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THE RELEVANCE OF PHARMACOPOEIAL PARTICULATE MATTER LIMIT TESTS

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ABSTRACT

The current pharmacopoeial limit tests for particulate contamination in parenteral solutions are critically reviewed and likely sources of contamination are then discussed in relation to the manufacturing processes and subsequent handling of the products. The site at which a particle may be lodged within the body and the possible reaction to the particle is described following recent reports in the literature which make a connection between the particle dimensions and host response. In addition, attention is drawn to the effects which may be due to the chemical identity of the contamination, suggesting that cellulose, asbestos and talcum require to be totally excluded from intravenous products. Inadequacies in the present

limit tests are emphasised, and directions for possible improvements are suggested.

INTRODUCTION

Although most pharmacopoeias and books of drug standards throughout the world express a pious hope that solutions intended for injection should not contain extraneous suspended particulate contamination, it has to be accepted that some contamination is inevitably present. The problem of defining what is allowable and what is not is a difficult one which, until instrumental methods of inspection became available, remained intractable.[1] In 1973 the British Pharmacopoeia[2] took the lead by imposing a limit test for particles in the majority of large volume (500 cm^3 or larger) solutions in current usage in the United Kingdom. The method was based on a decade of experimental work and clearly refers to the use of the Coulter Counter which, by this time, was readily widely available in industrial and hospital laboratories. However, despite the experience which had accumulated in the method and application it is doubtful if the Pharmacopoeia would have published a limit test if pressures had not been exerted following the so-called 'Devonport' incident and the resulting Clothier Report, in which the manufacturers concerned were criticised and a general tightening up of manufacturing practice and controls urged. As part of a similar world-wide movement towards improved quality a test for particulate matter was introduced in the United States Pharmacopoeia[3] in 1975, this time based on a microscope method for which there was relevant

experience in other fields, in particular monitoring contamination in hydraulic fluids and fuels. In Australia legislation has been introduced for a standard which refers to the H.I.A.C. light blockage method[4]. This is an instrumental principle of more recent introduction which also finds a wide application in areas outside the pharmaceutical field.

Quantification of the very small amounts of particulate matter to be found in pharmaceutical solutions for parenteral administration produced a revolution in their quality since, coincidentally, the methods for producing cleaner solutions and packaging them had already been developed and were available to the industry without inordinate costs. The pressure on the manufacturer in the shape of governmental legislation, inspection and control is increasing throughout the world, and there are now very few countries who cannot boast of a Medicines Inspectorate or some equivalent of the Food and Drugs Administration. In time it would seem probable that most books of standards and pharmacopoeias will have an objective standard for cleanliness of injection solutions which will be a mandatory requirement for small volume ampoules as well as the larger volume containers. It is possible, for example, that the World Health Organisation will become interested in the problem since many drugs used in under-developed countries are either made in or have originated from, the United States or European countries. It may be of interest to speculate that progress towards this end may not be entirely smooth. The European Pharma-

copoeia, for example, does not contain an objective standard of cleanliness at present. As a signatory to the European Convention the United Kingdom will have to conform to E.P. standards in the next (1978) edition of the B.P. It would appear that the lead set by the B.P. 1973 will be thrown away in order to come down to the standards of the lowest common denominator amongst the E.E.C member countries. This would appear to be a retrograde step at first sight and it is to be hoped that the members would attempt to come up to a standard, rather than compromise and allow the standards to drop.

Nevertheless, despite these minor and local set-backs in progress towards an overall improvement in quality, the movement is underway. It is therefore time to examine the situation and ask ourselves how relevant it is to the requirements of the patient.

CURRENT PHARMACOPOEIAL STANDARDS

In general terms it is convenient to compare the three main standards on the basis of the limiting number of particles per unit volume of 1 cm^3

British Pharmacopoeia 1973	not more than	1000	particles cm^{-3}	at	2.0μm
	" "	100	" "		5.0μm
U.S.Pharmacopoeia 1975	" "	50	" "		10μm
	" "	5	" "		25μm
Australian Specification	" "	100	" "		5μm
	" "	4	" "		20μm

As noted earlier, the British standard is based on the use of the Coulter principle although other methods are permissible. This method appears to work reasonably well and the manufacturers of the main instrument in this area have gone to some trouble to educate and train users, thereby assisting the intelligent application of the device, at least in the United Kingdom. Unfortunately this is not alway practicable in those other parts of the world where the B.P. standards also apply. The device depends on the presence of an electrolyte which, for some solutions such as dextrose injection, requires additional manipulation and risk of contamination as the electrolyte is added. It has also been criticised[5] on the grounds that the sample volume (0.5 cm^3) is small in relation to the volume of the container but this is hardly valid since repeat samples would always be taken to ensure statistical validity of the procedure and results. The H.I.A.C. device, and others using the light-blockage principle, do not require electrolyte and can sample the whole container contents if required. Until recently, there was some doubt about the ability of the instrument electronic circuits to respond with sufficient rapidity, thereby enabling valid counts at the 2.0 and 5.0 μm thresholds to be obtained. The manufacturers claim that this has now been overcome and this type of instrumental approach to the problem of measuring very small numbers of suspended particles may provide promise for the future, as is recognised by its utilisation in the Australian Specification.

It was therefore something of a surprise to find that the United States Pharmacopoeial authorities had fallen back onto the non-instrumental

microscope method developed some years previously for the evaluation of contamination in hydraulic fluids and aircraft fuels. The method involves filtration through a membrane surface and microscope counting of the collected material. By modern standards the procedure is elaborate, cumbersome and time-consuming and must be regarded as basically inapplicable on a routine basis. It is hardly objective since a great deal of operator selection and bias is inevitably involved, with attendant problems of fatigue and error. Some part of the objectivity could be restored if scanning microscopy were to be utilised for inspection of the membrane surface but the instrumentation here is generally expensive and relatively untried. The very complexity of scanning irregular particles of variable identity makes for difficulties of interpretation and there have been few reports of objective comparative trials of such devices. The pharmacopoeia does allow alternatives to be employed although the microscope becomes the reference method in the case of a dispute.

Inevitably the question of which standard is the most stringent will be asked. The answer can only be given by making some assumptions and extrapolations. The observation[1] that most size distributions of contaminating particles in intravenous fluids follow a log-log distribution law between the cumulative number and particle diameter threshold provides some assistance here. The B.P. limit test was based on a multipoint standard originally put forward in Australia. It was pointed out that this fitted a log-log plot and that the data could be manipulated to give a number, the S value, where

$$S = \frac{(\log \text{nos particles at } 1.0\mu m \text{ threshold} - 2.5)}{\text{slope of the distribution}}.$$

In fact, this equation fits a family of curves all passing through a common point. The S value for the earlier Australian standard and the current B.P. limit test is 0.5. This value would be obtained for any other linear distribution which passed through the common point of 316 particles at 3.16μm. This is not unreasonable since, in effect, it means that if a limit test were set at a S value of 0.5 instead of selected counts at selected thresholds, a sample with a low count of small particles would be allowed to have a higher count of larger particles, and vice versa, but the net effect would be to limit the total number of particles in the systems. The value of S which is obtained for a given distribution is a derived numberical integration of the total number of particles in the system. By making convenient assumptions that a straight line can be used to join any two points on a log-log distribution and the particles concerned are spherical, S values can be calculated for the various limits, as follows

B.P. 0.5

Australian 0.48

U.S.P. 0.67

Whilst this approach to comparing the various limit tests may be arguable, the fact that the U.S.P. test does not take into account particles below 10μm must be regarded as a weakness since, as the size threshold is reduced the number of particles present in a system generally increases with

a corresponding increase in the accuracy of the determination, quite apart from any physiological effects which may be ascribed to the smaller particles. The above comparison assumes the particles are spherical but if, as is possible, the particles were fibres with their effective linear dimensions at the prescribed threshold, the comparison becomes more difficult, and less valid.

Indeed, all the pharmacopoeias and legislated limit tests could be criticised for playing the 'number game' since, without limits of error, such limits have little real meaning. In an extreme situation with the B.P. test it would appear that a bottle with 999 particles cm^{-3} at 2.0μm is allowable whereas another with 1001 particles is not. Using an instrument to count the particles the limits of error within a single container are relatively small but the limits become appreciably larger when dealing with bottle-to-bottle variation within a single batch. There is limited information about the batch-to-batch variation which will clearly be much greater. The same problem is currently causing concern when discussing the permissible numbers of viable micro-organisms in solid unit doses and can only be tackled by accumulating sufficient information from all the manufacturers concerned and dealing with the problem from a statistical point of view once a norm has been established.

ORIGINS OF PARTICULATE CONTAMINATION.

Ideally a solution intended for injection into the body should be completely free from extraneous matter but this may not be realistic since it is virtually impossible to eliminate every trace of unwanted material. Good manufacturing practices can be designed to avoid contamination, viable or

inert, in the first place and careful attention to packaging can help to prevent particles being added to the product but it cannot be eliminated completely. Unlike microbial contamination which can be eliminated by sterilisation the only practical way of dealing with the particulate situation is to determine which materials are likely to be in any way harmful and design procedures which avoid the chance of these selected materials being present in the final product.

Sources and origins of contamination have been well reviewed elsewhere and this present review will be confined to drawing attention to more recent work which is relevant. Endicott[6], for example, categorised the sources of contamination under the following headings

1. The solution and its components
2. The package and its components
3. The process of manufacture and its variables
4. The devices used to administer the solution to the patient
5. Manipulations involving the medical team and the patient per se

1. The solution and its components

The preparation and storage of distilled water represents a continuous challenge and recent work on ultrapure water has demonstrated that organic constituents in the atmosphere can contaminate water very readily[7-10]. Of the known contaminants in air it would appear that hydrocarbons can condense on any exposed water surface and become dispersed into submicrometer sized

droplets with only slight agitation of the surface. Distillation or filtration methods do not appear to remove the droplets but may shear them to reduce the particle size temporarily. Since the droplets have a density below unity they do not settle out on centrifugation. In addition, it has been shown that the particle count increases after only a few days storage in sealed containers[10]. This observation may account for changes in the particle counts reported in parenteral products,[11] since it has been suggested that there may be many sub-detectable sized particles which can agglomerate or grow into sizes which are detectable.

The ability of Pseudomonas aeruginosa to grow and maintain high numbers of viable cells in distilled water constitutes a hazardous environmental situation, especially as cells grown under these conditions react quite differently to chemical and physical stresses than those grown in laboratory culture media.[12] The ability of micro-organisms to grow under unfavourable conditions is relevant to the use, and abuse, of spray cooled autoclaves, resulting in several authenticated cases of sterilised fluids subsequently becoming contaminated by viable organisms.[13,14].

2. The package and its components.

Even if it is possible to remove the majority of particulate contaminants from a solution, when it is packaged in a container the number and type of contaminants is increased. The commonest type of container is likely to be made from glass, either borosilicate glass (Type 1) or the soda-lime glass of Types 2 and 3, and manufacturers have long been aware of the shedding of

glass spicules when filled containers are autoclaved or stored for any length of time. Flakes can form in acid, alkali, water or alcoholic solutions and appear to be a result of an interaction between magnesium salts and silicate ions. The magnesium salts can be present in the stored solution itself or in the glass since concentrations of the order of 1 part per million are required. Similarly, only 20 ppm of soluble silica are required which may come from attack on the glass surface or be present as an impurity in the solution.[15]

A major source of glass particles comes from the breaking of glass ampoules and it has been suggested that these might be minimised by packing the ampoules under hyperbaric conditions so that the excess pressure blows the particles out when opening.[16,17] Other investigators[18] have examined alternative methods for opening ampoules, without finding a method which avoids glass particulate contamination altogether. It must be concluded that glass particles are a common contaminant but the actual numbers injected into patients must have been reduced considerably since the replacement of the all-glass syringe by the plastic disposable syringe.

Rubber, in one form or another, is the material of choice for closures on multi-dose containers, intravenous fluid bottles and plastic bags as well as plungers for disposable syringes. It must also be regarded as the principle source of extraneous matter in parenteral solutions - a conclusion drawn in the classical work of Garvan and Gunner[19,20,21] and adequately confirmed since that time. Rubber itself may be natural latex, synthetic neoprene or butyl polymers but it is hardly ever used on its own, requiring vulcanising agents,

activators, fillers, softeners, antioxidants, pigments, mould release agents and other materials Although rubber particles can be readily generated by simply moving a plug in the glass neck, it is the adjuvants which produce most of the problems. Particles may be contributed from the surface by leached or abraded materials, by cleaning techniques and rough container openings, by reactions between leached materials and the solution or by a direct reaction between the solution and the closure itself.[22] It would appear that many of the interactions reported are very subtle, often involving materials such as sulphur or zinc which dissolve to form insoluble salts or complexes with mater ials in the parenteral solution.[23] Waxes added to inhibit oxygen transport in the rubber formulation can roll out on the surface as small 'balls' and mould release agents such as silicones and zinc stearates are difficult to wash off and are transferred to the solution. A fresh rubber surface when releas ' from the mould will often carry an electrostatic charge which is sufficient to collect air-borne contaminants such as lint or fibres and these unwelcome contaminants are also difficult to remove.

The volume of air trapped in a moulded rubber product can be surprisingly high and the escape of air during the sterilization and sealing processes can be expected to cause fragmentation and rupture of the surface of the bung. The airspaces could presumably be the site of contamination of the interior of the bung by bacteria and fungi since it has been suggested that unless moist steam penetrates through the rubber completely these

bubbles could act as loci for subsequent contamination of the contents of the container, especially during the perforation by a needle.[19]

Protecting the solution from the rubber by inserting a plastic film such as teflon has been reported[11,22] to be a successful method of reducing the count and replacement of natural by butyl rubber may also assist in minimising the problem.

Plastic containers, especially those types blown without a seam and sealed without bringing the solution into contact with rubber, have been shown to be consistently cleaner[1]. These are mainly polyethylene ampoules made from a mixture of hard and soft polymers without the need for plasticisers. Most other plastics require plasticisers and this is especially true for the soft-walled polyvinylchloride bags widely employed for intravenous solutions and blood collection. Although the plasticiser used here, diethyl hexyl phthalate (D.E.H.P.), is apparently not released in significant amounts into physiological saline stored in P.V.C. bags[24], particulate matter in the colloidal size range has been identified as D.E.H.P.[25] These two statements are not necessarily contradictory since the limit of detection of the assay method was 0.24 p.p.m. whereas a particle count of 20,000 cm^{-3} corresponds to approximately 0.044 p.p.m. by weight; at least an order of magnitude lower. Blown plastic surfaces are often strongly electrostatically charged and attract air-borne contamination which can be released when solutions are placed in them. This has become a problem when considering

the manufacture and assembly of both parenteral solution bags and administration sets.

3. The process of manufacture and its variables

There are wide differences between similar products available from different manufacturers and variables during processing must be responsible for the differences. In the present state of technology it is reasonable to expect that solutions can be prepared with relatively few suspended particles above a size threshold of 5μm, the approximate equivalent spherical diameter of an erythrocyte when measured on a Coulter Counter, and there is a general movement towards this state. However, the improvement is unlikely to continue unless greater attention is paid to good manufacturing practices, in particular in the case of some of the smaller organisations who find the necessary capital costs difficult to find. In some ways the processes utilised in the pharmaceutical industry are not ideal for the purpose since they have been adapted from other industries. An example here is the pressure autoclave which was originally designed for the sterilisation of sealed cans. It has been suggested that it is unsuitable for the type of screw-capped glass bottle at present in wide pharmaceutical use since any imperfections in the cap and seal will result in contamination by the cooling water.[13, 14, 26-30] The presence of a head-space vacuum is often taken as an indication that the bottle has been sealed but, in fact, it also indicates that the bottle closure has loosened during the autoclaving to allow air to escape. Once the cap has been opened contaminated air can be drawn in and the possibil-

ity of a slow and insidious release of vacuum during storage must also be taken into account. The use of continuous autoclaves may reduce this problem on the large scale since it is possible to ensure that the cooling water is sterile but the capital investment involved here is sufficient to preclude their installation on a large scale.[1]

Filtration is, paradoxically, a source of particulate matter. A common filtration material used in chemical processing is the asbestos or Seitz type and, despite the fact that it is hardly ever used to-day for the final filtration or clarification of the parenteral solutions, asbestos particles and fibres have been reported in a number of injection products. There has been a tendency towards glass fibre filters but here again the possibility of small glass fibres passing through into the final solution must be taken into account. Final filtration through a cellulose-membrane filter is not a guaranteed method of removing small fibres from suspension and it would seem that adequate membrane or depth filtration is required at a much earlier stage in the production process. Filtration should not be regarded as an absolute process in which all particles above some pre-determined size are removed. On the contrary, it is substantially a statistical process in which the probability of a particle being removed requires consideration. Even membrane filters have a range of pore diameters and bubble-point tests only reveal the largest of these.[31,32] If simple straining were the only mechanism involved it is unlikely that the modified cellulose type of membrane filter would be entirely successful as a method of sterilisation since the absolute

removal of micro-organisms is required. In theory on this mechanism mycoplasms and viruses will pass through the usual sterilising grades of membrane filter but the possibility exists that they are also adsorbed on the pore walls, as are other colloidal materials.[33]

A recent study[34] of the packaging of labile materials such as antibiotics has demonstrated how bulk filling of dry powders appears to generate particles when compared to the process of terminal filtration of solutions followed by lyophilisation in the final container. This is not altogether surprising since every step in the extraction, compounding and filling stage can both generate insoluble particles by attrition and collect contamination from the air and containing vessels. Although lyophilisation in the final container is the preferred method of filling it is also clearly more costly and is not entirely devoid of problems since hazes can develop in some materials when they are reconstituted after freezing and drying.

4. The devices used to administer the solution to the patient

Large volume intravenous solutions require administration to the patient through cannulae and giving sets, usually made of plastics such as P.V.C. A number of workers[35-38] have demonstrated that administration sets may be sources of considerable amounts of particulate contamination. Although most of the particles are flushed out in the first 50 cm^3 or so of solution, in some situations the contamination levels may increase in an erratic fashion and the suggestion has been made that different results may be obtained by running either serum or blood through a set when compared with physiological

solutions[37]. In addition, particles emanate from cannulae, the number apparently increasing with the complexity of the structure[37]. Gross contamination of the solution may also be caused by coring of the rubber seal when the cannula is inserted.

Special problem may arise with some solutions. Sodium bicarbonate injections can be packed in plastic disposable syringes for emergency use and such solutions are often heavily contaminated with particulate matter. These originate from the plastic itself or insoluble carbonates are precipitated, especially if the original bicarbonate solution is stored in glass containers prior to filling the syringes.[39]

5. Manipulations involving the medical staff and the patient per se

Contamination of the intravenous fluid with viable micro-organisms often occurs during the setting-up at the bedside, followed by manipulation of the complete container, administration set and cannula.[40-48] This must also be true for the non-viable contamination although there is rather less direct evidence to support this contention. Contamination occurs as a direct result of poor aseptic technique by medical and nursing staff[48], and the evidence suggests that a surprisingly high proportion of the containers returning from the wards may be polluted, regardless of the type of container used[45]. Under these circumstances it is difficult to justify the cost of providing clean, sterile, products. The answer to some extent may lie in improvement of packs to make it more difficult to violate the integrity of the complete unit,

especially in situation s where additives are required[47], and to provide a better education of the staff who are involved at the patients' bedside.[43]

Once the cannula is in place a further opportunity for contamination occurs from the patient himself since tissue will be cored by the needle and often blood is aspirated back into the system[46]. It is doubtful if much can be, or needs to be, done about this situation but it does provide a complication when considering the use of terminal membrane filtration units immediately prior to the cannula[45].

It has recently been pointed out that the addition of drugs to the administration set is by no means the only source of contamination since the influx of unfiltered air during preparation or administration is bound to provide a break in the sterility[42]. Collapsible plastic bags and ampoules appear to have an advantage here since an airway is not required[43].

PARTICLE SIZE AND SITE OF LODGEMENT

At present there is no truly objective evidence that untoward clinical symptoms are produced by the introduction of excessive amounts of extraneous particulate material. However, the known biological responses to some particulate materials are clinically undesirable and needless.

Two main factors require to be taken into consideration when attempting to assess the importance of the contamination. The first concerns the chemical and physical identity of the contaminant since any biological response to the stimulus is conditioned to a large degree by the properties of the surface exposed to the biological environment. The second factor

involves the particle size and, to some extent, the shape, since these will influence the site of lodgement within the body. It has been suggested that blockage of a capillary might inhibit oxygenation or normal metabolic activity, resulting in cellular damage or tissue death. In practical terms the lung, for example, has adequate collateral circulation although the possibility of injury by occlusion might exist for more critical areas such as brain, kidney or eye. The inter-relation of the circulation system is such that most particles introduced into the intravenous blood supply will be strained out in the lung capillary beds. Unfortunately, there is also adequate evidence[49] to suggest that large A-V shunts do exist in the human lung, allowing the pulmonary capillary beds to byepass particles of up to 400 μm in diameter into the systemic circulation. Thus, it is apparent that the lung need not be the final resting place of the intravascular particles administered in parenteral solutions. Fluids administered interarterially can, of course, reach organs supplied by that segment of the arterial tree.

Excluding the actual physical effects caused by stoppage of the blood supply to specific tissues, any particle injected into tissues may elicit a response in the host. If a foreign particle lodges in a vessel an inflammatory reaction may result, similar to the type of response seen when a splinter of wood is driven into the tissue. The seriousness of the reaction depends on whether or not it is self-limiting, or progresses to a neo-plastic or cancerous response. It is even possible that the foreign material may produce a delayed response if there is an allergenic reaction, followed by reintroduction of the

same material at a later date. Thus, it would not be anticipated that all contaminants are equal in their effects on the body. However, irrespective of the secondary effects produced, it is probable that, once through the lung capillary bed, the final site of lodgement will be determined by the size of the particles concerned. In general the largest particles tend to lodge in the lung capillaries, the smaller ones in the spleen and hepatic lymphatic nodes and the finest in the liver. In this connection the suggestion has been made that small (<10 μm) silica particles are particularly active, producing a progressive, coarsely nodular cirrhosis of the liver, attended by extensive destruction of the parenchyma which may be followed by some regeneration in certain areas.[50]

Recent studies of clearance kinetics and distribution of particles have concentrated on the use of polystyrene divinylbenzene-polystyrene copolymer produced as spherical particles with a range of diameters and considered to be biologically inert. Schoenberg et al.[51,52] injected doses of up to 1.6×10^{12}/kg of particles varying from 0.514 to 1.17 μm and found that the efficiency of clearance was unaffected by either particle size or dosage. More than 90% of the particles injected were cleared within the first 90 minutes but the remainder could take up to 18 days. Later it was suggested that the slower rate of particle clearance represented equilibration between phagocytosis and re entry into the circulation from the reticulo-endothelial cells of the liver and spleen. Phagocytosis requires energy and the oxygen consumed in proportional to the number of particles ingested by

the cells and their size[53]. Using a range of particle sizes it has been shown that particles smaller than 0.264 μm produce no significant increment in leucocyte respiration. A similar conclusion was reached for the site of deposition, particles smaller than 0.4 μm being undetected in any tissue.

On the other hand, particles larger than 10 μm produced no histopathological effects except for infrequent myocardial degeneration but a positive correlation existed between the proportion of fibres between 20 and 320 μm in length and the incidence of mesothelioma[54]. A sigmoid relationship was postulated, linking the incidence of growth in vitro and particle length and it was further postulated that fibres smaller than 20 μm induced neither in vitro growth or in vivo mesothelioma. It has been suggested[55] that solid foreign bodies may induce two basic types of hyperplastic reaction, according to their size. The effect of fibres longer than 40 μm depends on the provision of anchorage, which predominantly stimulates mesenchyme, whilst fibres below 20 μm in length stimulate monocytes alone. A condition of persistent hyperplasia in fibroblasts or in cells of the reticulo-endothelial system respectively would then be a necessary (though possibly not sufficient) component of the mechanism of carcinogenesis by solid implants.

In opposition to this view, attention has been drawn to the fact that, irrespective of their mineralogical origin and nature, all fibres with a diameter of below 0.5 μm may produce tumours if injected into the pleural cavity of rats.[56] If substantiated in other species this must indicate an

increasing hygienic risk if fibres of such small diameters are to be manufactured and employed in, for example, the construction of glass-fibre sterilising filters where there would be more than a reasonable chance of fibre fragments passing downstream into the product.

The overall effect of particle size, irrespective of the chemical identity of the material constituting the contaminant, would therefore appear to be a little uncertain. It seems reasonable to conclude that caution is certainly required if the parenteral solution contains large fibres ($>10\mu m$), and rounded particles below about $0.5\mu m$ are unlikely to be a problem, although fibres of this diameter may well be.

CHEMICAL IDENTITY AND HOST RESPONSE

Whilst it is probably true to say that a very wide variety of materials have been reported in intravenous solutions as contaminants, relatively few have been implicated up to the present in a pathological response to the contamination. However, at least one of these, cellulose, has been associated with the production of granulomatous reactions and serious doubts exist about another, asbestos, and both of these materials can be considered to be sufficiently common as to constitute a possible threat to safety.

Cellulose

Garvan and Gunner,[19-21] in their pioneering investigations, were able to point to an association between granuloma production and the presence of cellulose or starch particles. In seven granulomas removed from a patient who had received only between 100 and 150 cm^3 of a heavily contaminated

saline solution two contained cellulose particles and two starch grains. Later work using rabbits showed that when granulomas were produced, every one contained a cellulose particle, usually of the naturally occurring bast fibres. Later work in America[57] supported the observations of Garvan and Gunner although the massive injections of ground filter paper and plastic materials were possibly on the heroic scale. The character of the pulmonary granulomatous lesions produced were typical of a classical foreign body reaction and did not differ according to the type of material associated with the reaction. A more recent systematic investigation of the effect produced by cellulose in mice suggests that the majority of injected particles of cellulose remained within the blood vessels although the numbers of particles entering the systemic circulation was not only governed by the limiting diameter of the pulmonary blood vessels[58]. This could be anticipated since A-V shunts are known to exist. Particles were detected in the sections of heart, kidney, brain and liver but granulomas containing cellulose particles were only detected twice in the kidneys and more frequently in the spleen, the remaining tissues being uncertain. This was attributed to the fact that such reactions required time to develop and further work is clearly required on this aspect.

Other evidence in this area tends to be somewhat circumstantial. For example, over a period of 20 years only six cases of embolism associated with cotton fibres were found post-mortem in patients, but in all cases the patient had received large volumes of intravenous fluids prior to death[59].

Using animals, evidence was obtained for changes in arterial walls produced by granulomata distending the lumina, tearing the walls and eventually resulting in scar tissue. This type of reaction has been identified in human cases of granulomata due to foreign bodies. Studies[60] in post-mortem examinations from children who had received large volumes of parenteral solutions provided evidence of a direct relationship between the volume of fluid administered and the number of cotton fibres per unit area of tissue examined. A more distinct correlation was also obtained between the degree of reaction identified and the duration of therapy. In one case it was suggested that the extensive multiple emboli found in the lung producing complete occlusion of the vessel lumen could have been produced by the associated cellulose particles providing a nidus for the infarcts. Cellulose particles have certainly been associated with emboli resulting in death following carotid angiography or selective arteriography[61].

The importance of avoiding microembolisation and infarction was emphasised as long ago as 1951 when it was pointed out that "the injection of even minute foreign bodies may be fraught with serious consequences and great care should be taken in the preparation of solutions or materials for intravenous injection in order to make certain they do not contain foreign substances"[62]. The problem has not diminished in the past 25 years and it has to be admitted that cellulose particles still constitute the commonest form of particulate contamination.

Asbestos

The strongly reactive nature of the surface of asbestos fibres makes it an effective filtration medium and its use, at least until very recently, was widespread in pharmacy as well as other industries.

The dangers associated with the inhalation of asbestos fibres, in particular crocidolite asbestos, have been appreciated since the early part of the present century and is the subject of environmental health legislation in almost every country in the world[63]. The dangers which may arise from its introduction into the body by the parenteral route are less certain.

In experimental animals it has been demonstrated that the injection of large quantities (typically 30-40 mg) of all varieties of asbestos can produce neoplasms at the injection site and along draining lymphatic channels[64-68]. Mesotheliomas of the pleura and peritoneum are common in those individuals exposed to asbestos dusts and these may be produced experimentally by pleural implants. However, tumours of this type may also be induced by implanting glass or aluminium oxide fibres similar in size to the asbestos fibres, suggesting that the tumorigenic potential of these particles is a consequence of their size and shape, not of their chemical composition[56,69]. It seems reasonable to ask if there is any property of the cells which would cause them to react to the dimensions of solid bodies. In fact, one candidate may have recently presented itself since it has been reported that fibroblasts, except under special conditions, require the support of a solid substrate for growth. This property has been termed

anchorage dependence[68] an growth does not occur unless the particles can attach themselves to solid particles above a critical dimension[70]. In addition it would appear that a minimum length of fibre is required to support anchorage-dependent growth of fibroblasts in tissue culture[71].

Whilst at present somewhat speculative, it may be that further work will show asbestos fibres are not unique in producing a potentially serious response in the body and that the response produced depends on having particles of certain minimal dimensions present. This consideration may call into question the value of studies where chrysotile asbestos particles have b been reported[72] in a number of widely employed parenteral drugs. The amounts of asbestos were estimated to exceed micrograms in some instances and this quantity is high in relation to levels allowed in other environmental circumstances. The significance of this amount of contamination may be evaluated in the knowledge that 10^{-9}g asbestos represents 10^{4} fibrils of a size seen typically in drug samples of 40 nm x 10 nm. If the fibroblast attachment reaction were the only problem then it may be that this type of contamination would not be important but the highly reactive nature of the asbestos surface may also mean that a response can be triggered, irrespective of the particle size. In this case, every particle must be regarded as a potential hazard.

Glass

Since glass particles are known to constitute an important source of contamination they have been investigated systematically. Results of these

investigations tend to point to a common conclusion in that occasional contamination arising from the use of glass ampouled solutions or all-glass syringes produces no significant pathological response in animals and truly massive doses are required to produce any reaction[73]. Very fine glass particles may produce a reverse silicosis rather than embolisms. Injection of silica particles produced no macroscopic evidence of disease at autopsy and microscopically both lungs and kidneys had capillary emboli composed of aggregates of silicotic particles which, however, later dispersed[50,74]. Distinctive fibrotic lesions were produced in the liver, spleen and lymph nodes of rabbits following intravenous injection of silicious dust suspensions where the particles were 0.8 μm, or less, in size.

No evidence for foreign body reactions, granulomata production or pyrogen reaction would be obtained after the injection of glass particles[75] and the relevance of recent reports[76] of excessive glass contamination arising from ampoules becomes uncertain.

Talcum

Talcum powder has been employed as a filter aid and mould-release agent for rubber goods. It is chemically inert but produces a rapid tissue reaction in the form of a foreign body granuloma. After entry into tissues foreign agents may produce a localised histocytic reaction[77] and a characteristic variety of this reaction produced by talcum is similar to silicosis granulomata[78]. Once the particles enter the tissues a reactionary productive inflammation is set up which becomes permanent and progressive and may be

provocative of almost insuperable complications. The nuclei of the granulomatous nodules produced by talcum particles contain lymphocites, epitheloid and giant cells. The phagocytes are immobilised without being destroyed so that they are not carried into the blood stream and this leads to a circumscribed granuloma production at the original site of injection.

The dangers associated with talcum introduced into the body by injection or from lubricated surgical gloves have been appreciated for over 40 years[79] and it is clear that under no circumstances must it be allowed to come into contact with parenteral products at any stage of production or manipulation.

Other, less significant, contaminants

Allergic reactions to nickel have been reported from infusion solutions[80,81]. Since nickel is a common constituent of many alloys and plated metal articles an anaphilactoid react may be provoked if a patient already has circulating antibodies. Embolic granulomatous lesions have been found in mice accidentally injected with fragments of hair and epidermal cells[82] and carbon black produced micro-emboli in rabbits.[83] Deep injection of rubber fragments produced by needle puncture of multi-dose closures has been reported[84] to produce reactions in man and this type of contamination will remain whilst the present systems of presentation and injection of solutions continues to be used.

DISCUSSION

Without attempting to become involved in an argument as to the usefulness of the official compendia, it would seem that we do need to

question some of the assumptions about the relevance to the pharmacopoeial limit tests when considering particulate contamination. If really pressed it may well be that we would finally have to admit that all a pharmacopoeia can offer is guidelines which provide some sort of measurement of quality or index of cleanliness which will be used by the manufacturer on one hand and government controlling agencies on the other. However, none of us must lose sight of the fact that neither situation is the case since, in the final analysis, the sole purpose of any book of standards is for the protection of the patient or recipient. The patient is uninterested in the technicalities and we need to ensure that the best possible product is always available in the light of current knowledge and experience.

Nevertheless, limit tests are not carried out immediately before a product is administered to the patient so that, in practice, the prescribed tests are only useful to the manufacturer and customer in the sense of the purchasing authority. It is unrealistic to expect that the patient will be protected up to the point of delivery. Indeed, evidence is beginning to accumulate which suggests that contamination, especially with viable micro-organisms, may often arise through faulty manipulation and technique by the medical and nursing staff. To go through the technically exacting process of producing a near perfect product, only to find it polluted at the point of administration, will be a discouraging and traumatic experience for any normal individual and it is only too easy to call for better education of the other members of the team. However, should we not be looking more

closely at the containers in which our solutions are provided so that it becomes more difficult for the often overworked medical staff to contaminate the product? As academic outsiders, it would seem to us that a complete re-examination of this particular problem is urgently required even if we ourselves do not have any suggestions to make. The compendia make few demands about the containers and yet it would seem that most of the problems associated with the contamination and use of parenteral solutions are concerned in the design of the equipment used to store and administer them.

The inescapable conclusion must be drawn that the interests of the patient will only be served in the short run by closer attention to the tests themselves to ensure that cleanliness is maintained. A good product will only be produced under good conditions since, as Miller[85] noted in 1966 - "quality cannot be tested into a product; it has to be built in."

Whilst it is true that clinical information about the harmful effects produced by particulate matter in intravenous solutions is sparse on the whole, there are some indications and pointers. Rather than limit tests the pharmacopoeiae should be publishing specifications designed to ensure that all particles have been eliminated completely. This is an ideal which will not be fully realisable. Nevertheless, by proceeding in systematic steps it should be possible to get closer to the ideal.

The first step in this direction has to be to design tests eliminating the contaminants which, by their chemical identity, elicit foreign body

reactions in the host. Here the main contestants appear to be cellulose, asbestos and talcum. The first of these is at present extremely common and represents the biggest technical problem. Fortunately, cellulose particles and fibres are generally birefringent and samples from a batch may be detected using visual inspection under polarised light. This method will only detect the largest and possibly the most hazardous of the fibres and is utilised in the B.P. monograph. However, the inspection method required to be combined with the U.S.P. requirement that every container showing signs of contamination must be rejected. In order to detect the smaller fibres the filtration and microscope inspection method requires to be used and here a simple modification of the U.S.P. method would be adequate for the purpose. Similarly, the asbestos and talcum particles could be detected using the appropriate identification procedures. In these cases containers would have to be selected from a batch, with all the attendant problems of selecting representative samples. From this aspect alone better guidance is required from the pharmacopoeia as to the actual meaning of "statistically sound sampling plans". The identification of samples within a batch which contains asbestos or talcum provides a considerable difficulty, especially as only very small quantities are likely to be involved. It might be that this would be a suitable project for the application of academic talents. Whichever method is devised, it will be unlikely that it will be readily available to the smaller producer. On present technology any method is going to require a concentration stage, probably by centrifugation or membrane filtration, followed by

scanning electron microscopy with x-ray scattering analytical facilities, or transmission electron microscopy and selected area electron diffraction.[86]

The division of contamination into intrinsic and extrinsic types enables a distinction to be drawn between materials which may be either added accidentally or left in during the manufacturing process.[1] Both are influenced by the manufacturing practices of a producer but the material left in the product, the intrinsic contamination, is generally smaller in size and is suitable for instrumental analysis. The extrinsic material such as lumps of rubber or plastic, fibres and the like, tend to be much larger in size and smaller in number, making instrumental detection less likely and visual inspection necessary. There is no question that visual inspection at all stages of each individual ontainer will always be required in order to eliminate the chance contamination. However, by far the best method of monitoring the intrinsic material and the manufacturers' good manufacturing practice, or lack of it, will have to be instrumental and this is where the official guidelines require sharpening.

Nevertheless, these improvements in quality control procedures are still some distance away. It should not be unrealistic to expect that solutions for parenteral use should not even come into contact with undesirable materials and this places the problem into the realm of good manufacturing practice. The patient or customer requires better protection and we need to ask if the pharmacopoeiae are providing this. On the face of the present tests there must be reasonable doubt. Confidence in any counting procedure

can only be achieved if the results have real meaning. Doubts have to be expressed about methods which yield low counts per unit volume since slight variability in the count can represent a wide experimental error. There is need to count at lower size thresholds where the counts would be anticipated to be higher. The instrumentation is already available for this purpose. The Coulter principle appears to be valid down to equivalent spherical particles of 0.5μm and the new generation of H.I.A.C. instruments appear to be capable of operating down to 1μm. The next improvement in pharmacopoeial limit tests must be to count at lower thresholds, say at 1μm and 5μm in order to build up experience of the methods. These counting procedures are, in effect, monitoring the effectiveness of a manufacturers' "good manufacturing practices". Already experience with the B.P. method suggests that some manufacturers are quite capable of producing solutions with counts as little as 20% of the allowable limit. In this situation we can say that one manufacturer is better than another so it is to the obvious benefit of the consumer if standards can be improved by tightening up the official specifications.

CONCLUSIONS

To summarise, it is possible to conclude that the patient requires better protection from hazard during intravenous therapy. From a fundamental point of view, the design of the container and the administration apparatus requires re-thinking so that contamination during and after preparation and immediately before administration becomes more difficult, if not impossible.

Certain types of contamination should be eliminated completely and this will require more fundamental investigations to devise suitable tests. In the meantime, the present pharmacopoeial tests require improvement in their objectivity and this will only be achieved by using the newer generation of instruments to count at lower size thresholds. Once experience has been built up, allowable limits should be reduced to ensure that manufacturing practices, equipment and materials are maintained only at the highest standards of the day.

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