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*Short Communication*

# Changes in plasma and erythrocyte chlorpromazine concentrations during sample storage

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## Introduction

The development of sensitive and specific methods for the measurement of plasma concentrations of major tranquilizers such as chlorpromazine (CPZ) has permitted the study of relationships between effects and concentration. Correlations between plasma concentrations and therapeutic and toxic effects suggest a role for such measurements in the clinical management of schizophrenic illness and other psychiatric disorders [1].

An appreciation of the effects of different methods of sample handling on apparent drug concentrations can help to establish optimal analytical data. Accordingly a gas chromatography (GLC)–mass fragmentographic method for the determination of CPZ has been applied to the study of the effects of different methods of sample storage.

## Experimental

Plasma CPZ concentrations were determined in physically healthy inpatients receiving therapeutic doses of chlorpromazine hydrochloride. Heparinized venous blood samples were obtained in evacuated tubes (Venoject; Kimble-Terumo).

*Refrigeration of whole blood*

To test the effect of refrigerated storage of whole blood on plasma and erythrocyte CPZ determinations, heparinized blood from nine patients was stored upright in collection tubes in the dark at 4°C for 24 or 168 h prior to centrifugation. After centrifugation at 850 ***g*** for 15 min, aliquots of plasma and packed cells were immediately frozen at −70°C prior to extraction and analysis.

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*Storage of frozen plasma*

The effect of storage of frozen plasma on CPZ determination was assessed using heparinized blood specimens from another nine patients. These were immediately centrifuged as previously described and the resulting plasma was stored in the dark at −70°C for 48 or 168 h prior to extraction and analysis.

*CPZ assay*

Plasma (2.0 ml) in a screw-capped glass culture tube (Corning) was extracted with 10 ml of nanograde petroleum ether (Mallinckrodt; boiling range 30–60°C) after the addition of 2 ml of 0.5 M NaOH and 200 ng of trideuterated CPZ as internal standard. Chlorpromazine labelled with three deuterium atoms in the methyl group was the generous gift of G. Sedvall. The culture tubes were agitated horizontally in a shaker (Eberbach) for 10 min and then centrifuged at 850 ***g*** for 10 min. Packed erythrocytes were also extracted with petroleum ether after being lysed with an equal volume of distilled water, being made basic with 0.5 M NaOH, and the addition of internal standard. After extraction and centrifugation, the petroleum ether (9 ml) was removed and taken to dryness under a stream of nitrogen. The residue was dissolved in 0.3 ml of methanol immediately prior to analysis. Recovery of CPZ from spiked samples ranged from 70 to 80%.

Chlorpromazine concentrations were measured by GLC–mass fragmentometry by a modification of the method of Alfredsson *et al.* [2, 3]. All samples were assayed in duplicate. Paired determinations agreed within 5% and were averaged.

Statistical comparisons were made with one-way analysis of variance for repeated measurements. *Post hoc* comparisons of means were performed using the Neuman Keuls method. Two-tailed levels of significance are reported.

## Results

The erythrocyte/plasma CPZ ratio is found to decrease significantly during the refrigerated storage of patients' blood samples, particularly for periods greater than 24 h as illustrated by Fig. 1 and the data in Table 1. There is, however, considerable interindividual variation in the changes with time. The overall decrease in ratio reflects a tendency for plasma CPZ concentrations to increase with a concomitant tendency for erythrocyte CPZ concentrations to decrease.

Storage of plasma at −70°C did not consistently alter the determined CPZ concentrations. The mean concentration at 0 h was 153 ± 143 ng $ml^{-1}$ (range 24–421 ng $ml^{-1}$) and those at 48 h and 168 h were 150 ± 133 ng $ml^{-1}$ (range 30–397 ng $ml^{-1}$) and 147 ± 133 ng $ml^{-1}$ (range 32–398 ng $ml^{-1}$), respectively.

## Discussion

Prolonged storage of whole blood samples in a refrigerator, prior to centrifugation, is associated with a tendency for plasma concentrations to increase whilst erythrocyte concentrations decrease. As a consequence the erythrocyte/plasma CPZ ratio is found to decrease. This may reflect the release of drug bound to erythrocytes into plasma. Also, the observations may in part be due to different conversion, in plasma compared to erythrocytes, of CPZ to metabolites, and vice versa in the case of CPZ sulfoxide [4]. A previous study [6] has indicated that apparent stability of CPZ during frozen storage is different in erythrocytes compared to plasma.

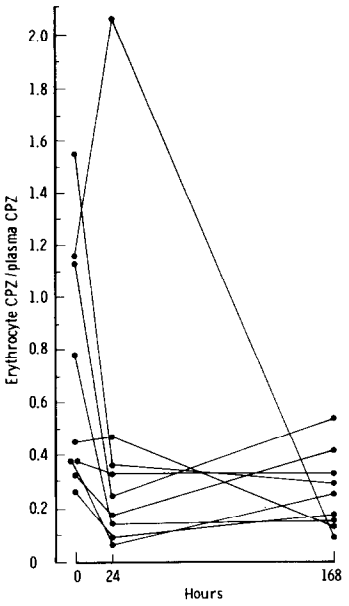


**Figure 1**
The effect of duration of refrigeration of whole blood on erythrocyte/plasma CPZ ratio in samples from nine patients.

**Table 1**
Concentration of chlorpromazine (CPZ) in plasma and erythrocytes and ratio of concentrations after refrigeration of whole blood for varying duration. The samples are from nine patients.

| | Period of refrigeration | | |
|---|---|---|---|
| | 0 h | 24 h | 168 h |
| Plasma CPZ (ng ml$^{-1}$) | | | |
| Mean | 415 | 412 | 543 |
| S.D. | 880 | 864 | 1139 |
| Range | 23–2682 | 20–2682 | 24–3532 |
| Erythrocyte CPZ (ng ml$^{-1}$) | | | |
| Mean | 182 | 160 | 150 |
| S.D. | 333 | 314 | 347 |
| Range | 9–1052 | 5–972 | 6–1070 |
| Erythrocyte CPZ/Plasma CPZ | | | |
| Mean | 0.71 | 0.45 | 0.26 |
| S.D. | 0.45 | 0.62 | 0.15 |
| Range | 0.27–1.50 | 0.09–2.06 | 0.09–0.54 |

For erythrocyte CPZ/plasma CPZ: $F = 3.75$, df = 2, 16, $p < 0.05$; values at 168 h different from those at 0 h, $p < 0.05$.

The erythrocyte/plasma CPZ ratios found in the present study are in general agreement with those reported elsewhere [2, 5–8]. There is considerable interindividual variation in these ratios, as with other neuroleptics. Serial determinations of CPZ in blood samples from the same individual indicate that plasma and erythrocyte-bound drug are in rapid *in vivo* equilibrium [2]. However, some workers have suggested a closer relationship between clinical response and erythrocyte neuroleptic concentration than plasma neuroleptic concentration [9]. Therefore the clinical utility of erythrocyte

determinations remains to be confirmed; accordingly it is an area for further investigation.

The relative stability of CPZ in frozen plasma under these conditions should be viewed in the context of other conflicting reports. For instance, Curry [10] reported that plasma CPZ was relatively stable when samples were frozen. However, Linnoila and Dorrity [7] noted a 33% average decrease in plasma CPZ over a 168-h period, which was independent of the storage temperatures tested (between 4° and −20°C) and the type of storage container. In their study blood was collected in EDTA tubes rather than heparinized tubes; storage at −70°C was not tested. More recently, Gupta *et al.* [11] found that plasma CPZ was stable at −20°C for at least one week. The use of assay methods with differing specificity, and therefore differing discrimination of CPZ from metabolites, may be one contributor to the disagreement in the literature.

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