regrowth of a peripheral nerve leads to a remarkable overproduction of Schwann cells in the distal nerve stump reaching a point at which these cells are applied in multiple layers around each single axon. Also, in crush lesions of the abnormal peripheral nerve roots of dystrophic mice, where a paucity of Schwann cells and myelin segments provides much less degenerating material than in normal nerve, an increase in the number of myelin segments and, presumably, Schwann cells is induced by crush injury[7]. The importance of the axon in provoking Schwann cell proliferation is also indicated by observations, on the proximal stump of severed or crushed sciatic nerve, that proliferation occurs independently of widespread nerve fibre or myelin degeneration and in conditions in which nerve regeneration is most actively occurring[8].

Our observation of the axon–Schwann cell interaction resulting in mitogenesis illustrates one use of cultures containing either 'pure' Schwann cell populations or bare neurite preparations. These preparations may be useful in further analysis of the interaction between neurite and Schwann cell, as well as in studies of the basic properties of the Schwann cell itself.

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Biological reactivity of PVC dust

CONCERN has been expressed about the biological potential of polyvinyl chloride (PVC) and its associated monomer vinyl chloride monomer (VCM)[1]. During the industrial processing of PVC, workers can be exposed to varying quantities of this material in the form of a dust. At the present time, however, there is little detailed biological or biochemical information on the effects of inhaled or ingested PVC, or on the reactivity of this dust material. The biological reactivity of other dusts (silica, asbestos) have been studied by a haemolysis technique[2] which is useful for assessing the degree of membrane-induced damage by a variety of toxic materials. Other *in vitro* screening systems using lung[3] and other cells[4] have also provided useful information on structural and biochemical changes induced by particulate matter. Here we report on the haemolytic potential of PVC dust, comparison of which is made with the highly haemolytic and biologically reactive chrysotile asbestos A (UICC standard reference sample) and the effect of PVC on lung fibroblast cultures.

The haemolysis technique used has been described in detail previously[2]. The degree of haemolysis was expressed as a percentage of the totally lysed sample and the results presented as the means and ranges (if any) of, at least, quadruplicate assays. Two samples of PVC were tested (KM 1 and KM2) both of which were obtained as finely-divided dried powders which had been formed by standard processing procedures for use in fabrication work.

The first sample (KM 1) was highly haemolytic at relatively low concentrations, 100% haemolysis (over 50 min) being achieved by between 7.5 and 10 mg of dust (Fig. 1*a*). With increasing concentrations of PVC, haemolysis seems to be reduced, but this effect is most likely the result of some of the released haemoglobin binding to the dust, which is then spun down into the pellet. Sample KM 2 PVC was found to be practically non-

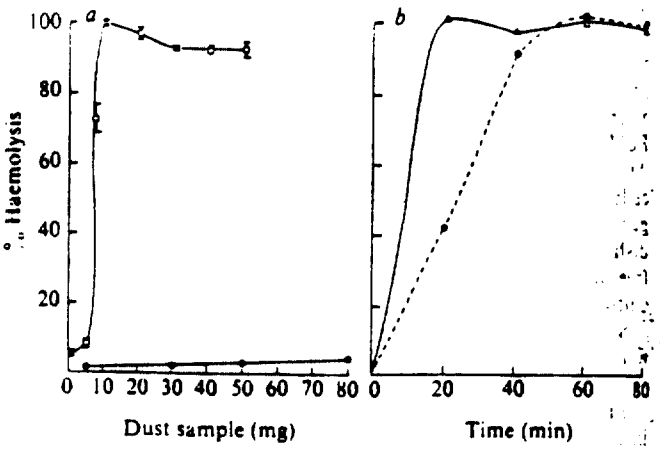
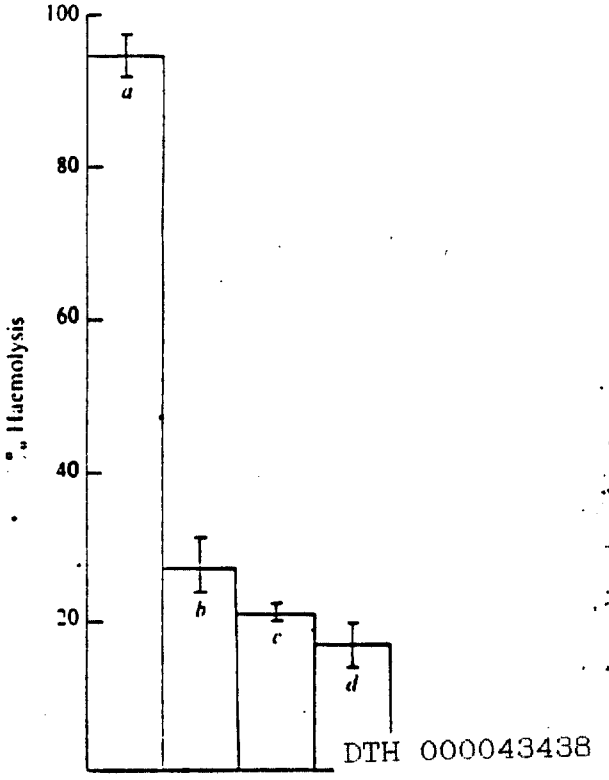


Fig. 1 Haemolysis by PVC dusts and chrysotile asbestos. *a*, Haemolytic potency of KM 1 (○) and KM 2 (●) PVC samples after 50 min; *b*, change in haemolysis with time by 7.5 mg samples of UICC chrysotile asbestos A (△) and KM 1 (●) PVC. Briefly, a 1% (v/v) suspension of packed rabbit erythrocytes in veronal buffered saline, *p*H 7.4, was used in all the experiments. The incubation mixture normally consisted of dust sample with 1 ml 1% erythrocyte suspension + 3 ml veronal buffered saline. After incubation and agitation for 50 min (or varying time intervals) at 37 °C the samples were centrifuged at 2,000 r.p.m. for 20 min and the extinction of the supernatant read at 541 nm. Controls consisting of a totally lysed sample (1 ml 1% erythrocyte suspension + 3 ml water) and a fragility control (1 ml 1% erythrocyte suspension + 3 ml veronal buffered saline) were treated identically in each experiment.

haemolytic. A sample of 100 mg of KM 2 PVC gave an equivalent haemolytic effect to 1 mg of sample KM 1. The haemolytic activity with time of a sample of KM 1 PVC (7.5 mg) was compared with an equivalent mass of chrysotile asbestos A (Fig. 1*b*). It is evident that the asbestos is a faster haemolytic agent, although total lysis is achieved by PVC after 1 h.

During the processing of different forms of PVC, a variety

Fig. 2 Haemolysis by washed samples of KM 1 PVC dust (7.5 mg) and the resulting supernatant fluids from these washings compared with the haemolytic activity of the untreated dust sample. KM 1 PVC *a*, Untreated; *b*, washed once; *c*, washed twice; *d*, washed three times; *e*, wash 1; *f*, wash 2; *g*, wash 3.



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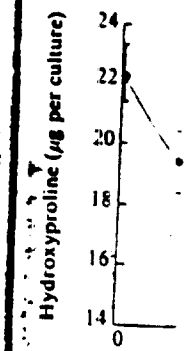

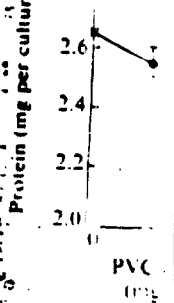

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of agents are used which could contribute to the haemolytic effect of the dust. Therefore, samples (7.5 mg) of KM 1 PVC were washed once, twice and three times (5 s each wash, in 3 ml veronal buffer on a whirlimix; followed by centrifugation, 2,000 r.p.m. for 20 min) and the haemolytic potency of the washed dust samples and the supernatant fluid were compared with the lytic potential of an untreated PVC sample (Fig. 2). After a single wash, the haemolytic potency of KM 1 PVC is reduced by over 60%, and subsequent washes reduce the activity of the dust even further. Thus the removal of some surface-associated material, which must exist in a reasonably soluble form, considerably reduces the biological potency of this PVC dust. It is also evident that this material, once removed from the dust surface and diluted out in solution, has very limited haemolytic activity (Fig. 2).

The effect of KM 1 PVC was studied on the levels of cell mat DNA, RNA, protein and hydroxyproline (assessment of collagen) in lung fibroblast cultures maintained *in vitro* for 24 d. The methods of isolation and cell culture and the analyses for DNA, RNA protein and hydroxyproline have been detailed previously[2,4]. Before addition of different concentrations of KM 1 PVC (50–200 μg ml^{-1} culture medium or 0.5–2.0 mg per culture) the dust sample was first washed in a balanced salt solution containing antibiotics (see legend to Fig. 3). Other methods of dust sterilisation (heat, autoclaving, radiation)

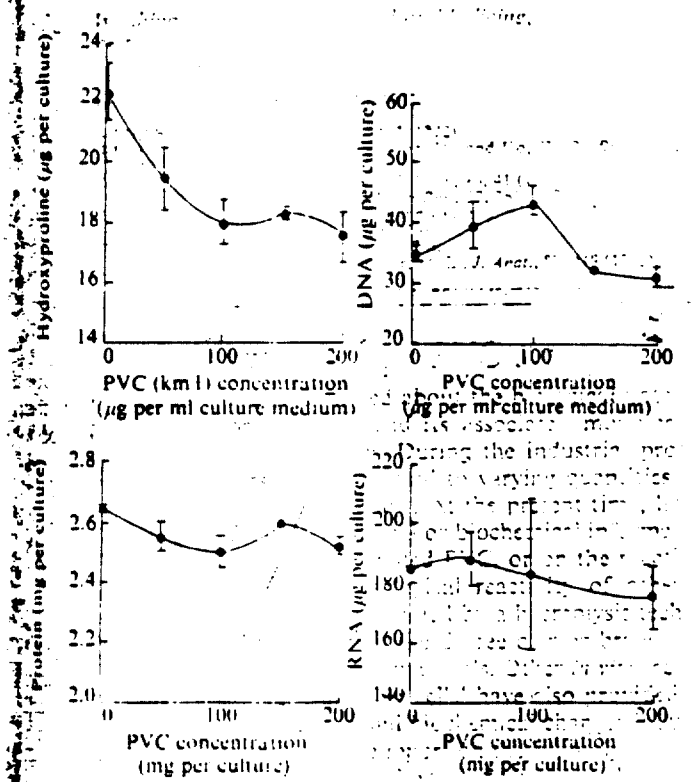

Fig. 3 The effect of different concentrations of KM 1 PVC on lung fibroblast cultures. The PVC sample was first washed with an antibiotic solution (penicillin (100 units) streptomycin (100 μg) in 1 ml) and then added as a single dose to 3-d-old cultures (in logarithmic growth). Cultures were maintained in 10 ml of 20% foetal bovine serum plus Waymouth's medium containing additional ascorbic acid[5], changed twice weekly and removed for analysis on day 24.

were not considered to be feasible and while the washing pro- ...ly reduces the ability of this PVC sample ... (Fig. 2) fibroblast cultures treated with the ... lower levels of cell mat hydroxyproline after 24 d (Fig. 3). Se... fluctuation in DNA levels was apparent with different dust co...trations but the significance of these is doubtful without ... experimentation. There is little change in the level of ... protein or RNA in the PVC-treated cultures.

From these prelim... findings we conclude that certain forms of PVC dusts exhibit a high haemolytic potential because of the presence of a readily soluble, surface-associated agent. Exposure to this type of PVC dust may thus constitute an additional health hazard because of the increased biological activity of the dust. Work is in progress to determine the nature of the haemolytically-active agent by assessing a wide range of PVC dusts of which complete knowledge has been obtained of the chemical processing. The possibility that VCM is the active agent has been explored, but both samples tested were found to contain immeasurable amounts of VCM (<1 p.p.m.). Nevertheless, the present study indicates that the introduction of a washing procedure after the processing of KM 1 PVC dust would certainly reduce or abolish the haemolytic activity of this material.

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Mechanism of induction of haemolytic anaemia by phenylhydrazine

A compound with the optical spectrum of a ferrihaemochrome was produced when ferricyanide-oxidised phenylhydrazine was added to a solution of ferrihaemoglobin[1]. The three isomers of methylphenylhydrazine similarly resulted in ferrihaemochromes, but 4-hydrazinobenzoic acid did not[2]. The induction of haemolytic anaemia by a substituted phenylhydrazine was related to the reactivity of its oxidised form with ferrihaemoglobin to produce a ferrihaemochrome[2,3]. Further studies of the reaction of oxidised arylhydrazine with ferrihaemoglobin have established that the formation of a ferrihaemochrome-like product and the character of the optical spectrum of this product depend on the nature and position of substituents on the benzene ring of phenylhydrazine.

Substituted phenylhydrazine hydrochlorides were obtained from commercial sources or were synthesised by standard procedures. Each compound was purified by recrystallisation from 2 N HCl or ethanol, and the structure and purity of the recrystallised product were confirmed by its NMR spectrum, infrared spectrum, melting point, and analyses for C, H, N, and Cl. Oxyhaemoglobin solutions were deoxygenated by the passage of oxygen-free[4] nitrogen or helium, and the resulting ferrohaemoglobin was oxidised to ferrihaemoglobin by the addition of excess ferricyanide. To a solution of ferrihaemoglobin in excess ferricyanide, a solution of arylhydrazine hydrochloride in water or ethanol was added, and the optical spectrum that resulted was recorded with the Cary Model 17 instrument. The spectra obtained with 3- and 4-chlorophenylhydrazine were similar to that previously reported with unsubstituted phenylhydrazine[1]. The spectra obtained with 3,4- and 3,5-dichlorophenylhydrazine were distinguished by a more prominent absorption band in the red than the others. 2-Chloro- and 2,3-, 2,4-, and 2,5-dichlorophenylhydrazine resulted in spectra with a single broad maximum at around 540–550 nm and without a band in the red. 2-6-Dichlorophenylhydrazine, 2,6-dimethylphenylhydrazine, 2-hydrazin...