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A surface-active agent involved in PVC-induced haemolysis

MANY different forms of PVC powders are manufactured, the variety of the polymers depending on the initial constituents in the reaction mixture. Suspension homopolymers and paste (emulsion or microsuspension) polymers form 91% of the total PVC produced in the US. The latter products range in particle diameter from 0.5 to 30 μm and generally have ionic detergents added during their manufacture, whereas suspension homopolymers have no ionic detergent and are of larger particle diameter (60–180 μm). A preliminary investigation[1] had shown that some forms of PVC particles produced haemolysis *in vitro* and it was suggested that this effect was due to some residual component of the preparation (not vinyl chloride monomer) located on the surface of the particle. The following data support these findings and suggest that the surface-active factor is ionic detergent, used in the manufacture of the paste polymers.

A range of PVC samples was tested for haemolytic activity; fourteen of these were obtained from normal production processes and were used without further treatment; eight samples were specially prepared so that the associated detergent and other factors were different in each case; and a further four samples (20–23) were normal production materials which had been subsequently treated with components (other additives) of the reaction mixture. The results shown in Table 1 are for representative samples of the different types of PVC preparation. Suspension homopolymers have no haemolytic activity unless experimentally treated with detergent (compare samples 19 and 3). A considerable mass of this type of polymer is necessary to produce a significant haemolytic action, and the detergent, its relative concentration and the presence of other additives are also critical factors (compare sample 19 and samples 9 and 8).

All the paste polymers of normal commercial origin or those experimentally produced showed some haemolytic activity although this was low when the detergent code C (a derivative of a sulphated oleate ester) was present (samples 12, 20, 21 and 23). Particularly strong haemolytic potential was found for PVC paste polymers containing code B detergent, sodium dodecyl benzene sulphonate (samples 10, 14 and 18) and in two samples of unknown composition. Paste polymers containing the detergent sodium lauryl sulphate (code A) produced significant haemolysis but were not as active as samples containing detergent B. The haemolytic activity of paste polymers with code A detergent was dependent on: the particle size of the PVC sample (samples 17 and 7 at 10 mg exposure); the total concentration of the detergents associated with the particle surface (samples 15 and 7); and the presence of additives and other detergents (samples 7, 20 and 22).

It has been reported previously[1] that PVC-induced haemolysis could be markedly reduced if the sample was first washed with water or saline and that once removed, the surface-active factor was too dilute or did not retain activity in solution. The present studies have, however, indicated

Table 1 Haemolytic potential of different PVC samples

PVC sample code number	Sample description	Particle size (μm)	Detergent code	% Haemolysis produced by 5 mg	10 mg	20 mg	50 mg
3	SH (NP)	60–120	Absent	—	1	—	1
8	SH (EP)	60–120	B	—	1	—	2
9	SH (EP) low detergent	60–120	A	—	1	—	3
19	SH (EP)	60–120	A†	5	7	13	83
5	Vinylchloride–Vinylacetate copolymer	60–180	Absent	—	0	—	0
7	Paste polymer (NP)	0.5–30	A†	10	99	—	90
15	Paste polymer (EP) low detergent	0.5–30	A†	20	38	100	94
17	Paste polymer (EP, slightly larger particle size distribution)	0.5–30	A†	13	33	99	96
12	Paste polymer (NP)	0.5–30	C	7	10	—	—
20	Paste polymer (as 12 plus other components)	0.5–30	C+A†	7	12	—	—
21	Paste polymer (as 12 plus other components)	0.5–30	C*	5	15	—	—
22	Paste polymer (as 12 plus other components)	0.5–30	C+A	8	81	—	—
23	Paste polymer (as 12 plus other components)	0.5–30	C*	4	7	—	—
10	Paste polymer (NP)	0.5–30	B	100	97	—	—
14	Paste polymer (EP)	0.5–30	B	99	100	—	—
18	Paste polymer (EP) plus sulphosuccinate	0.5–30	B	100	98	—	—
24	Paste polymer (NP)	Unknown	Unknown	99	99	—	—

Haemolysis experiments were carried out as described previously[1] in quadruplicate.
SH, Suspension homopolymer; NP, in normal production; EP, experimentally produced; A, sodium lauryl sulphate; B, sodium dodecyl benzene sulphonate; C, sulphated oleate ester–sodium or potassium; †, at least two additives present; *, single additive present. PVC sample codes are Chemical Industries Association identification numbers.

that some samples (10, 14, 18 and 24) still retain haemolytic potential after washing in buffered saline and that the first wash from 3 of these samples (10, 18 and 24) retains haemolytic potential (45–100%). It is possible that after centrifugation, some very fine particles of PVC remain in the wash material and so account for the haemolytic activity in the wash. Diameter size distribution data suggest that 30–50% of particles in some samples are below 2 μm. The results obtained by further processing the wash material through a 0.2-μm filter before testing for haemolytic potential would support this hypothesis. The wash from one sample (18) still produced 100% haemolysis after passage through the 0.2-μm filter although this effect was reduced to 4% by passage through a 0.01-μm filter. It could also be argued, however, that detergent in the washings binds to the Millipore filter thus reducing the haemolytic potential of the filtered washings.

Most of the paste polymers containing the detergent sodium dodecyl benzene sulphonate (10, 14 and 18) retained high haemolytic activity (75–100%) after washing in saline, an effect particularly pronounced with sample 18 which also contained sulphosuccinate. This effect was not found with PVC samples containing sodium lauryl sulphate (10–20%) which is more readily solubilised. The haemolytic potential of the former samples, however, was totally reduced if they were first treated with ethanol.

Thus, in summary, it is evident that suspension PVC homopolymers have little, if any, haemolytic potential, whereas paste or emulsion powders produce haemolysis probably due to the presence of ionic detergent on their surface. The extent of reaction of these latter PVC polymers is dependent on their particle diameter and size distribution, the concentration and type of detergent and the presence of other processing additives. The results also indicate that certain detergents such as sodium dodecyl benzene sulphonate form a tight association with PVC particles and in this form they are highly reactive membrane-lytic agents. The physiological implications of the above findings are as yet unclear and therefore need assessment by further experimentation.

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Pioneer neurones in an insect embryo

Insect sense organs are produced by small groups of specialised epidermal cells[1]. The receptor neurones differentiate at the surface, so developing sensory axons grow inwards from the epidermis to the central nervous system (CNS). How do they find their way? During larval life the axons of newly differentiated sense cells combine with those of neighbouring receptors and thus are guided to the nearest branch of a peripheral nerve which carries them to the CNS[2]. At metamorphosis the axons of adult sensory neurones reach their central destination by growing along persistent larval nerves which are associated with the developing imaginal disks[3–5]. Thus with pathways to the ganglia already established, growth along existing nerves ensures the delivery of each generation of sensory axons to within a few hundred micrometres of their central targets.

Fig. 1 One of two pairs of axons (arrowed) seen in transverse section through the base of the antenna at the level indicated in the inset. At 105 h of embryonic life at 30 °C. Scale: 1.25 μm.

Just how the connection between the surface and the CNS is first established, whether by an outgrowth of nerves from the centre or by pioneering axons which grow inwards from the surface has never been shown, although some descriptions of embryonic development imply that the first axons to enter the developing appendages are growing outwards from the CNS[6,7]. Presumably these early centrifugal axons would provide a route for the later differentiating sensory fibres to follow in growth to the centre. Here, however, I report observations on the embryonic nervous system of *Locusta migratoria* which show that the first pathways between the epidermis and the central ganglia are formed by axons which grow inwards from peripheral neurones which differentiate early in embryonic life.

Before it rotates round the egg at blastokinesis[8] the locust embryo consists of a segmented band of cells lying on a bed of yolk with its ventral side uppermost. The ganglia develop as a strip on either side of the midline and the base of each appendage is a focus which axons must pass on their way inwards or outwards from the CNS. Timed embryos (30 °C) from a laboratory culture of *Locusta* were processed for electron microscopy[9] and transverse sections were cut through the base of the antennae and the limb buds to look for the early appearance of peripheral nerves.

An initial search revealed that from 110 h of embryonic life onwards, two pairs of axons appear at the base of the antenna (Fig. 1). Where do these axons come from and are they growing into or out of the CNS? Serial sections cut parallel to the long axis of the antenna show that each pair of nerves is produced by a pair of neurones at its distal tip (Fig. 2). From 95 h onwards the two cells of each pair put out processes which grow together to the base of the antenna and enter the embryonic deuterocerebrum. Further series of sections in both planes confirm that there are no other axons in the antenna at this stage, so the two pairs of distal neurones initiate the development of the antennal nerve. During subsequent development the axons of differentiating receptors accumulate about the two sets of pioneering fibres to form two bundles of nerves in the lumen of the antenna. Although the first pairs of axons are naked, the developing bundles become enclosed in the sheathing process of glial cells which appear at intervals along the antenna.

Slightly earlier in development, single pairs of axons appear at the base of each of the three thoracic limb buds. As in the antenna, each pair of nerves is produced by a pair of peripheral neurones. In the limb bud the single pair of cells lies at the anterior margin of the distal end of the lumen (Fig. 2) and from 90 h onwards both of these neurones put out processes which grow inwards to the CNS. Fifteen hours later the two nerves with an expanded growth cone enter the embryonic neuropile from the base of