, resulting in a number of metabolites, several of which have been proposed as biomarkers for benzene exposure [4,5]. Unmetabolised benzene is also excreted in urine, and since no physiological source of benzene has yet been identified, it is a fully specific biomarker. Furthermore, good correlation between the benzene concentration in urine and the level of air exposure has been shown

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even at low exposure levels [6,7]. One of the few disadvantages is that less than 0.1% of the total uptake of benzene is excreted unmetabolised in urine [8]. This puts high demands on the sensitivity, detection limit and selectivity of the analytical system.

In our laboratory, we have previously used the concept outlined by Ghittori et al. to determine benzene in urine [9]. After dynamic headspace, the benzene was preconcentrated on a solid adsorbent (Tenax®), followed by thermal desorption, gas chromatographic separation and flame ionisation detection (FID). However, although we used a 60-m dimethylpolysiloxane phase capillary column, at low benzene concentrations, we sometimes had problems with interfering compounds. The aim of this study was to achieve higher selectivity and a lower detection limit by changing to mass selective detection and by modifying the headspace procedure. This would facilitate analysis of urine from occupationally and environmentally exposed subjects.

2. Experimental

2.1. Principle

The determination of benzene in urine is here based on dynamic headspace and preconcentration on a solid sorbent of the analyte, which is subsequently thermally desorbed and analysed by capillary gas chromatography and mass selective detection.

2.2. Equipment

2.2.1. Headspace equipment

The dynamic headspace equipment (Fig. 1) consisted of a 150-ml Erlenmeyer flask, with a Teflon®/silicon-septum sealed cap penetrated by two 2-mm O.D. steel tubing. The flask was placed on a magnetic stirrer device. Two drying devices were connected between the headspace vessel and the adsorbent tube used for collection of the benzene vapours. The first consisted of a permeable membrane dryer (Permapure MD-125-12T, Perma Pure Products, Toms River, NJ, USA), with a tubular Naphion® membrane, which is a copolymer of

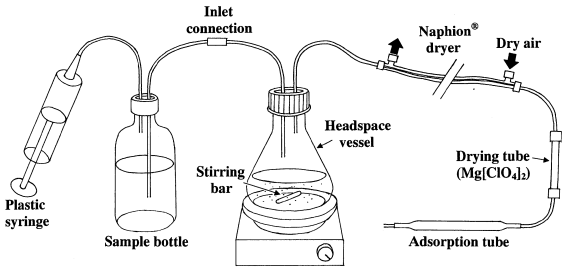

Fig. 1. Headspace equipment. The figure shows the transfer of urine from a sample bottle to the headspace vessel. During the headspace procedure, the gas supply is connected at the inlet connection.

tetrafluoroethylene and perfluoro-3,6-dioxa-4-methyl-7-octene-sulphonic acid. Water molecules in the headspace gas hydrate the sulphonic acid and are transported through the membrane along the humidity gradient to the dry gas outside the membrane. Nonpolar substances like benzene are retained quantitatively inside the tubing. The second dryer was a glass tube filled with about 600 mg of magnesium perchlorate-hydrate, which does not adsorb nonpolar hydrocarbons. The gas flows were controlled by flow meters. The adsorbent tube was made of glass and filled with about 900 mg of 2,6-diphenyl-*p*-phenyloxide (Tenax® TA 60–80 mesh). The cartridge was sealed with glass PTFE coaxial valves [10].

2.2.2. Thermal desorption and GC–MS

A custom-made thermal desorption unit was used for desorption of benzene from the adsorbent tube [11]. The adsorbent tube containing collected benzene was inserted in a tubular oven, and the analyte was cryo-focused on glass beads in glass-lined tubing (GLT) held at −196°C (Fig. 2a). After desorption was completed, the temperature was raised, and the analyte was transferred to a Varian 3400 gas chromatograph (Walnut Creek, CA, USA) via GLT tubing without splitting the sample (Fig. 2b). The column was a 5% phenyl 95% dimethylpolysiloxane phase capillary column (CP-Sil-8 CB-MS, 60 m×0.32 mm I.D., phase thickness, 1 μm) from Chrompack (Bergen op Zoom, The Netherlands). Helium was used as a carrier gas, and the inlet pressure was 0.11 MPa. A Finnigan MAT

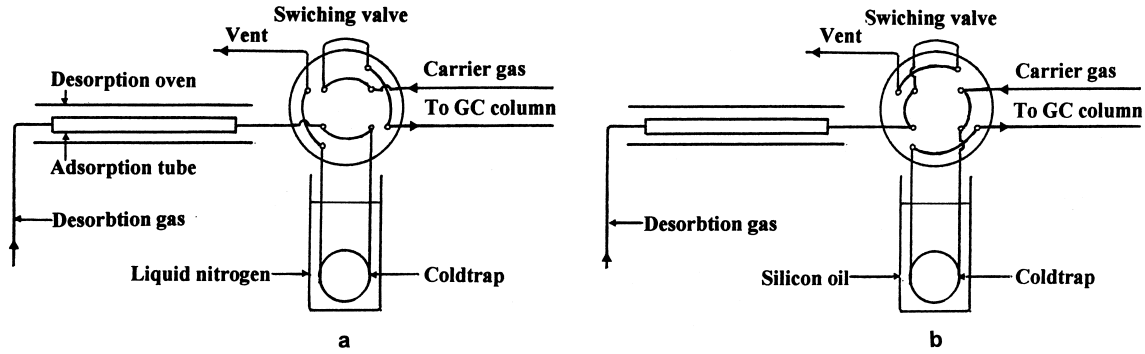

Fig. 2. Thermal desorption unit. (a) shows the desorption of the adsorption tube and the subsequent cryo-focusing on the coldtrap. The coldtrap is submerged in liquid nitrogen (−196°C). (b) shows the injection into the GC system by raising the temperature of the coldtrap to 150°C.

ion trap detector Model 700 (San José, CA, USA) mass spectrometer was used as a detector.

2.3. Chemicals

Benzene (p.a.), anhydrous sodium sulphate (p.a.) and magnesium perchlorate hydrate (p.a.) were purchased from Merck (Darmstadt, Germany), and methanol (HPLC grade) was purchased from Rathburn (Walkerburn, UK). Tenax® TA (60–80 mesh) from Chrompack was used as an adsorbent. Reference substances, 2-methylbutylaldehyde, 3-methyl-2-butanol and 3-methyl-2-butanone, were purchased from Fluka (Buchs, Switzerland), and *n*-butanol was purchased from Merck. Nitrogen, purchased from AGA (Göteborg, Sweden), was further purified by a cartridge filled with a molecular sieve and activated charcoal.

2.4. Procedures

2.4.1. Sample collection and storage

Urine samples were collected in 250-ml polyethylene bottles and then immediately transferred to 125-ml glass bottles (referred to below as sample bottles) sealed with Teflon®/silicon-septa (Supelco, Bellefonte, PA, USA). The bottles were kept in a refrigerator (+4°C) if they were analysed within a few days or frozen (−20°C) if stored over longer periods. The volume of urine in the 125-ml sample bottles normally was about 110 ml, which permitted duplicate analyses of two 50 ml aliquots. Before analysis, the sample bottles were brought to ambient temperature.

2.4.2. Preparation of standards

Urine standards were prepared by spiking urine from non-smoking persons with methanol solutions of benzene using a Hamilton syringe. The methanol solutions were prepared by diluting a weighed amount of benzene in methanol and then further diluting it to the appropriate concentrations. These solutions were injected into the 125-ml sample bottles containing 110 ml of urine through a Teflon®/silicon-septa. The spiking concentrations ranged from 20 to 4000 ng/l. The urine used for the standard preparation was analysed before spiking, and the calibration curve was corrected for the benzene content in this urine.

The recovery of benzene was investigated by comparing detector responses of spiked urine samples with those obtained from tubes spiked with gaseous standards. The calibration gas was prepared in a 1-l gas cylinder made of stainless steel. Benzene diluted in methanol was put into a glass tube, which was sealed and weighed by difference on an analytical balance. The glass tube was then put into the cylinder, which was pressurised with nitrogen and the weight of the diluent gas was determined with a toploading balance [12]. The benzene concentration prepared in this experiment was about 250 $\mu g/m^3$. Known volumes of the calibration gas were passed through the adsorbent tubes, and afterwards, the tubes were purged with 100 ml of nitrogen. The

preparation procedure has previously been verified by comparing tubes with benzene from the calibration gas with a certified reference material for benzene on Tenax® (CRM 112, Community Bureau of Reference, Brussels, Belgium) [10]. To check the concentration of the calibration gas used in the recovery experiment, a comparison was made between adsorption tubes spiked with a known volume of calibration gas and adsorption tubes spiked with liquid standard. The difference between benzene values obtained from gas or liquid standards was less than 5%.

2.4.3. Sample work-up

After addition of 20 g of anhydrous sodium sulphate and a magnetic stir bar to the headspace vessel, 50 ml of urine was transferred from the sample bottle as shown in Fig. 1. Using a plastic syringe, 50 ml of air was injected slowly into the headspace of the sample bottle, displacing the same volume of urine through a second needle connected to the headspace vessel via PTFE tubing. The adsorbent tube was already connected, and the magnetic stirrer stirred the transferred sample. Sodium sulphate was added to give a salting-out effect, thus facilitating the release of benzene from the urine solution. The mean volume transferred in this way (determined from weighing water) was determined to be 50.1 ml (RSD 0.01, $n=3$). After transfer of the sample, the inlet tubing was connected (at inlet connection in Fig. 1) to a gas cylinder filled with nitrogen, purified as described above. Nitrogen was swept over the urine surface at a flow-rate of 200 ml/min for 15 min. Table 1 shows that this time period is sufficient. The water-saturated headspace gas containing benzene vapours was dried in two steps before passing the collecting adsorbent. These drying procedures were not sufficient for the subsequent GC–MS determination of benzene. To decrease the effect of water on the MS response, the adsorbent tube, after completed headspace work-up, was purged with dry, purified nitrogen for 10 min at a flow-rate of 200 ml/min. The whole sample work-up was performed at room temperature.

2.4.4. Gas chromatographic determination

The adsorption tube was desorbed at 160°C for 8 min with helium at a flow-rate of 20 ml/min. After desorption was completed, the temperature of the trapping device was raised to 150°C, and the analyte was transferred to the gas chromatograph. The initial temperature of 50°C for the GC oven was maintained for 3 min, raised to 80°C at 2.5°C/min, then raised to 200°C at 20°C/min and maintained there for 3 min. The ion trap was operated in the full-scan mode with a scan rate of 1 scan/s over a mass range of 40 to 160 m/z. The detector temperature was 225°C.

3. Results and discussion

3.1. Sample collection and storage

Losses during collection and the initial transfer from the polyethylene bottles to the sample bottles were investigated as follows. From a batch of spiked urine (benzene concentration 304 ng/l, SD 23 ng/l, $n=2$), a volume of 175 ml was poured into each of twelve 250-ml polyethylene bottles. The contents of six of the polyethylene bottles were immediately transferred to sample bottles. The remaining six plastic bottles were stored at ambient temperature

Table 1
Transfer of benzene during the dynamic headspace procedure[a,b]

Desorption period (min)	Detector response (area units)	Totally transferred benzene (%)
0–5	67862	87.1
5–10	8805	11.3
10–15	1279	1.6
15–20	nd[c]	–

[a] Benzene was transferred from a spiked urine sample (1530 ng/l) and collected on adsorbent tubes.
[b] During the experiment, the tubes were changed every 5 min.
[c] nd=Not detectable (less than 100 area units).

and also transported in a car for about half an hour. After 1.5 h, the contents were transferred to sample bottles. The analysis of all samples was made on the next day and quantified using a calibration curve based on spiked urine samples. The loss during the direct transfer to sample bottles, imitating the collection procedure, was found to be 6% compared to the original spiking concentration. Storage and transportation of the polyethylene bottles at ambient temperature for 1.5 h increased the loss to 12%. This is in agreement with a calculated loss of about 10% to 13% to the headspace of the polyethylene bottles, based on reported water/air partition coefficients [13,14]. The experiment showed that the loss is reasonably low if the transfer to the sample bottles is made quickly after the voiding. In the field situation, however, the voided urine is at body temperature, which might make the initial losses somewhat higher than those obtained at ambient temperature. The previously developed analytical system with flame ionisation detection [10] was used in this experiment as well as in the stability test experiment below.

Sample stability was investigated by filling 18 sample bottles with urine spiked with benzene to 875 ng/l. Six bottles were analysed immediately, six after being stored for three days at about +4°C and another six after being stored for one month at −20°C. Compared to the unstored bottles, the recoveries were 99% and 98%, respectively.

3.2. The dynamic headspace benzene transfer step

In a study of the transfer process of benzene from the urine sample to the collecting adsorption tube, tubes were changed every fifth minute. This experiment was conducted on our original analytical system with a flame ionisation detector. Results are shown in Table 1. After 10 min, 98% of the totally transferred amount of benzene was collected on the adsorption tubes, and after 15 min, the transfer was considered to be complete.

The possibility of breakthrough on the adsorption tube, which would lead to underestimation of the benzene concentrations, was investigated by connecting two tubes in a series and performing the dynamic headspace procedure. No significant breakthrough could be observed at urinary benzene concentrations ranging from 20 to 4000 ng/l.

3.3. GC determination

Before we had introduced the third drying step (purging the adsorbent tube with nitrogen), the response of the mass selective detector decreased during the analysis of urine samples. The response of a gaseous standard was about 50% of the expected, if it was analysed immediately after one urine sample and even lower if consecutive urine samples were analysed. After using the dry carrier gas, the response slowly recovered. Accordingly, we suspected that the decrease was caused by traces of water contaminating the detector. After including the additional drying step in the analytical procedure, the response was stable. To investigate possible losses during this additional drying step, three tubes spiked with 6.4 ng benzene (corresponding to a urinary concentration of 128 ng/l) were purged with helium for 10 min, and the results were compared with tubes ($n=3$) that were not purged. The difference was small and not statistically significant.

The thermal desorption of benzene from the adsorbent was almost complete (>99%) in 5 min, but the desorption time was extended to 8 min to ensure complete desorption and to decrease the risk of carryover. The second desorption step, transfer of the analyte from the GLT tubing to the analytical column, was completed within the 3 min the column was held at a low temperature (50°C) before the temperature programme was started. This is illustrated by the narrow peak width in Fig. 3, showing a chromatogram obtained from a urine sample of a non-smoking individual with no occupational exposure.

As shown in Fig. 3, benzene coelutes with other volatile compounds from urine. The major interferents were identified as 3-methyl-2-butanone, 1-butanol, 2-methylbutylaldehyde and 3-methyl-2-butanol, all known to be normal human metabolites [15]. The identification was confirmed by comparing MS spectra from urine samples with spectra from pure compounds. A 5% phenyl 95% dimethylpolysiloxane stationary phase was used, which is slightly more polar than the commonly used dimethylpolysiloxane phases. However, according to our experience, the interferents are the same, but the eluting order on the dimethylpolysiloxane phase is different. Headspace techniques generally decrease

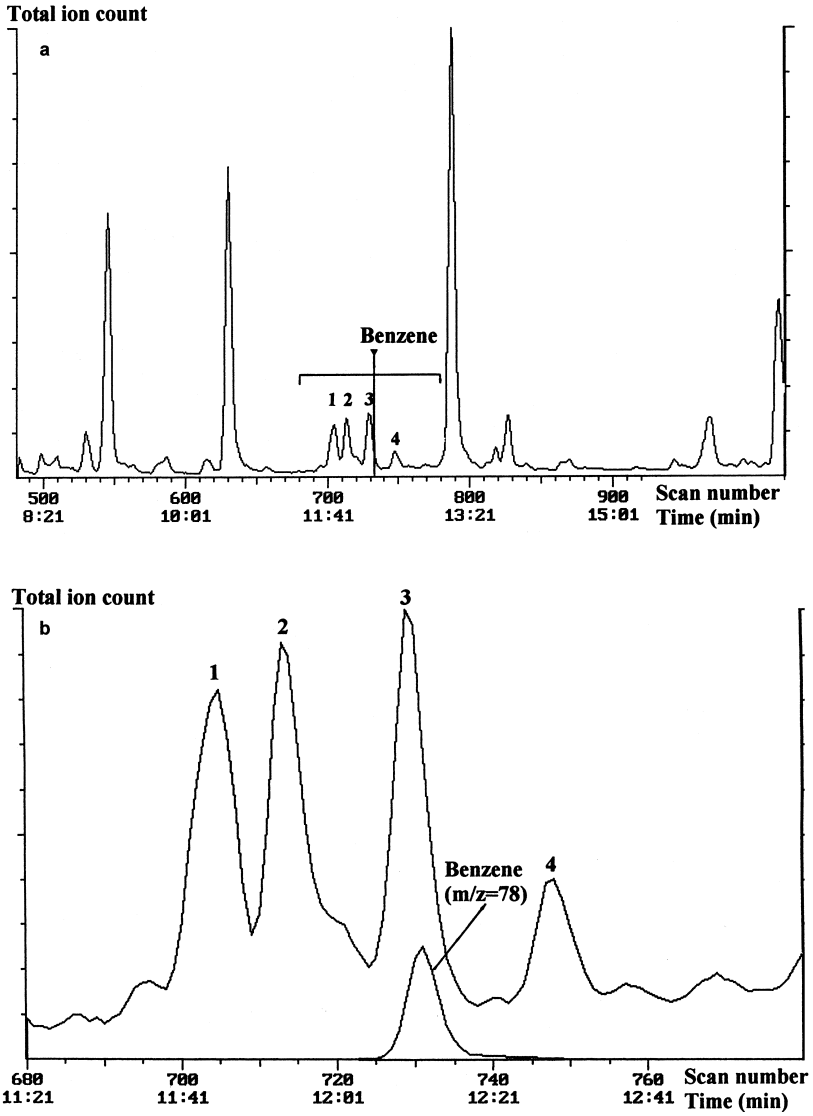

Fig. 3. Chromatogram from the analysis of urine from a non-occupationally exposed, non-smoking subject. (a) shows the total ionisation chromatogram. The bracket shows the region where benzene elutes, and the arrow indicates the retention time for benzene. In (b), this region is magnified. The main interferents are (1) 3-methyl-2-butanone, (2) 1-butanol, (3) 2-methylbutylaldehyde and (4) 3-methyl-2-butanone. Below the total ionisation chromatogram, the $m/z=78$ peak (magnified 8 times compared to the other peaks) is shown. The benzene concentration is about 35 ng/l. The signal-to-noise ratio for the $m/z=78$ peak is 370.

the interference from a biological matrix, but there are obviously still problems with evaporation of other volatile compounds from urine samples. These problems are more pronounced as the concentration of the analyte decreases. It has been suggested that the number of interferents will increase 6 to 10 times per magnitude of decrease of the detection limit in biological systems [16]. It should be noticed that the use of a photoionisation detector instead of an MS detector would not solve the specificity problem,

although this detector has some selectivity towards aromatic compounds like benzene. All identified interferents are ionised by the 10.2-eV lamp commonly used, and ketones are even ionised by a 9.5-eV lamp [17].

The combination of the high separation efficiency obtained with a capillary GC column and the selectivity in MS detection using a suitable mass fragment offers a specific determination of benzene. The dominating mass fragment of benzene is the molecular peak with a m/z ratio of 78. None of the identified interferents, including the most critical interferent, 2-methylbutylaldehyde, produced fragments with this ratio.

3.4. Quantitation

Linearity was assessed by duplicate analyses of 8 spiked urine standards ($n=16$) in the range of 20 to 4000 ng/l. The linearity was good in the concentration range studied, with a slope of 4970 (SD 106), an intercept of 224 000 (SD 168 900) and a correlation coefficient of 0.993. The 95% confidence interval of the intercept included the origin. Repeatability was calculated from the pooled RSD of these duplicate analyses [18]. At lower concentration levels (20–200 ng/l) the RSD was 0.082 and at higher levels (500–4000 ng/l), 0.059. This is in agreement with earlier reports [9,19].

The recovery of benzene was investigated in the concentration range of 20–4000 ng/l by comparing detector responses of spiked urine samples with those obtained from tubes spiked with gaseous standards. Results are shown in Table 2. The average recovery (83%) is in agreement with the results of Ghittori et al., who found recoveries in the range of 80% to 84% [9]. The lower recoveries for concentrations above 500 ng/l may indicate poor linearity of the detector. Another explanation could be that the sweep gas flow-rate was too low during the headspace procedure at these high concentrations, resulting in a decreased transfer rate. Breakthrough is a less plausible explanation according to the results obtained from the breakthrough experiments.

Limit of detection (LOD) and limit of quantification (LOQ) were determined according to a method proposed by NIOSH [18]. The method is based on spiked samples below and above the expected LOD and includes both the sample preparation (dynamic headspace) and the GC analysis. According to this procedure, LOD was determined to be 6.5 ng/l and LOQ to be 22 ng/l. The LOD of the instrumental analysis alone was at least an order of magnitude lower. This shows that the sample preparation is the major source of variability, as is often the case in trace analysis.

Table 2
Recoveries of benzene from spiked urine samples[a]

Concentration (ng/l)	Recovery (%)		
	Sample 1	Sample 2	Mean
20	61	69	65
50	90	74	82
99	89	89	89
198	92	92	92
496	106	99	103
1002	83	76	79
1876	74	66	70
4096	79	74	76

[a] The recoveries of benzene were compared to gaseous benzene standards collected on the adsorbent tube and then analysed in the same way that the urine samples were. The urine analyses were performed in duplicate.

The most common analytical approach for determining benzene in urine is either static or dynamic headspace in combination with capillary gas chromatography with FID or PID detection [9,19–21]. Using static headspace, the injected gas volume is restricted to a fraction of a millilitre, if capillary columns are used, which makes it difficult to obtain a low detection limit. Dynamic headspace has the advantage that the analyte can be preconcentrated from a large sample. The detection limits reported [9,19] have been in the range of 40 to 50 ng/l. Our approach, using dynamic headspace in combination with the more specific mass selective detector, resulted in a LOD about ten times lower and is expected to be sufficient for the detection of benzene in occupationally unexposed non-smokers. Analysis of urine from two such subjects gave values in the range of 11 to 14 ng/l. Concentrations below detection limits of about 50 ng/l have been reported by others [7]. To allow quantitation of non-smokers in the general population, the procedure needs to be somewhat further improved.

3.5. General remarks

Since benzene has a relatively high vapour pressure, the collection and transfer of samples are critical steps. Open handling should be avoided, and the number of transfer steps should be reduced to a minimum.

One general problem in estimating environmental exposure of benzene from biological samples is that smokers will have increased levels of benzene. This problem, of course, occurs using any biomarker of benzene.

One drawback with the method presented here is that the headspace procedure is time-consuming, tedious and the major source of variation and that mass-selective detection may still be considered somewhat too sophisticated for routine analysis. Substituting the headspace procedure with a procedure that could be automated would be a worthwhile goal.

4. Conclusions

The method for the determination of benzene in urine permits reliable quantitation of occupational exposure and, in most cases, of environmental exposure as well. The repeatability is acceptable, as is the selectivity guaranteed by the combination of capillary GC and MS detection. Samples can be stored frozen for at least a month.

Acknowledgements

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