Letters to the Editor

Distribution of some phenothiazines in red blood cells and platelets

SIR,—Chlorpromazine and some other phenothiazines are known to cause haemolysis of red blood cells *in vitro* and *in vivo* (Chaplin, Crawford, Cutbush & Mollison, 1952; Freeman & Spirtes, 1962). They also liberate 5-hydroxytryptamine (5-HT) from platelets *in vitro* (Bartholini, Pletscher & Gey, 1961; Paasonen, 1964). We have shown previously (Ahtee & Paasonen, 1965) that chlorpromazine is active in causing haemolysis but that its sulphoxide and a quaternary phenothiazine *N*-hydroxyethylpromethazine (NHP) are not. On the other hand, both chlorpromazine and its sulphoxide release 5-HT from platelets while NHP does not. The purpose of this work was to study to what extent these three phenothiazines are absorbed by the red blood cells and platelets.

Male rabbits under ether anaesthesia were bled from the carotid artery by means of a polyethylene cannula. The blood was mixed with 1/9 volume of 1·5% ethylenediaminetetra-acetic acid in 0·7% sodium chloride. In *in vitro* experiments, samples of whole blood were incubated in air with or without chlorpromazine hydrochloride (May & Baker), the sulphoxide (Rhône-Poulenc) or NHP (Orion), with gentle shaking at 37°. After incubation, the plasma, platelets and red cells were separated at 4° and the phenothiazines estimated spectrophotometrically according to Salzman & Brodie (1956). NHP was extracted from sodium carbonate-bromothymol blue solution into chloroform, transferred into 1·25N sulphuric acid and measured at 250 mμ. No recovery corrections have been made. As a value for platelet volume, 1 $\mu l/10^8$ platelet was used. The standard error of mean is given to indicate the distribution.

The distribution of chlorpromazine was estimated 0, 15, 30, 60 and 180 min after incubation with blood. In 6 experiments, immediately after the addition of 10^{-4}M/litre (31·9 μg/ml) of chlorpromazine, both the platelets and the red cells took up more of the drug per volume than was present in the plasma. The concentration of the drug in the platelets was 287 ± 42 μg/ml, which was about 10 times as high as that in the red cells (29·9 ± 3·7 μg/ml). The content of chlorpromazine in the platelets increased to 488 ± 84 μg/ml during the first hr of incubation. It then decreased to, or below, the starting level. The amount of chlorpromazine in the red cells decreased, while that in the plasma increased, during the first hr, to about 22 μg/ml. Both concentrations remained about the same during the remainder of the incubation. The total amount of chlorpromazine did not change.

Fig. 1 shows the distribution of the three phenothiazine derivatives after incubation of whole blood for 1 hr with the corresponding compound in 3 or 4 experiments. Platelets took up 5 to 22 times more chlorpromazine and its sulphoxide than was present in plasma. The uptake of NHP by platelets, on the other hand, remained relatively low. Although the concentration of chlorpromazine in the red cells was slightly higher than in the plasma, the concentration of sulphoxide and especially that of NHP remained far below the plasma levels.

In *in vivo* experiments three conscious rabbits received, 2 hr after the arterial cannulation, 10 mg/kg of chlorpromazine i.v. within 1 min. Blood samples were collected 2, 5, 10 and 15 min after the injection was started. The first sample contained 2·71 ± 0·45 μg/ml of the drug in the red cells and 1·46 ± 0·24 μg/ml in the plasma. This means that 1 min after the injection only about 1/100th of the injected phenothiazine is present in the red cells and plasma.

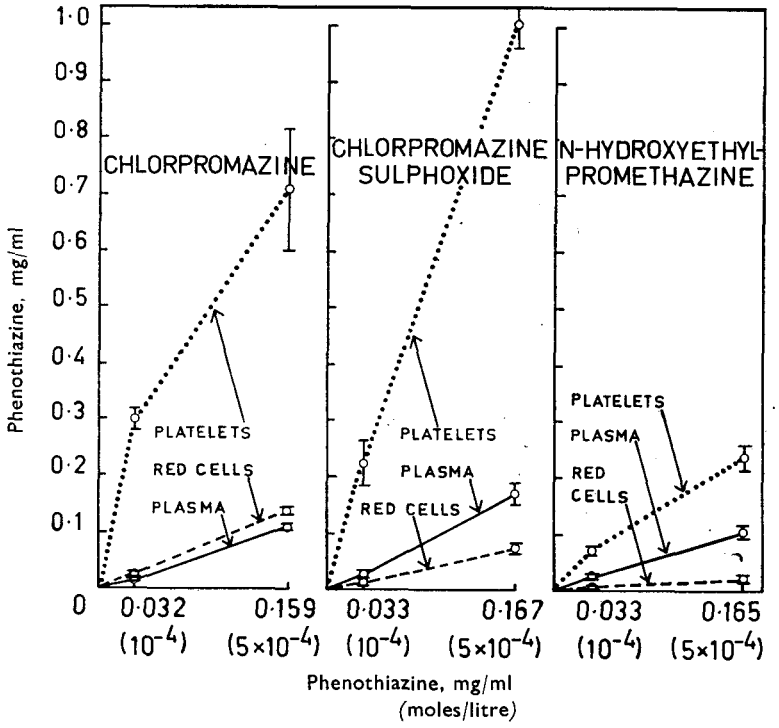

Fig. 1. The amount of phenothiazines in platelets, red blood cells and plasma after 1-hr incubation of whole blood with phenothiazines.

The amount of chlorpromazine in both components fell in 10 min to about 1/4th of the values mentioned. Due to the small amount of platelets in each sample there was not enough chlorpromazine for an accurate quantitative analysis.

The above results show that the red cells, and especially the platelets, absorb phenothiazines from plasma *in vitro* and *in vivo*. In the same volume the total surface of platelets is about 5 times as great as in the red cells. The 10 times higher concentration of chlorpromazine in platelets cannot therefore be explained solely by a difference in the surface area of these two cells. The fall of chlorpromazine in platelets and red cells during the incubation is probably due to the cellular damage it caused (Telkkä, Nyholm & Paasonen, 1964; Paasonen, 1965). The haemolysis may also mask a possible time dependent uptake of the drug in some of the red cells.

The uptake of chlorpromazine, its sulphoxide and NHP by platelets and red cells is in some way related to the ability of the drugs to liberate 5-HT from platelets and to cause haemolysis of red cells. However, haemolysis is not caused by the sulphoxide or NHP even when their content in red cells is higher than a haemolysing concentration of chlorpromazine. The uptake of these three phenothiazines by the red cells is better related to their tranquillising action.

Acknowledgements. This work was supported in part by the U.S. Public Health Service Research Grant M-5832, from the National Institute of Mental

Health, and the Sigrid Jusélius Foundation. We are indebted to May & Baker Ltd., Orion Oy. and Rhône-Poulenc for the drugs used.

Department of Pharmacology,
University of Helsinki,
Helsinki,
Finland.
November 16, 1965

LIISA AHTEE
M. K. PAASONEN

References

Ahtee, L. & Paasonen, M. K. (1965). *Annls Med. exp. Biol. Fenn.*, **43**, 101–105.
Bartholini, G., Pletscher, A. & Gey, K. F. (1961). *Experientia*, **17**, 541–542.
Chaplin, H. Jr., Crawford, H., Cutbush, M. & Mollison, P. O. (1952). *J. clin. Path.*, **5**, 91–101.
Freeman, A. R. & Spirtes, M. A. (1962). *Biochem. Pharmac.*, **11**, 161–163.
Paasonen, M. K. (1965). *J. Pharm. Pharmac.*, **17**, 681–697.
Salzman, N. P. & Brodie, B. B. (1956). *J. Pharmac. exp. Ther.*, **118**, 46–54.
Telkkä, A., Nyholm, M. & Paasonen, M. K. (1964). *Experientia*, **20**, 27–28.

The effect of (+)-amphetamine on various central and peripheral catecholamine-containing neurones

SIR,—In previous experiments with rat brain (Carlsson, Lindqvist, Dahlström, Fuxe & Masuoka, 1965), support was obtained for the view that substances of the amphetamine group are capable of causing the release of extragranular catecholamines, that is, of catecholamines located intraneuronally outside the storage granules. Furthermore, it was found that in large doses these drugs may also cause the release of catecholamines from the granules. In the present work the effect of (+)-amphetamine has been further examined for its effect on extragranular amines. Special attention has been paid to the sensitivity of different catecholamine neurone systems to this drug.

Adult, male Sprague-Dawley rats, 200–300 g, were used. Since the extragranular amine fraction normally seems to be very small, the experiments were made on animals whose amine stores had been emptied by reserpine. Loading of the extragranular space was then brought about by means of the monoamine oxidase inhibitor nialamide, followed by the catecholamine precursor L-3,4-dihydroxyphenylalanine (L-dopa).

In vivo *experiments.* The animals were treated with reserpine, 10 mg/kg, i.p., 20–22 hr before being killed, nialamide, 100 mg/kg, i.p., 4 hr before death, and dopa, 25–50 mg/kg, s.c., 30 min before death. (+)-Amphetamine was administered in various doses (calculated as the base) 15 min before the dopa.

Dopamine, noradrenaline and 3-methoxytyramine were measured fluorimetrically (Bertler, Carlsson & Rosengren, 1958; Carlsson & Waldeck, 1958; Carlsson & Lindqvist, 1962; Carlsson & Lindqvist, 1963; Carlsson & Waldeck, 1964).

Fifteen animals were taken for the cellular localization of monoamines in the brain, heart and vas deferens (Falck, Hillarp, Thieme & Torp, 1962; Falck, 1962; see review by Hillarp, Fuxe & Dahlström, 1965), one control group (4 animals) and two groups receiving (+)-amphetamine (0·75 mg/kg, 5 animals, and 0·4 mg/kg, 6 animals).

In vitro *experiments.* Brain slices of the neostriatum, hypothalamus, neocortex and the vas deferens of reserpine-treated rats (10 mg/kg, 12–18 hr before killing) were incubated for 30 min (Hamberger & Masuoka, 1965) with α-methylnoradrenaline, 1 or 0·03 μg/ml. In the test experiments the slices were preincubated for 15 min with (+)-amphetamine (0·0075–0·75 μg/ml), whereupon the α-methylnoradrenaline was added to the medium.