REFERENCES

Cannon, J. G. (1975) in: Calne, D. B., Chase, T. N., Barbeau, A. (eds) Advances in Neurology. Vol. 9, Raven Press, New York, pp 177–183

Cannon, J. G., Costall, B., Laduron, P. M., Leysen, J. E., Naylor, R. J. (1978) Biochem. Pharmacol. 27: 1417–1420

Cools, A. R. (1981) TIPS July, 178–183

Dandiya, P. C., Sharma, H. L., Patni, S. K., Ghambhir, R. S. (1975) Experientia 31: 1441–1443

Feenstra, M. G. P., Rollema, H., Dijkstra, D., Grol, C. J., Horn, A. S., Westerink, B. H. C. (1980) Naunyn-Schmiedeberg's Arch. Pharmacol. 313: 213–219

Feenstra, M. G. P., Homan, J. W., Dijkstra, D., Mulder, T. B. A., Rollema, H., Westerink, B. H. C., Horn, A. S. (1982) J. Chromatogr. 230: 271–287

Feenstra, M. G. P., Rollema, H., Mulder, T. B. A., Westerink, B. H. C., Horn, A. S. (1983a) Life Sci. 32: 459–465

Feenstra, M. G. P., Rollema, H., Mulder, T. B. A., Westerink, B. H. C., Horn, A. S. (1983b) Life Sci. 32: 1313–1323

Horn, A. S., Feenstra, M. G. P., Grol, C. J., Rollema, H., Van Oene, J. C., Westerink, B. H. C. (1981) Pharm. Weekbl. Sci. Ed. 3: 145–165

Kebabian, J. W., Calne, D. B. (1979) Nature (London) 277: 93–96

Köhler, C., Fuxe, K., Ross, S. B. (1981) Eur. J. Pharmacol. 72: 397–402

Leysen, J. E., Gommeren, W. (1981) J. Neurochem. 36: 210–219

Rollema, H., Westerink, B. H. C., Mulder, T. B. A., Dijkstra, D., Feenstra, M. G. P., Horn, A. S. (1980) Eur. J. Pharmacol. 64: 313–323

Seeman, P. (1980) Pharmacol. Rev. 32: 229–313

Stoof, J. C., Kebabian, J. W. (1981) Nature (London) 294: 366–368

Westerink, B. H. C., Horn, A. S. (1979) Eur. J. Pharmacol. 58: 39–48

Westerink, B. H. C., Dijkstra, D., Feenstra, M. G. P., Grol, C. J., Horn, A. S., Rollema, H., Wirix, E. (1980) Ibid. 61: 7–15

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Chlorpromazine enhances haemolysis induced by haemin

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Chlorpromazine, a cationic phenothiazine drug generally used as an antipsychotic tranquillizer, binds to cell membranes and membrane lipids (Seeman 1972; Römer & Bickel 1979; Farah & Kellaway 1981). It can cause shape change in red blood cells (Deuticke 1968; Kanaho et al 1981). Between 10^{-4} and 10^{-5} M chlorpromazine protects human erythrocytes from hypotonic lysis. However at 10^{-3}M or higher, this drug can disrupt membranes (Seeman 1972; Seeman & Kwant 1969). Haemin or ferriprotoporphyrin IX has been reported to cause lysis of erythrocytes and blood stream forms of *Trypanosoma brucei* (Meshnick et al 1977; Chou & Fitch 1980). Here we report that haemin-induced lysis of human, mouse and rabbit erythrocytes is enhanced by chlorpromazine in the micromolar range.

Method

Blood was collected from healthy human donors, and mice and rabbits fed nutritionally sufficient diets. Blood was centrifuged, the buffy coat layer removed and the red blood cells were washed three times with 310 mOsm phosphate-buffer-saline (PBS). The erythrocytes were freed of reticulocytes by passage through a cellulose CF-11 column (Richards & Williams 1973). A 10% red blood cell suspension in PBS buffer pH 7·4, 4 °C, was the stock for the time-course and fixed time haemolysis experiments. Lysis of 0·25% red cell suspension was monitored at 540 nm after sedimentation of the cells and cell debris. Complete haemolysis was measured by the absorbance of haemoglobin at 540 nm after sedimentation of cell debris following freeze-thaw lysis of red blood cells. Mouse red blood cell membranes were prepared and extracted for lipids by the chloroform–methanol method (Bligh & Dyer 1959). Liposomes were made from the extracted lipid suspension (1 mg ml^{-1}) by sonication with Branson sonicator at 50 °C for 15 min.

Haemin was recrystallized from that sold by Sigma Chemical Co. (St Louis, Mo., U.S.A.). Chlorpromazine and phospholipids were also from Sigma, but perazine was donated by Yoshitomi Pharmaceutical Co. (Osaka, Japan). Difference spectroscopy was performed on a Shimadzu UV 240 spectrophotometer with a matched pair of quartz tandem cells (Hellma (England) Ltd.) in a thermostated cell holder. The procedure for two-component difference spectroscopy was as described previously (Jearnpipatkul & Panijpan 1980). For the three-component (haemin-chlorpromazine-lipid) difference spectrum, one chamber of the control cuvette had haemin and lipid, the other chlorpromazine, whereas the sample cuvette had the three components in one chamber and the other chamber contained buffer; all components must have the same concentration X pathlength in both cuvettes.

Results

Fig. 1 shows the lysis after 1 h incubation of human, mouse and rabbit red blood cells by haemin in the absence and presence of 5×10^{-6} M chlorpromazine. Fig. 2 shows the 1 h haemolysis of mouse red blood cells at 7 μM haemin and different concentrations of chlorpromazine. Chlorpromazine enhanced haemolysis due to haemin. That haemolysis induced by haemin was

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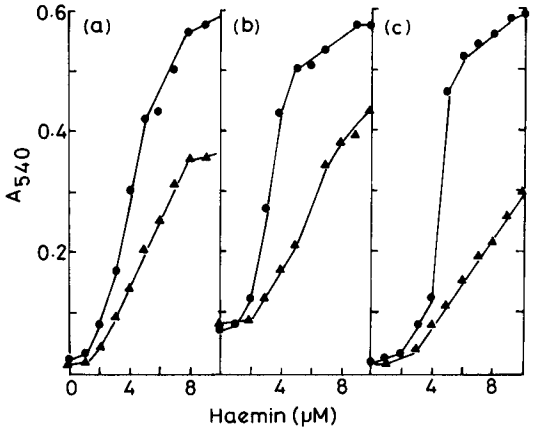

FIG. 1. Change in absorbance at 540 nm (reflecting the released haemoglobin after sedimentation of unlysed red blood cells and cell debris) after 1 h haemin-induced haemolysis of 0·25% red blood cell suspension in PBS pH 7·4, 37 °C in the presence (●) and absence (▲) of chlorpromazine 5 μM. Erythrocytes from (a) human, (b) mouse, (c) rabbit.

more rapid in the presence of chlorpromazine is shown in Fig. 3.

Fig. 4 shows the difference spectra arising from the interaction between chlorpromazine and haemin, haemin and lipids, and also that between chlorpromazine-haemin complex and lipids extracted from normal mouse red blood cells. Although haemotoporphyrin and protoporphyrin IX could be shown to interact with chlorpromazine by difference spectroscopy, no enhanced haemolysis in the absence of intense broad spectrum light was observed for both the drug and the two ironless porphyrin compounds at 5×10^{-6} M. Chlorpromazine alone at 5×10^{-6} M or lower did not cause haemolysis.

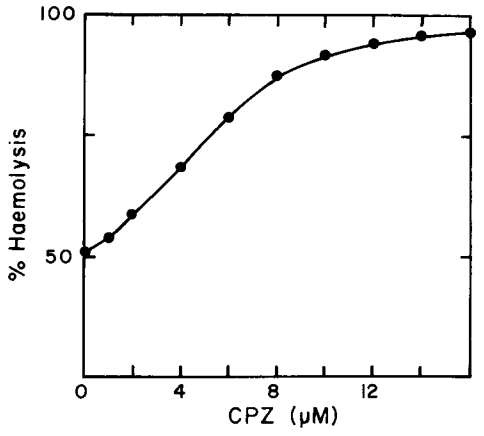

FIG. 2. Percent haemolysis as followed by absorbance at 540 of 0·25% suspension of mouse red blood cells after 1 h incubation with 7 μM haemin and different concentrations of chlorpromazine.

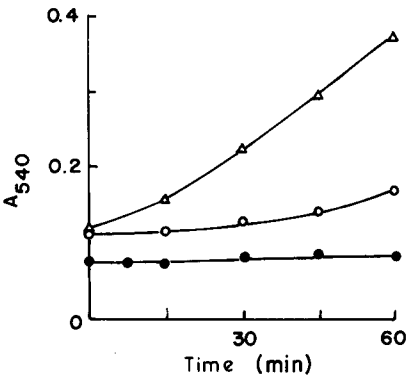

FIG. 3. Time-course of haemolysis of mouse red blood cells (0·25%) in the presence of PBS (●——●), 4 μM haemin in PBS (○——○) and 4 μM haemin and 4 μM chlorpromazine in PBS (△——△).

Discussion

Haemin can form aggregates at μM concentrations (Brown et al 1970). Probably, chlorpromazine interacts with haemin to affect the size distribution and/or the structure of the aggregate. The enhanced haemolysis is at least partly due to the formation of a haemin-chlorpromazine-lipid complex because the difference spectra in the haemin-chlorpromazine-lipid mixture was not the simple linear combination of the difference spectra of the haemin-lipid, haemin-chlorpromazine and chlorpromazine-lipid systems. That the haemin-chlorpromazine-induced haemolysis arises from water influx into the red blood cells is indicated by the microscopic observation of red cell swelling, disrupting and disappearing when haemin-chlorpromazine solution was applied. The haemolysis observed is not the same as hypotonic lysis because there was no resealing of the red cell ghosts (Seeman et al 1969). At complete haemolysis the sedimented cell debris had very little

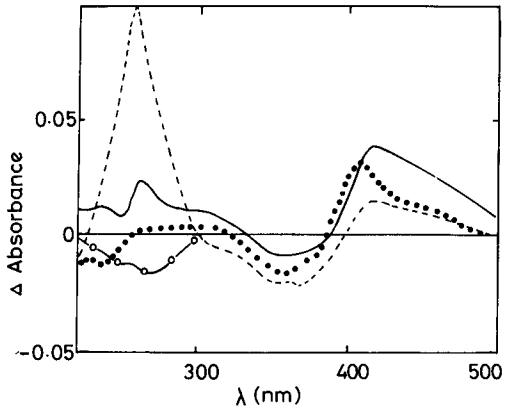

FIG. 4. Difference spectra of haemin-chlorpromazine (——), haemin-lipid (●●●●), chlorpromazine-lipid (○——○) and chlorpromazine-haemin-lipid (----) systems in PBS. Chlorpromazine and haemin concentration were 10^{-5} M and the extracted mouse red blood cell lipid concentration was 0·1 mg ml^{-1}.

colour and haemoglobin released was the same as in freeze-thaw lysis. All the observations above have also been obtained for perazine which has an *N*-methylpiperazine side-chain.

Chlorpromazine has been implicated in an immune haemolysis which does not appear to be related to the presence of haemin (Beutler 1969). Spontaneous release of haem and haemin from denatured haemoglobin has been associated with some haemolytic anaemias and the production of Heinz bodies (Jacob & Winterhalter 1970). We have tested chlorpromazine for its antimalarial activity on in-vitro cultures of the blood stages of *Plasmodium falciparum*, a human malaria. At chlorpromazine concentration of 5×10^{-5} M and above the inhibition of growth was 100% and at 1×10^{-5} M growth was inhibited by 40%. Haemin-mediated mechanism may be one of the mechanisms involved (Jearnpipatkul & Panijpan 1980; Chou & Fitch 1981; Orjih et al 1981). Phenothiazine neuroleptics also have been found to have protozoacidal effects on *Leishmania donovani*, a human pathogen (Pearson et al 1982).

REFERENCES

Beutler, E. (1969) Pharmacol. Rev. 21: 73–103

Bligh, E. G., Dyer, W. J. (1959) Can. J. Biochem. Physiol. 37: 911–917

Brown, S. B., Dean, T. C., Jones, P. (1970) Biochem. J. 117: 733–739

Chou, A. C., Fitch, C. D. (1980) J. Clin. Invest. 66: 856–858

Chou, A. C., Fitch, C. D. (1981) Ibid. 68: 672–677

Deuticke, B. (1968) Biochim. Biophys. Acta 163: 494–500

Farah, F. H., Kellaway, I. W. (1981) J. Pharm. Pharmacol. 22: 66P

Jacob, H. S., Winterhalter, K. H. (1970) Proc. Natl. Acad. Sci. U.S.A. 65: 697–701

Jearnpipatkul, A., Panijpan, B. (1980) Chemico-Biol. Interact. 33: 83–90

Kanaho, Y., Sato, T., Fujii, T. (1981) Mol. Pharmacol. 20: 704–708

Meshnick, S. R., Chang, K-P., Cerami, A. (1977) Biochem. Pharmacol. 26: 1923–1928

Orjih, A. U., Banyal, H. S., Chevli, R., Fitch, C. D. (1981) Science 214: 667–669

Pearson, R. D., Manian, A. A., Harcus, J. L., Hall, D., Hewlett, E. L. (1982) Ibid. 217: 369–371

Richards, W. H. G., Williams, S. G. (1973) Annal. Trop. Med. Parasitol. 67: 249–250

Römer, J., Bickel, M. H. (1979) Biochem. Pharmacol. 28: 799–805

Seeman, P., Kwant, W. O. (1969) Biochim. Biophys. Acta 183: 512–519

Seeman, P., Sauk, T., Argent, W., Kwant, W. O. (1969) Biochim. Biophys. Acta 183: 476–489

Seeman, P. (1972) Pharmacol. Rev. 24: 583–625

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Design and evaluation of a miniature air-suspension coating apparatus

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The air-suspension coating technique has been preferred to conventional pan coating technique in developing a dosage form since Wurster's disclosures (1953, 1957) because of its simplicity, rapidity and uniformity of the final product. Several papers have been published regarding the design and utility of laboratory air-suspension coating devices (Wilhelm & Valentine 1951; Wurster 1959; Singiser & Lowenthal 1961; Caldwell & Rosen 1964). All of them require a minimum of nearly a kilogram of material for optimum efficiency of their working although Wolkoff et al (1968) have designed an assembly requiring gram quantities of material. During our work on the formulation of sustained release tablet dosage forms of new drugs, synthesized material was often in short supply and a much simpler and smaller coating model was badly needed. This communication deals with the design and evaluation of such a unit developed in our laboratory.

A schematic diagram of the miniature air-suspension coating apparatus is shown in Fig. 1. The body of the apparatus was a glass tube of 2·6 cm internal diam. and length of 11·0 cm the lower end of which was fitted through a B_{24} standard joint to a glass tube of 2·1 cm internal diam. The lower end of the latter was tapered to allow the entry of hot air into the column. In between the cone and the socket of the joint, a stretched muslin cloth screen provided the base of the column, on which the tablets were fluidized with minimal attrition. The tip of the column was also covered with muslin cloth. Coating solution present in a burette was sprayed from the top so that the spray tip was 4·5 cm above the muslin screen. A coating solution was not sprayed from bottom as in Wurster apparatus since the coating materials were blocking the muslin screen and obstructing the entry of hot air. The air supply provided by an air compressor (K. G. Khosla & Co. Pvt. Ltd, New Delhi) was heated by passing it through a heat exchanger controlled by a dimmerstat. The output of the compressor was adjusted in such a way that it was just sufficient to fluidize the tablets between the muslin base and the spray tip.

Tablets of equal weight were prepared using a single

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