interest. The results of Sulpizio et al (1978) suggest that the fenfluramine-induced hyperthermia in the rat may be a good test for the 5-HT uptake inhibition by drugs. The present results indicate that the test is not as universal as originally thought, since it yields false negative results for some compounds, such as zimelidine and Org 6582. On the other hand, the hyperthermia test employing PCA seems in more cases to give results parallel to those obtained in in vitro tests for 5-HT uptake inhibition.

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The reduction of tuberculin-induced pleurisy in the guinea-pig by a gold salt, chloroquine and D-penicillamine

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It is well established that the phenomena of delayed hypersensitivity play a major role at the synovial membranes of the affected joints in rheumatoid arthritis. In the search for models in which this type of hypersensitivity is involved and reduced by antirheumatic agents, we have studied the effects of chloroquine, D-penicillamine and a gold salt, sodium aurothiopropanol sulphonate, on a classic model of delayed hypersensitivity, purified-protein-derivative (PPD) pleurisy in the guinea-pig (Allen & Apicella 1968).

Material and methods

Sodium aurothiopropanol sulphonate (Sarbach); chloroquine diphosphate (Rhône Poulenc); D-penicillamine (Fluka).

Dukin-Hartley guinea pigs of either sex, 300–400 g, and male Swiss mice, 23–25 g, were used.

Tuberculin-induced pleurisy in guinea-pigs. 80 mg of killed *Mycobacterium tuberculosis* (Difco) was ground in a mortar and was then suspended in a mixture of 10 ml of incomplete Freund's adjuvant (Difco) and 10 ml of a 0·2 M phosphate buffer solution, pH: 7. 0·5 ml of this suspension was injected into the thigh muscles of the guinea-pigs.

Five to 7 weeks after the sensitization, batches of animals were made homogeneous in weight and sex (50% males and 50% females). PPD (Institut Pasteur) 10 μg 0·1 ml was injected into the pleural cavity of the animals lightly anaesthetized with ether. 48 h later, the animals were killed and the volume of the pleural exudate was measured. The pleural cavity was rinsed with 2 ml of medium 199 (Institut Pasteur) to recover the cells remaining on the walls, and the leucocytes of the exudate were counted with a Coulter Counter (Coultronics, model ZF). The samples were spread on slides and stained with Hemacolor (Merck), and the numbers of mononuclear cells, neutrophils, and eosinophils determined.

The antirheumatic agents were dissolved in 0·9% NaCl, and D-penicillamine and chloroquine diphosphate were injected subcutaneously, the gold salt intramuscularly. Controls received the solvent alone. The agents were administered 24 and 2 h before and 2 and 24 h after the challenge.

Action of antirheumatic agents on circulating blood cells. Groups of animals containing 50% males and

* Correspondence.

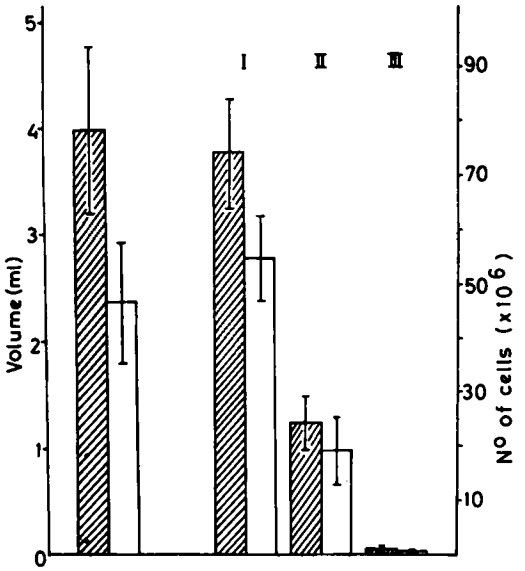

FIG. 1. Action of 25 mg kg^{-1} (× 4) of D-penicillamine in PPD-induced pleurisy in guinea-pigs sensitized to tuberculin, all parameters measured at 48 h, 24 animals per group. Hatched columns controls; open columns animals treated with D-penicillamine. First column = exudate. I. Mononuclear leucocyte count per animal. II. Neutrophil count per animal. III. Eosinophil count per animal. Bars represent standard errors.

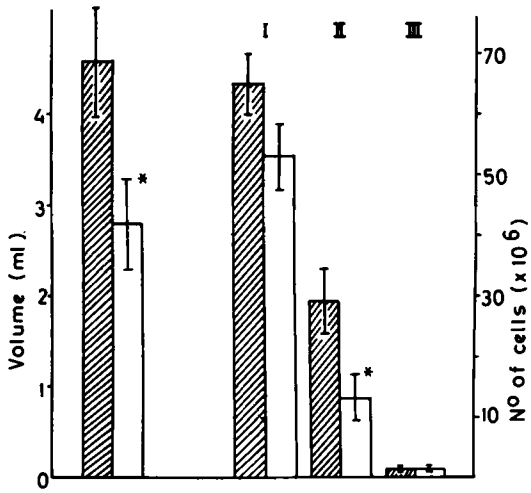

FIG. 2. Action of 50 mg kg^{-1} (× 4) of D-penicillamine in PPD-induced pleurisy in the guinea-pig. Key as FIG. 1. 28 animals per group.* $P < 0.05$ compared with controls.

50% females were treated with the same doses and in the same experimental conditions as those used for the tuberculin pleurisy. The control batch, received 0·9% NaCl subcutaneously. Three hours after the fourth injection, the animals were anaesthetized with ether, and after cardiac puncture blood was drawn into citrate. The red cells and the leucocytes were counted with a Coulter Counter. Slides were made up and stained, and the percentages of mononuclear cells, neutrophils, and eosinophils were determined.

Toxicity in mice. Groups of 10 to 16 mice received four doses of antirheumatic agent by the same routes and in the same conditions as used for the guinea-pig pleurisy. The lethal doses were determined.

The results of each experiment were analysed by Student's *t*-test.

Results

Pleurisy. D-Penicillamine at 25 mg kg^{-1} had no significant effect although the action on the exudate was evident (Fig. 1). At 50 mg kg^{-1} the amount of exudate and the number of neutrophils of the inflammatory focus were significantly reduced (Fig. 2). Sodium aurothiopropanol sulphonate at 10 mg kg^{-1} (corresponding to 3·3 mg kg^{-1} of Au) clearly reduced the volume of exudate and the number of neutrophils and mononuclear cells of pleural cavity (Fig. 3).

Chloroquine caused a significant decrease of exudate and of the number of mononuclear cells (Fig. 4).

None of the compounds changed the number of red cells or leucocytes in the circulating blood (Table 1).

Toxicity in mice. D-Penicillamine 500 mg kg^{-1} (× 4), did not induce any mortality. With the gold salt, 25% of the animals died after 90 mg kg^{-1} (× 4). With chloroquine diphosphate, 8% mortality was found for 100 mg kg^{-1} (× 4).

Discussion

Delayed hypersensitivity pleurisy in the guinea-pig, initially described by Allen & Apicella (1968), is the transposition to the pleural cavity of the classic tuberculin model that is generally employed on the skin. The use of the pleural cavity, permits a detailed study of the various components of the inflammatory reaction. Thus, Yamamoto et al (1976) were able to demonstrate the presence of factors inhibiting the migration of mononuclear cells. We have already described the effect of cyclophosphamide, desonide, levamisole and phenylbutazone on this pleurisy (Tarayre & Lauressergues 1979, 1980). In addition, Blackham & Woods (1979) published preliminary results for the action of certain compounds in this model.

The mouse toxicity revealed that with the gold salt and especially with D-penicillamine the doses used were well below those that caused mortalities. The doses of chloroquine had a smaller margin for toxicity.

In addition to reducing the exudate, the three antirheumatic agents also acted on cellular phenomena, although this effect was not produced on the same types of leucocyte. At the doses used, the actions on the leucocytes of the inflammatory focus were not related to modifications of the numbers of circulating blood cells.

The action of D-penicillamine at 50 mg kg^{-1} on the volume of exudate and on the number of neutrophils in the pleural cavity was marked. Chwalińska-Sadowska &

Table 1. Action of the compounds on the erythrocyte and differential leucocyte count in the blood of guinea-pigs. Means are followed by standard errors—In brackets number of animals used.

Groups	Doses mg kg^{-1} (× 4)	Erythrocytes 10^6 mm^{-3}	Leucocytes 10^3 mm^{-3}	Neutrophils (%)	Eosinophils (%)	Mononuclears (%)
Controls	—	4·51 ± 0·09 (14)	7·40 ± 0·8	37·5 ± 4·9	0·5 ± 0·1	62·0 ± 4·8
D-Penicillamine	50	4·77 ± 0·09 (14)	6·7 ± 0·6	43·1 ± 3·5	0·9 ± 0·3	56·0 ± 3·6
Na aurothio-propanol sulphonate	10	4·51 ± 0·12 (14)	6·6 ± 0·8	36·9 ± 3·7	0·6 ± 0·2	62·5 ± 3·7
Chloroquine diphosphate	30	4·58 ± 0·09 (14)	8·3 ± 0·8	35·3 ± 4·4	0·8 ± 0·2	63·9 ± 4·5

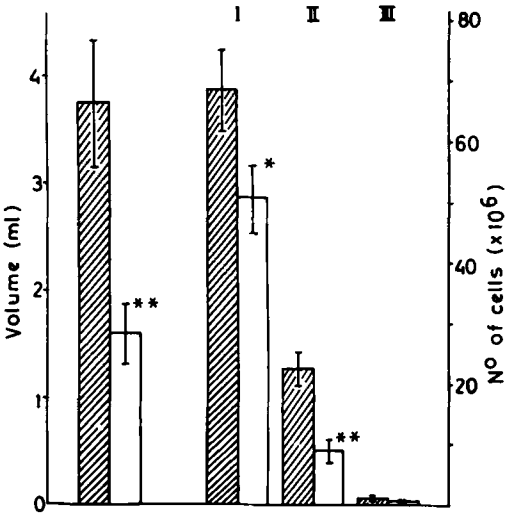

FIG. 3. Action of sodium aurothiopropanol sulphonate (10 mg kg^{-1} × 4) in PPD-induced pleurisy in the guinea-pig. Key as Fig. 1. 34 animals per group.* $P < 0{\cdot}05$** $P < 0{\cdot}01$ compared with controls.

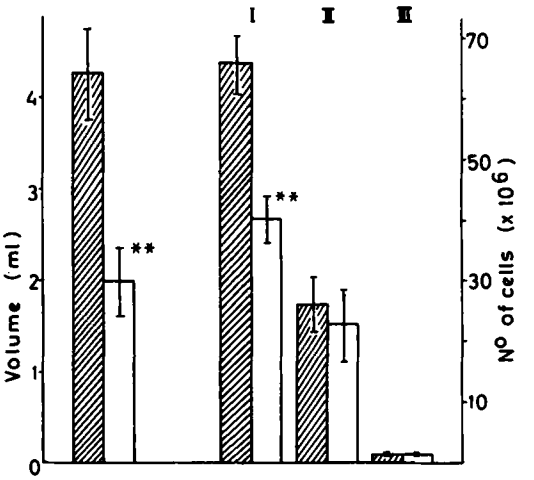

FIG. 4. Action of chloroquine diphosphate (30 mg kg^{-1} × 4) in PPD-induced pleurisy in the guinea pig. Same legends as Fig. 1. 36 animals per group.** $P < 0{\cdot}01$ compared with controls.

Baum (1976) reported that the thiol derivative inhibited the chemotaxis of neutrophils. In experiments on the same model, Blackham & Woods (1979), though they too found that the compound had an anti-exudative action, saw no effect on the total number of leucocytes of the inflammatory focus (the various classes of leucocytes were not given). On the other hand, they found that the compound inhibited the liberation of a lysosomal enzyme, β-glucuronidase, measured in the exudate. Skosey & Chow (1980) thought that this action on the liberation of lysosomal enzymes could be attributed to a 'scavenger' effect of the product on the free radicals derived from the metabolism of oxygen. De Vore et al (1979), however, suggested that this effect results from the action of D-penicillamine on lymphocytes. Despite many contradictory studies, it is now thought that D-penicillamine has an inhibitory effect on T lymphocytes. Thus Hunneyball et al (1978) found that it reduced the delayed-hypersensitivity skin reactions during the inhibition of monoarticular arthritis in the rabbit. Ziff & Lipsky (1980) attributed the clinical action of D-penicillamine to a specific 'immunosuppressive' effect on lymphocytes.

Sodium aurothiopropanol sulphonate significantly decreased the volume of exudate and the number of neutrophils and mononuclear cells in the pleural cavity. An inhibitory action by gold salts on the migration of mononuclears (Ho et al 1978), or of mononuclears and neutrophils (Vernon-Roberts et al 1973) has been demonstrated. There is also a decrease of the phago-

cytosis by macrophages and neutrophils (Vernon-Roberts et al 1973; Jessop et al 1973; Sliwinsky & Guertin 1979). Thus the release of lysosomal and non-lysosomal enzymes may be reduced, although direct inactivation of the enzymes has also been demonstrated (reviewed by Lewis et al 1980). In the experiments of Blackham & Woods (1979), the gold salt not only acted on the exudate and on the total number of leucocytes, but also reduced the level of β-glucuronidase. Skosey & Chow (1980) thought that, as with D-penicillamine, gold salts inhibit the release of enzymes through a 'scavenger' action on free radicals. Many studies have shown that gold salts decrease the lymphocytic response to mitogens (Cahill 1971; Lies et al 1977; Harth et al 1977). Ziff et al thought that this 'immunosuppressive' action on lymphocytes came about through an effect on the macrophages, where the gold binds preferentially (Lipsky & Ziff 1977; Ugai et al 1979; Ziff & Lipsky 1980).

Unlike Blackham & Woods (1979), who, with chloroquine, obtained no change in the parameters studied, we found that chloroquine reduced the exudate and the number of mononuclears of the inflammatory focus. Several studies have showed that this antimalarial acts on T lymphocytes, opposing their stimulation by specific antigen and by mitogens (Hurtvitz & Hirschhorn 1965; Panayi et al 1973). Likewise, Rybczyńska et al (1978) found that in mice it prolonged the survival of a cutaneous allograft and also hindered the local graft-versus-host reaction. Stabilization of lysosomal membranes by the compound may be involved in this action (Weissmann 1965; Weissmann et al 1967; Ignarro 1971; Lie & Schofield 1973). Though an inhibition of neutrophil migration and of phagocytosis has been demonstrated (Ward 1966), not much work has been done on mononuclear cells (Tarayre & Lauressergues 1978). In addition, the quinoline derivative antagonizes certain actions of prostaglandins (Manku & Horrobin 1976; Famey et al 1977), which may perhaps participate in pleurisy (Willoughby et al 1977). The relation between all the reported properties of chloroquine and its action on nucleic acids (Cohen & Yielding 1965; Whichard et al 1972; Field et al 1978) is poorly understood.

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