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Contamination of Pharmaceutical Products

IRA FLAUM

Received from the *Food and Drug Administration, Department of Health, Education, and Welfare, Brooklyn, NY 11232.*

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The ubiquitous problem of contamination of pharmaceutical products is well known to anyone involved in drug manufacture, control, or distribution. In fact, the types of contaminants, their relative hazard to human health, the sources of contamination, and the methods for prevention and detection of such contamination are so varied and numerous as almost to defy compilation. This paper attempts to condense some of the more pertinent literature as well as to put forth possible paths of action with the hope of reducing the future incidence of contaminated drugs.

An indication of the importance of this problem is the fact that the Federal Food, Drug, and Cosmetic Act of the United States (1) appears to place even more emphasis on what should not be included in a drug or its packaging than on what should. In making this point, Wright (2) mentioned five specific conditions listed in the Act as constituting adulteration and not related to the potency of the active ingredients. Although the Act does require drugs to meet standards of potency, quality, and purity of active ingredients, these standards are more measures of ingredients that do not belong in a drug than of those that do.

The point is reinforced when one examines the fact that official compendial standards (3, 4) invariably provide for only one assay method but usually several other tests for the identity of specific expected impurities as well as the presence of materials not included in the formula.

NATURE OF CONTAMINANTS

Mechanical Contamination—Mechanical contaminants are foreign extraneous materials that get into drugs (5, 6). They may be easily seen and explained, such as an iron screw in a drug substance for ingestion or chips and fragments broken from processing equipment, and can be incorporated into products during manufacturing. They may be environmental such as metal fragments, wood slivers, rust, rubber particles, plant fibers, lint, hair, glass, ashes, starch, talc, or asbestos. They may come from the packaging containers such as pieces of glass, plastic, or metal.

A persistent contamination problem continues to be that of metal particles in ophthalmic ointments which result from the stamping and thread cutting operation (7). The potential hazard to sight is obvious. Recognizing this problem, the USP set limits for the number and size of discrete metal particles allowable in ophthalmic ointments (8).

A problem creating much current concern is that of particulate matter (9–29), especially asbestos (30, 31) in parenterals. The inhalation of fibers of the chrysotile form

of asbestos has been associated with serious health hazards, including pulmonary fibrosis and cancer. It is now known that much less exposure than that received by asbestos workers, as little as that received by a person living in a household with an asbestos worker, is associated with neoplastic disease.

To remove foreign material, asbestos filters have been used extensively at many stages of the pharmaceutical manufacturing process, including the final filtration of parenterals. Because of its rapid filtering properties, minimizing bacterial growth, the chrysotile variety is most often chosen. When used in this way, chrysotile fibrils tend to flake off into solutions, with the possibility of finding their way into the human bloodstream and settling at points in the body.

The suspicion that these particles may be foci for disease is supported by experiments with animals and indications of increased GI cancer among asbestos workers. In view of this hazard, the Food and Drug Administration (FDA) ruled that by September 14, 1976, parenteral manufacturers must eliminate asbestos or other fiber-releasing filters from their manufacturing process unless it is impossible to produce a particular drug otherwise. In that case, subsequent filtration with a nonfiber-releasing filter is required (32, 33).

Coincidentally, talc, widely used in the pharmaceutical industry as a tablet excipient, often contains asbestos minerals. There are no current regulations concerning the purity of talc used in this manner. Blejer and Arlon (34) suggested, based on findings relative to talc-coated rice, that analyses for asbestos contamination be performed on talc that can be expected to be significantly exposed to humans. Ominously, they also suggested that studies be made to determine the carcinogenicity of pure talc itself.

Starch, plant fibers, glass particles (35), lint, and undissolved crystalline material from the formulation are other common particles found as contaminants (36–39). Cellulose fibers or particles of powdered plastics, injected intravenously, can cause granulomas and microthrombi in the lungs of rabbits and dogs (6). Autopsies on patients who had received large amounts of intravenous fluids indicated similar lesions (40–42). The particle sizes involved in these observations are about 50 μm, the limit of unmagnified visibility. Medical disagreement surrounds the question of what size particle is hazardous. It is clear, however, that the risk with particulate matter is real and that parenteral drugs should contain as few extraneous particles of any kind as possible.

Chemical Contamination—Chemical contamination occurs in many ways (43). Material obtained from natural sources may contain important contaminants, even after purification. Natural pituitary hormone preparations, corticotropin (adrenocorticotropic hormone), and human growth hormone (HGH) have been found to contain the vasopressor antidiuretic hormone (ADH). This finding explains the occurrence of water retention among some patients receiving 48-hr intravenous infusions of corticotropin or human growth hormone (44).

Incorrect ingredients are sometimes added to the product by mistake, such as the use of an excipient not called for in the formula (2). The addition of an incorrect physiologically active ingredient is, of course, more serious. In deliberate ingredient substitution, there is a different kind of contamination, one commonly known as "sophistication." This substitution would ordinarily be done for economic reasons, such as the substitution of a cheaper synthetic for a "natural" ingredient (synthetic B vitamins for yeast extract), or for reasons of convenience based on the greater availability of one excipient over another.

Because of limitations in purification steps, precursors, reactants, and intermediates from the synthesis of the drug may occur in the final product beyond limits defined by safety and regulations. Soluble equipment components and interaction and degradation products also may be found (45). To control these impurities, tests are instituted, either as called for by the official compendia or as deemed necessary by the manufacturer. These may be tests for specific anions, specific cations, nonspecific impurities, or drug congeners. For example, the USP monograph for chlorpromazine requires a test for an objectionable congener, 4-chlorpromazine, a structural isomer.

Similarly, limit tests are often devised for contaminants expected in very small amounts in the final product, originating from some vessels, catalysts, pipes, stirrers, filters, or other utensils used to process or handle the drugs. These are usually tests for specific metals or for general classes of compounds such as heavy metals (46) or halides.

If nonmetallic processing equipment is used, tests for oxidizable or foreign organic substances may be required.

Drug breakdown and interaction (47, 48) may necessitate tests such as those for salicylic acid in an aspirin product (2). Peculiar and original formulations may often lead to unforeseen contamination problems. Wright (2) cited the reaction between the active ingredient in isoproterenol injection and an added antioxidant, sodium bisulfite, in which the isoproterenol side-chain hydroxyl group is replaced by a sulfo ($HOSO_2$—) group, creating a therapeutically inactive compound. Wright (2) also described the effect of warm humid storage conditions on tetracycline hydrochloride capsules containing citric acid. Not only is tetracycline degraded, but the situation is aggravated by the fact that one degradation product, epianhydrotetracycline, is quite toxic.

Breakdown and contamination after packaging may occur from reactions between liquid drugs and rubber closures (49) or plastic containers (50, 51). Sulfides, added to rubbers as vulcanizers, react with mercury preservatives to form mercuric sulfide. Plastics may release salts into solutions or absorb active ingredients.

Crude drugs (52), although rarely dispensed in this country, have been contaminated with organochlorine pesticides. Noguchi *et al.* (53) found cyclodiene insecticides and benzene hexachloride isomers, including chlorophenothane (DDT), in many Far Eastern commercial crude drugs and in their processed products.

Herlihy (54) described an isolated incident of the contamination of a medical oxygen cylinder. Although the source of the problem was not found, the experimental combustion of polytef yielded products whose IR spectra were identical to the contaminants.

During the last decade, there has been increased awareness of another problem, that of cross-contamination. This problem may be described as the unintended,

unexpected presence of one drug in another, generally resulting from the spread of dust particles arising from the manufacture of other drugs in the area or from equipment not completely cleaned after use in the manufacture of other products (55).

Historically, the increased attention paid to this problem began with attempts to control penicillin cross-contamination (56). The unusual concern shown penicillin is a result of its allergenicity. An individual receiving very small doses of penicillin as a contaminant in another drug may become sensitized, setting up an allergic response or even a severe anaphylactic shock when a full dose (or less) is adminstered (57). Thus, as a result of an ad hoc Advisory Committee on Penicillin Contamination convened by the FDA Commissioner in 1965 (58, 59), penicillin limits were fixed at 0.5 IU (0.3 μg) for oral drugs and at 0.05 IU (0.03 μg) for parenteral drugs for the highest single recommended dose. Proposed new regulations would reduce the tolerance to zero (60). Ironically, penicillin's allergic properties may be traceable to penicillin itself being contaminated. It has been suggested that macromolecular proteinaceous and nonproteinaceous substances, as well as polymers of penicillin, present in trace amounts are important in precipitating allergic reactions (61).

Penicillin is usually used as a very fine, dry powder, easily becoming airborne and migratory (62). It is particularly difficult to clean a penicillin production facility because the drug clings electrostatically to processing equipment (63). Also, the weight of a penicillin tablet is typically about 70% active ingredient, giving a very high yield of penicillin in the dust produced by the tableting operation (55).

Some investigators agree that, because of its high potency and ease of dissemination, if penicillin could be prevented from contaminating other products in a plant, cross-contamination in general within that plant would be under control (56, 64). In this regard, penicillin may be considered as a test substance, since it is possible to detect and quantitate it at very low levels by the simple and specific agar plate-zone inhibition technique (65).

Microbiological Contamination—Regarding microbiological contamination, injections, intravenous infusion fluids, and drugs intended for ophthalmic use are required to be sterile; the presence of even one live microorganism, of any type, is not tolerated (66–68). The literature is replete with unfortunate violations of this requirement (69–74).

The great danger in contaminated intravenous infusion fluid was painfully learned during the "Devonport Incident" in England (75–80). At least four, and possibly six, people collapsed and died as a direct result of receiving 5% dextrose infusions following surgery. A committee of inquiry deduced that an autoclave that left air in the chamber during the pressure cycle was at fault. The dextrose bottles were stacked in three layers, and the effect was that the bottles in the lowest layer were not sterilized. Bacteriological control samples were taken only from the uppermost layer, preventing the detection of the problem. More than 1 year passed before the solutions were administered, allowing heavy bacterial growth and accumulation of endotoxin. The cloudy solutions that resulted were not recognized by the hospital staff. Eventually, *Klebsiella aerogenes*, other Enterobacteriaceae, *Erwinia herbiocola*, *Pseudomonas thomasii*, and coryneform organisms were identified in the bottles.

The microbiological condition of drugs not intended to be sterile represents a different problem (81–91). About 200 cases of salmonellosis in Sweden in 1966, traced to thyroid tablets contaminated with defatted thyroid powder containing over 30 million bacteria/g, proved to be one stimulus to study in this area (92). Yet a random sampling of 660 production batches of various drugs marketed in Sweden revealed remarkably high levels of bacteria and fungi not apparently associated with disease. Counts of up to millions per gram were common. Of 160 tablet batches, 38 contained coliform bacteria, an indication of poor sanitation somewhere in the manufacturing procedure.

Just why one set of conditions represents a hazard to health and another does not is defined by certain parameters, namely, the intended use of the drug, the pathogenicity and virulence of the organisms in relation to the immunological state of the patients, and the total numbers of organisms present in the preparation. Other parameters to be considered include the possibility of changes in pharmacological activity, side effects, and shelflife resulting from the presence of microorganisms.

Ignorance of some of these parameters resulted in reduced sight and the loss of an eye from the use of an ophthalmic ointment containing two broad spectrum antibiotics and a steroid (92). The manufacturer failed to use a preservative, wrongly thinking that the low water content and two antibiotics would prevent bacterial growth. What resulted were tubes heavily contaminated with *Pseudomonas aeruginosa* in pure culture. Since 1965, the USP has required sterility in solutions and ointments for ophthalmic use (66–68).

PREVENTION OF CONTAMINATION

The prevention of contamination, particularly cross-contamination, is a problem whose solution is best summed up by Elias' (93) three I's: "The integrity and identity of the product are assured by isolation." However, this simple statement should not obscure the fact that the problem is multifaceted (63).

With particular reference to the prevention of microbiological contamination, Moller (94) listed the following key areas: premises, personnel, raw materials, working processes, formulation, and control. Molin (64) added equipment and packaging. The proper control of these factors will serve equally well to prevent chemical and mechanical contamination.

Premises—"Premises" refers to the layout and general construction of the plant (94–101). Production areas and adjacent formulation, storage, and cleaning facilities, as well as product transportation routes, should be separated from each other as much as possible (64). They also should be isolated from similar areas used in making other products. In fact, the current dominant guiding principle in plant design calls for the subdivision of production areas into cellular units where it is possible for personnel to pass physically through production departments without going through the actual working areas. Also, admission to various areas is possible only through lock arrangements, with concurrent facilities for the changing of clothes.

The importance of this cellular isolation varies with the

particular procedure involved. For the production of some drugs, separate rooms are required. For others, such as the redoubtable penicillin, separate rooms and, additionally, equipment not used for any other product are necessary. The isolated rooms are kept at negative air pressure relative to the surrounding areas to prevent the escape of particles to other parts of the plant.

An additional important feature in a modern, well-designed plant is a suction system with inlets to remove dust at crucial points in the manufacturing process such as tableting and coating.

Also important are the materials used for the interior surfaces of the production buildings. Smooth-surfaced, easily cleaned, and seamless polyurethane is a popular and serviceable material.

The judicious use of underpressure and filtered, recirculated air throughout the plant helps prevent cross-contamination and bacterial spread (102), as does the HEPA filter, which is usually used where sterile air is a requirement (103–105).

The last decade has seen a great increase in clean room design utilizing the principle of laminar air flow in the pharmaceutical industry (106–115). HEPA-filtered air is blown uniformly through a work area or entire room and exhausted on the opposite side. This system can be done horizontally or vertically. The resultant sweeping effect leads to a considerable improvement in air cleanliness over that achieved by conventional HEPA filtration. However, despite its proven utility, the laminar flow technique must be examined carefully for effectiveness in a particular situation; it should not be thought of as a panacea (116).

The general subject of "clean room" technology was discussed previously (117–125). Kinsky (126) described the conversion of ordinary office space into a sterile filling and sealing facility. Three rooms were constructed: a gown room, a sterile storage room, and a main production room. The original sheetrock walls and ceiling were replaced by plaster, sealed, and finished with epoxy paint and sheet metal. The original vinyl tile floor was replaced by sheet vinyl curved upward at the walls to form a cove molding. Horizontal laminar air flow was provided in the main production area. The storage and gown rooms, thought not to require full recirculated laminar flow, were utilized for the removal of air and the introduction of makeup air. A smoke generator and photometer[1] were then used to evaluate the efficiency of the system and to check for leaks. Finally, plate counts of sampled air and control production samples confirmed the suitability of the complex for the beginning of routine sterile production.

Federal Standard No. 209B (127), whose provisions must be met by U.S. Government contractors manufacturing under conditions requiring clean rooms, is an often-quoted document which has become widely accepted as a guide to clean room requirements and standards.

Personnel—Personnel immediately involved in the production process, their very presence and what they do, are crucial to the quality of the product (128). People who move into, out of, and about production areas are inevitably carriers of potential contamination (129). Walking, talking, and breathing can spread bacteria and chemical dust. People who work in production areas should be free from communicable disease and be subject to essentially the same health requirements as are food workers. Pharmaceutical workers should have the proper facilities and follow prescribed routines for the changing of clothes, shoes, and gloves.

Just as important, they should have the proper knowledge and education. A program of continuing education, such as that introduced by the Swedish Drug Industry Association, is useful (94). Austin (130) described various approaches effective in the training of clean room personnel. Lecture techniques, slide shows, and on-site demonstrations are recommended. However, knowledge and education count for nothing if the proper motivation is not present. Neglect and indifference can overwhelm the most sophisticated system. Failure to check a weight and careless cleaning and inspection of a mixer are examples of behavior that are greatly influenced by the employer–employee relationship. A strong sense of responsibility must be instilled in people who work with pharmaceuticals.

Raw Materials—Perhaps the most essential single factor that can guarantee the quality of a drug product is high quality raw materials (64). All materials used in formulations should be obtained from reliable sources, quarantined after receipt at the plant, and tested prior to use (56). They should be kept in sealed containers, in isolated quarters, and not opened except in formulation or weighing rooms.

For sterile production, the most important single raw material is undoubtedly water. Tap water is well recognized as a carrier and incubator of microorganisms. This fact, plus its mineral content, makes it unsuitable for all pharmaceutical production (131). Distilled water, if not properly collected and stored, may also harbor bacteria. Rubber and plastic connections in a still may be sources of infection, necessitating either frequent sterilization of the system or the introduction of all-glass equipment. For nonsterile preparations, demineralization, followed by filtration[2] and continuous holding at 80°, was described (94). This system resulted in sterile and pyrogen-free water.

Working Processes—The systematic study of new working processes and procedures is necessary to discover potential trouble spots (132, 133). Weak points might be an inadequate drying time or temperature for granulations, water condensation on the surfaces of ointments or solutions, and taps containing residual water after cleaning (94).

An interesting method for discovering potential trouble is to substitute a broth or buffer solution for the product and to pass it through the process (64). Sampling at different stages is performed to locate sources of contamination.

A key area of concern that could be considered a processing problem is the production schedule itself (56). Drugs that are in any way incompatible should not be scheduled in succession on the same equipment. For example, barbiturates stimulate the metabolic breakdown of anticoagulants in the body, reducing their efficiency. Therefore, it would be prudent not to schedule anticoagulant manufacture immediately after the production of

[1] Royco.

[2] Millipore.

a barbiturate. If cross-contamination has to occur, it should be kept as harmless as possible.

Tablet production is inevitably dusty. Some steps that should be taken to control it (55, 134) include: (*a*) limiting the weighing area to the ingredient that is actually being weighed, (*b*) maintaining negative pressure in the vicinity of tablet machines, (*c*) avoiding hand-scooping of granulations by elevating the drums and using continuous flow into the hoppers, (*d*) cleaning containers that accumulate dust before removing them from the area, (*e*) thoroughly cleaning each piece of equipment between runs of different products, and (*f*) providing facilities for the disposal of operators' clothes that have accumulated dust.

Otto (135) described the large-volume parenteral manufacturing procedure with emphasis on minimizing particulate matter. The solution itself, ambient air, the interior glass surface, and the closure affect the residue of particles in the final product. Recommended steps are: the proper filtration of the solution, efficient cap and bottle wash and rinse procedures, a short time lapse between washing and final production steps, inspections and quality control checks (136) during filling (137), and the use of enclosures to limit fallout from the air. It is currently anticipated that the Code of Federal Regulations will soon require stringent procedures to be followed in the manufacture of large-volume parenterals.

In the production of sterile solids for injection, the filtration prior to recovery of the solid is the last step to reduce particulate matter. Hammer (138) discussed factors in subsequent steps that influence the final particulate content: spray or drum drying (139) usually results in fewer particles than the recrystallization procedure, simpler machinery with fewer moving and grinding parts generates less particulate matter, large room-size laminar flow units throughout the manufacturing procedure reduce particulate fallout from the air, and shrink-wrapped vials (140) contain fewer particles than those shipped in corrugated boxes and result in less contamination after washing.

The difficulty of the problem can be seen in the fact that, even with proper knowledge and consideration of these factors, particulate contamination was reduced by only about half in the Hammer (138) study—and at great expense.

Formulation—The protection of a nonsterile formulation against microbial growth both during and after its preparation is affected by the control of certain factors (94). For granulations, these factors may be the lowering of pH, the change or elimination of the granulating liquid, and the introduction of preservatives. For liquid preparations, a pH change, the use of preservatives, or a change in osmotic pressure may be necessary.

Basically, what a good master formula must do is carefully spell out every step in the procedure with respect to the equipment used, the order of addition of ingredients, the duration of mixing, safety precautions, *etc.* The batch master formula is the key to a contamination-free operation.

Control—Once a satisfactory system is achieved, it is a permanent function of the quality control system to maintain it (94).

To guard against error in labeling or processing, assay, identity, and limit tests should be scrupulously performed on all finished products. Although acceptance of protocol for raw materials received from suppliers is permissible according to good manufacturing practices (141, 142), additional identity tests should be instituted (142). For sterile productions, every raw material and finished product, as well as some intermediates and the production environment, should be checked for all microorganisms (143, 144) according to a fixed schedule. Tap water, demineralized water, and distilled water especially should be checked regularly.

The method, described earlier (64), of broth substitution for the product is a useful periodic check on the microbiological cleanliness of a procedure. However, as a routine in-process test, it is not generally acceptable because of its cost, incompatibility with normal production runs in terms of the duration of the run, and the danger of accidentally leaving a bacteriological medium where it may become a substrate for microbial growth.

Hess *et al.* (145) published a generalized scheme and routine procedures for determining low level microbiological contamination in topical preparations used on open skin wounds or mucous membranes. These preparations are unique in that, while they must be initially sterilized and contain preservatives that will maintain their freedom from pathogens, they are not required to remain sterile. However, they must not support the growth of high numbers of microorganisms of any kind (146).

Another indispensable part of the control system is the use of trained, on-site inspectors, who visually check every part of the production, packaging, and labeling procedure.

Equipment—Equipment should be constructed so as to facilitate cleaning and sterilization, especially between changes in product, to prevent the seeding of new batches with the residue from the old (64).

Machines such as mills and granulators with bushings and packing glands are usually difficult to clean. For this reason, they should be dismantled to an extent sufficient to permit proper cleaning.

Packaging—The packaging step in the production procedure is particularly important. Miller and Korczynski (147) outlined various aspects of drug and cosmetic packing from the point of view of a contract packing firm not involved in the prior manufacturing steps. The great variety of products handled requires that the firm carefully analyze the sterility requirements and problems of each product. Products are classified into groups and subgroups according to dosage form, label claim, intended use, and stability. For instance, a sterile parenteral is quarantined until tested whereas ointments, creams, liquids and powders for external use are merely screened (based on results from the first few lots) for an increase in microorganisms and the absence of undesirable microorganisms following handling and packaging.

DETECTION OF CONTAMINATION

The detection of drug contaminants is a broad problem, often requiring great ingenuity and imagination. Much depends on the amount of detailed information available to the analyst. Alexander (148) summarized his own experience at FDA in analyzing drugs for impurities. As an example, he cited a customer complaint of a manufacturer producing drugs and pesticides in the same apparatus

without proper cleaning of the equipment between product runs. Knowledge of what contaminant to expect simplified the chemist's task in detecting insecticide contamination of the drugs.

Once an analyst knows what to look for, the procedures used depend on the nature and level of the contamination, the nature of the drug, the equipment and instrumentation available, and the amount of time available.

Perception of the contamination problem has been influenced greatly by the development of modern sophisticated techniques and instrumentation (149). Contamination at the parts-per-million and parts-per-billion level can be detected with TLC (150–153), GLC (154–156) with its sensitive detectors, mass spectrometry (157–159), neutron activation analysis (160, 161), and microcrystalline tests (162–164).

Although it is impossible to describe here all of the methods that might be used to detect trace materials, the information relating to specific approaches to the problem will be reviewed.

Chemical Contaminants—An FDA procedure describes the general method for detecting and measuring penicillin contamination in drugs (65). The procedure is divided into three sections. In Section A, several microbiological assays are described using the method of bacterial growth inhibition on agar plates. Section B describes several methods of preparing samples for assay. Section C lists, in tabular form, the appropriate combination of assay and sample preparation for specific drugs. These methods comprise the basic approach to the detection and quantitation of penicillin as a cross-contaminant.

After the development of these methods, many semisynthetic penicillin and cephalosporin antibiotics were introduced. A study was made to determine if the existing methods were satisfactory for their detection (165). It was discovered that new methods were needed to detect ampicillin in other drugs and antibiotics.

Using high-speed liquid chromatography (HSLC), Bracey (166) determined trace ampicillin in nitrofurantoin capsules. The powder from 10 capsules is suspended in water, filtered through glass wool, and extracted with chloroform. A portion is injected into a liquid–liquid chromatograph with a UV detector. A strong anion-exchange column and a mobile phase of pH 6.5 phosphate buffer are used. With this procedure, the sensitivity limit is 0.1 mg or more/dose, which is too low to be of much use. It is suggested that the method, with proper sample preparation, could be applied to the determination of ampicillin in other drugs.

Although penicillin levels of 1 μg/g can be detected conveniently with microbiological methods, a rapid chemical method is advantageous where other antibiotics are present or the growth of the test microorganism is affected by the drug substance itself. A TLC method was developed (57). Two grams of sample is shaken with 10 ml of water, and the pH is adjusted to 6.5–7.0. After centrifugation, the supernate is washed with chloroform and acidified with phosphoric acid (pH about 2). Free penicillinic acids are extracted with methylene chloride, which is then dried by being passing through anhydrous sodium sulfate.

The combined extracts are evaporated to dryness, and the residue is extracted with isopropyl acetate. After evaporation to about 0.05 ml (a 90% yield is expected), 5 μl is spotted on TLC plates; penicillin G spotted in amounts from 0.5 to 5 μg is used as a standard. Any of four separate solvent systems are used, yielding different R_f values. The penicillin compounds may be visualized or monitored in different ways. No specific penicillin spray reagents are indicated, but several alternative sprays are listed.

An alternative to spraying is the bioautographic process of Stahl (167). The material in the penicillin-suspected spots is brought into contact with an agar gel inoculated with a microorganism, and the observed biological activity represents proof of the presence of penicillin. If it is assumed that the weakest standard spot represents the lower level of method sensitivity, 2.5 μg/g is the least amount that can be detected.

Specialized methodology for the determination of antibiotic dust dissemination has been published (168, 169). The general procedure involves passing a known volume of air through a 0.22-μm pore size filter[2]. Any residue is dissolved in a buffer, and portions are incubated on agar plates containing a suspension of test organism. Zones of growth inhibition as diameters in millimeters are measured and compared with those obtained from known concentrations of the same antibiotic, *i.e.*, a standard curve. The sensitivity of the procedure is 0.001 unit for penicillin and 0.004 μg for chlortetracycline.

An adaptation of this method was made by Garth *et al.* (170) for the purpose of making an environmental survey of a six-story building that housed the laboratories of the National Center for Antibiotics and Insulin Analysis. Qualitative and quantitative tests for penicillin and tetracycline were performed on air samples from various parts of the building. Initial tests were made using agar plates, and the results were used as a guide for the vacuum sampling of air at points in the building thought to be the most easily contaminated.

The results (170) of the tests indicated that the incidence of contamination by antibiotic dust was negligible other than in the actual laboratory rooms where testing was performed. The exception to this finding was a cleanup area where soap powders, antiseptics, and antibiotic-contaminated glassware were processed. The investigators concluded that persons in the building not testing antibiotics were not exposed to them by the atmosphere whereas antibiotic analysts were exposed minimally. While, strictly speaking, this paper did not concern pharmaceutical contamination, the general approach can certainly be applied analogously to a drug plant.

A comprehensive approach to the detection of foreign active drug ingredients in pharmaceutical preparations was made by Cieri (171). General procedures were discussed where the suspected contaminant was extracted with a suitable solvent and separated from the other extracted ingredients, usually by column chromatography. TLC was used to detect the contaminants, with a spiked sample being simultaneously run through the identical procedure for comparison. Specific methods were detailed for the detection of the following drugs: barbiturates, diethylstilbestrol, reserpine, cardiac glycosides, quinine or tropane alkaloids, pyrilamine or chlorpheniramine, phenylethylamines, steroids, methapyrilene or its salts, acetaminophen, and meprobamate. The author suggested the pos-

sibility of scraping the spots from the TLC plates and, after suitable extraction, using UV absorption as an identity confirmation.

Senov *et al.* (172) and Edmond (173) also described the use of TLC for the quantitative evaluation and determination of drug purity.

Adapting the Cieri TLC procedures, an FDA regulatory analyst detected drug residue in cotton balls that had been used to swab various parts of a drug plant. Positive results were obtained from acetaminophen, saccharin, and quinidine sulfate. Additionally, acetaminophen was confirmed on the basis of its UV absorption maximum.

Expanding on the problem of pharmaceutical factory dust, Page (174) published a general scheme for its analysis, concentrating mainly on ingredients found in relatively high dose level dosage forms. Direct IR spectra of the dust is occasionally useful, as is X-ray diffraction or X-ray fluorescence of the residue, following ignition at 600°. More often, purification is required. A subsample of the dust is mixed with aluminum oxide, and the mixture is then added to an aluminum oxide chromatographic column. Elution of the drugs is accomplished with chloroform, followed by methanol and, finally, water. Water deactivates the column. The separated eluates are then resolved by TLC using one or two of the solvent systems described. The TLC spots can be identified either with fluorescence quenchers or with spray reagents, with the probable detection limit for fluorescence quenchers being about 100 ng. Several confirmation tests are suggested, using IR, UV, GLC, or HSLC.

Another approach to the factory dust problem, using combined GLC–mass spectrometry, will be discussed later.

The manufacture of counterfeit drugs and illegal distribution of legitimate drugs are major societal and enforcement problems. Reynolds *et al.* (175) described the use of neutron activation analysis to identify the source of a drug product. The approach is based on the fact that natural trace elements may be present in a drug, giving it a characteristic "fingerprint" and establishing its origin. Manufacturers may also deliberately add trace elements to a drug to achieve the same effect. Neutron activation analysis, effective for about two-thirds of the chemical elements, is used to determine the patterns of trace elements. A nuclear reactor activates the sample, creating radioactive nuclides. After radiochemical separation, the radioactivity is measured directly or by direct γ-ray spectrometry with a scintillation detector and multichannel analyzer. The latter was used in this case. Five samples of dextroamphetamine tablets or capsules, along with several common tablet and capsule excipients, were analyzed for contaminants. Seventeen elements were detected in amounts ranging from less than 1 ppb to more than 0.1%. The results indicated that the tablet and capsule samples were readily distinguishable.

Microbiological Contaminants—The USP (176) describes detailed tests, where called for in compendial monographs, for the presence of viable bacteria, fungi, and yeasts. Procedures are given for opening containers, sampling, preparation of media, and incubation. The technique of membrane filtration (177) is also described. Other methods are acceptable, but the results of the official procedure take precedence in the event of contradictory findings. No sampling and testing format can guarantee that untested units of a given batch are sterile. This fact recalls one aspect of an expression often used in the pharmaceutical industry: "You can't test in quality."

Holdowsky (178) solved the problem of the determination of viable sensitive bacteria in antibacterial drugs. USP sterility tests could rarely recover bacteria from antibiotics that inhibited their growth unless a deactivator was available (*e.g.*, penicillinase for penicillin). The membrane filter was used to separate the bacteria from the antibiotics. Of 15 dihydrostreptomycin samples contaminated by the method, only one could be confirmed by the official USP test.

Wargo (89) performed sterility tests on topical ointments, utilizing a technique developed by Tsuji and Robinson (179). The ointment is dissolved in a mixture of isopropyl myristate, carbon disulfide, and xylene and then passed through a sterile membrane filter. The filter is divided in two, and retained bacterial contaminants are incubated on agar and in a fluid medium. Although only 11% of 180 previously unopened tubes were contaminated in this study, 93% of 80 tubes used in a patient care area were nonsterile. Isopropyl myristate is less toxic to microorganisms than the Tsuji and Robinson mixture of solvents. Therefore, the USP adopted a method using isopropyl myristate alone as the extracting solvent.

Mechanical Contaminants—The quantitation and identification of particulate matter in parenterals are relatively new fields and the subject of much recent study (180–186). The USP XIX (187) established an official procedure and particle-count limits for large-volume injections for single-dose infusions. Standards are met if the product contains "not more than 50 particles per ml that are equal to or larger than 10.0 μm and not more than 5 particles per ml that are equal to or larger than 25.0 μm."

The USP method is essentially a membrane filtration, followed by microscopic examination of the filter for particulate count and size. Other methods are allowed providing they are of equivalent reliability. Several instrumental methods have been developed, but their limitations must be considered. For instance, microscopic air bubbles, electrical disturbances, physical vibrations, or the formation of particle agglomerates may give rise to erroneous results (188–193).

A conductometric particle counter[3] was described (194, 195). An electrolyte solution containing the particles to be counted is sucked through a capillary across which resistance is continuously measured. When a particle passes through the capillary, a resistance change occurs and an impulse proportional to the particle size is generated and recorded. The use of "discriminators" that eliminate impulses below a threshold level permits the simultaneous counting of particles above given sizes (196).

An automated counter[4] was used to count particles of the 2–12-μm diameter range in intravenous infusions (19). Eighteen samples contained from 217 ± 49 to 6110 ± 343 particles/ml above 2.7 μm in diameter. Tests after autoclaving several times showed that, when new silanized rubber stoppers were used, it could not be concluded that

[3] Celloscope 202.
[4] Coulter.

repeated autoclaving increased the particle count. In fact, the count seemed to decrease, perhaps because of fragmentation into particles smaller than 2.7 μm. Other tests led to the conclusion that patients would receive fewer particles if the first 50–100 ml of intravenous solutions was used to flush out particulate matter present in intravenous administration sets.

A new instrument, the prototron, utilizes a laser light-scattering principle. It was used to count and size particulate matter in normal saline and 5% dextrose solutions (198). From three to five times as many particles were found greater than 1 μm than were found greater than 5 μm. This result is fairly typical of the skew in size distribution that other researchers have reported. Fortunately, it is felt that emboli are more likely to form with particles greater than 5 μm, the diameter of an erythrocyte being about 4.5 μm.

The determination of the silting index was proposed as an alternative to particle counting (199). The silting index is dependent on the flow rate decrease resulting from the tendency of particles to clog a filter. This decrease is, in turn, proportional to the number of particles as well as, to a certain extent, the size distribution of the particles. Values derived from three runs per sample were within 15% of the true value with 90% confidence.

The identification of particles requires the skilled use of a microscope (200). Oblique reflected light is used for opaque materials (metals and gelatinous debris) (201). Most common particulates of higher birefringence (fibers, starch crystals, plant tissue, and mold) are accessible to transmitted light microscopy techniques, which include polarized light, phase contrast, and differential interference contrast.

SUGGESTED APPROACHES TO THE CONTAMINATION PROBLEM

At this point, we might ask ourselves: "What is a rational basis for sampling to detect drug contamination?" The deciding criterion seems to be a logical, systematic approach that would yield the maximum, meaningful, practical amount of information for remedial action after subsequent analysis.

Many factors govern this criterion. Several questions need to be answered. Does the investigation have a clinical background or is it a general inquiry to determine the extent of suspected problems? If there has been a precipitating incident, is the source of the problem at the plant or a result of contaminated and/or mislabeled raw material? Might there be a repacking or storage-related problem? Are there initial laboratory findings to serve as a basis for further investigations? What is the potential health hazard? Are there specific, sensitive analytical procedures available for practical analyses?

In answering these and other questions, comprehensive scientific knowledge, experience, and sleuth-like qualities are required of the investigator. A routine, fixed approach does not exist, but certain recurrent themes will govern the approach to be taken.

For a plant survey, without clinical or laboratory findings as a starting point, a complete picture of the physical layout, production processes, manufacturing scheduling, flow of materials, in-process controls, and cleaning routines should be obtained. Personal observations should be made to see if the prescribed routines are being followed. Deficiencies can then be identified and potential problem areas can be investigated in depth.

Observers should be as unobtrusive as possible and not disturb the normal routine. It is important that the plant personnel not be so aware of being watched that they alter their normal procedures. What is remarkable, however, is the extent to which people, out of ignorance or habit, will continue to do things the way they have in the past, even though they know they are being observed[5].

Environmental analyses may provide indicators of trouble spots. For sterile production, testing for airborne bacteria by the use of agar plates left in fixed positions for various times has become an accepted, useful procedure.

However, the use of routine procedures for estimating airborne, particulate contamination risks during production and for determining the effectiveness of prophylactic measures (202) has been essentially neglected, as indicated by the paucity of literature. A start was made by Buogo and Eboli (203), who adapted an electrostatic bacterial air sampler to collect micronized drug particles. With a highly susceptible strain of *Sarcina lutea* seeded on Mueller–Hinton medium, penicillin, tetracycline, paromomycin, and sulfapyrazine were identified and quantitated. Penicillin G was detected at a level of 0.3 $\mu g/m^3$ of air. When 20–40 min had elapsed after the drugs were sprayed into the air, small quantities of penicillin were still detectable.

Ernerot and Molin (204) used an air sampling method[6] and two sedimentation methods (particle-fallout photometer and the analysis of penicillin fallout on petri dishes). Penicillin powder was artificially dispersed at regular intervals and under different conditions of ventilation in a "clean room," and the fallout pattern was observed at various locations in the room. Similarly, a penicillin vial filling room, penicillin tableting area, general tableting department, and granulation room were surveyed for particle fallout. The results from the different methods used correlated well and provided useful information about the dispersion pattern of chemical dust in the room.

A point to be kept in mind is the advantage of sampling product material at the earliest production stage after the suspected occurrence of contamination, *i.e.*, at that point where contamination will be the most concentrated. This is important for the analytical chemist as well as for pinpointing the trouble area. Those who have attempted to detect parts-per-million levels of estrogen in a multivitamin tablet, for instance, will attest to the great difficulty that may be encountered in recovering trace drugs from complex mixtures.

Nevertheless, the demonstration of a contaminant in the dosage form to be administered provides the most information about the extent of the health hazard as well as the

[5] The following incident involving an FDA investigator may serve as an extreme example. One of two weighers, who were supposedly checking each other's work, left the weighing area to obtain dextroamphetamine sulfate. He returned with a handscoop filled with white powder. After weighing the prescribed amount, he took the excess powder back to where he had obtained it, accompanied by the investigator. The material was returned to one of two adjacent, open drums, both containing white powder. There was no check of the drum from which the powder came or was returned to, and a potent drug was carried open, some distance through material storage areas, with the possibility of airborne cross-contamination. The chief weigher remained at the weighing scale throughout, apparently unaware of the multiple dangers in the procedure.

[6] Royco particle counter.

strongest legal evidence[7].

As an example of the solution of a sequential cross-contamination problem, Banes (205) cited the classic case of diethylstilbestrol contamination of isoniazid tablets. Initially, suspicions were aroused by the appearance of premature sexual maturation among young girls being treated for tuberculosis with isoniazid tablets. After ruling out environmental factors as a cause, isoniazid therapy was discontinued and the symptoms disappeared. Animals were fed the tablets and showed a positive estrogen response. Chemical analysis of the tablet batch then yielded an extract whose IR and UV spectra and chromatographic properties were identical to those of diethylstilbestrol. Production records revealed that isoniazid had been compressed immediately after a high potency diethylstilbestrol batch. This evidence and the observation of lax cleaning procedures and, eventually, the discovery of other batches sequentially contaminated with diethylstilbestrol represented an irrefutable chain of evidence.

A final philosophical point should be made. Surely, many of the cited examples of contaminated pharmaceuticals are real and severe dangers to the public. However, what of an innocuous condition represented by a trace of ascorbic acid in an aspirin tablet? It might be argued that the expense of eliminating the possibility of this occurring is not worth the doubtful benefit. It must be decided what kind of general attitude should be fostered in pharmaceutical manufacturers, whether they are small private label houses with limited funds or large multinational corporations. Aside from the fact that the consumer of an item so important as a drug is entitled to receive no more and no less than what he or she is paying for and not a mixture of traces of everything present in a plant, an attitude of scrupulous attention to detail, anticipation of problems, and even seeking out of problems is well worth whatever it costs if one "Devonport Incident" is avoided.

[7] The solution of a sampling and detection problem that arose at this agency is presented, not as an exact model to be followed but as a particular solution to a special situation. Hopefully, it will provide some insight into similar and related problems facing drug manufacturers and regulatory agencies.

During an inspection of a large ethical drug manufacturing plant, an FDA inspector observed substantial concentrations of dust throughout various parts of a general tablet compression room. There was dust on the walls of the room, on vacuum air returns atop the room partitions, and at the fresh air return. In instances where hand loading of granulations into the hoppers was observed, considerable dusting occurred despite the operator's care and the use of vacuum equipment. Nine tablet compression machines were separated only by 1.7-m high partitions.

The inspector felt that, to reduce the chance of cross-contamination under the prevailing manufacturing conditions, ceiling-high partitions between the compression machines should be installed. Although the amount of dust was small, it was felt that if this dust could be shown to contain drug material that had migrated from tableting cubicles some distance away, the potential of cross-contamination would be demonstrated. In addition, records were obtained of the firm's tablet compression schedules for the 2-week period prior to sampling. The New York FDA laboratory was then given the assignment of determining the drug content of cotton swabs used for the sampling of the various dust deposits. To complete the picture, a comprehensive listing of the firm's tableted drugs was obtained.

The method of analysis adopted was combined GLC–mass spectrometry. The procedures used in general by this author were as follows. The contents of a swab plus any loose powder were extracted into alcohol, filtered through Whatman No. 41 paper, and brought to volume. A portion of the alcohol solution was cleaned up (as found necessary) using acid and basic washes and reextractions. Eventually, a very small volume of chloroform extract was obtained, and a portion was injected into a system consisting of a Barber-Coleman 5000 gas chromatograph interfaced to an A.E.I. M.S.-12 mass spectrometer through a Markey glass frit separator.

The column liquid phase was 10% DC 200 on a support of 80–100-mesh Gas Chrom W (HP) with the column effluent split between a flame-ionization detector and the mass spectrometer source. As a peak appeared on the GLC recorder, the total ion current monitor of the mass spectrometer indicated a peak and the mass spectrum was scanned. GLC retention times and mass spectra were compared with standards, and identifications were made. The areas under the GLC peaks could be used for approximate quantitation.

Seven swabs were analyzed. All seven contained phenobarbital, and four contained caffeine. These positive results were obtained from widely disparate parts of the room. While the origin of the phenobarbital and caffeine as to date of manufacture and compression machine used could not be proven, a serious dust problem was demonstrated. Upon being presented with the findings, the firm acceded to the agency's recommendations.

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