Editorial

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Here's another issue of JHS loaded with lot of valuable information, kindly flip a few pages to believe us……………

Wound microbiology may be considered a complex and sometimes misunderstood area in clinical medicine, not least because a wound provides an environment in which the microbial ecosystem is very dynamic and unstable.

The majority of dermal wounds are colonized with aerobic and anaerobic microorganisms, often referred to as the "indigenous" or normal micro biota that originate predominantly from mucosal surfaces such as those of the oral cavity and gut. These micro biota play an important role in preventing colonization by pathogens of significant virulence (colonization resistance).

Papanicolaou stain (also **Papanicolaou's stain** and **Pap stain**) is a multichromatic staining cytological technique developed by George Papanikolaou, the father of cytopathology. Pap staining is used to differentiate cells in smear preparations of various bodily secretions; the specimens can be gynecological smears (Pap smears), sputum, brushings, washings, urine, cerebrospinal fluid, abdominal fluid, pleural fluid, synovial fluid, seminal fluid, fine needle aspiration material, tumor touch samples, or other materials containing cells.

Our In Profile section highlights **Margaret Pittman**, known worldwide for her pioneering research into the microbiology and immunology of infectious diseases. Her work in developing a vaccine for whooping cough remains the scientific basis (with later improvements) for protecting the children of Arkansas and the world from this potentially deadly disease.

Our Bug of the month is *Toxoplasma gondii*, an obligate, intracellular, parasitic protozoan that causes the disease toxoplasmosis. Toxoplasmosis is considered to be a leading cause of death attributed to foodborne illness in the United States. More than 60 million men, women, and children in the U.S. carry the *Toxoplasma* parasite, but very few have symptoms because the immune system usually keeps the parasite from causing illness. Toxoplasmosis is considered one of the Neglected Parasitic Infections, a group of five parasitic diseases that have been targeted by CDC for public health action.

If you are convinced that drinking a can of soda or two per day is not so bad, then think about it again! Two new studies have linked soda consumption with deteriorative effects on the kidneys.

Isopropyl alcohol is a secondary alcohol, a structural isomer of propanol that is widely used as an industrial solvent and as a preservative and antiseptic in the clinical environment. Since 2009, when the World Health Organization published its guidelines on hand hygiene for health care professionals, there has been a marked upswing in the distribution and use of alcohol-based products because of the numerous advantages they offer over traditional handwashing.

The hand rubs that have achieved the greatest commercial success are those that contain isopropyl alcohol.

Tickle yourself enjoying the jokes in our Relaxed Mood section.

Our JHS team is thankful to all our readers for their ever increasing appreciation that has served as a reward & motivation for us. Looking forward for your continuous support.

Wound Infection and Modern Biocides

Introduction

Wound microbiology may be considered a complex and sometimes misunderstood area in clinical medicine, not least because a wound provides an environment in which the microbial ecosystem is very dynamic and unstable.

The human body contains an estimated 1014 microbial cells and these outnumber mammalian cells 10-fold. These micro biota are necessary for health but have the potential for causing disease given the opportunity. Infections occur when microorganisms overcome the host natural immune system and subsequent invasion and dissemination of microorganisms in viable tissue provoke a series of local and systemic host responses.

Wound microbiology

The majority of dermal wounds are colonized with aerobic and anaerobic microorganisms, often referred to as the "indigenous" or normal micro biota that originate predominantly from mucosal surfaces such as those of the oral cavity and gut. These micro biota play an important role in preventing colonization by pathogens of significant virulence (colonization resistance). The role and significance of microorganisms in wound healing have been debated for many years. Some consider the microbial density to be critical in predicting wound healing and infection, while others consider the types of microorganisms to be of greater importance. However, these and other factors such as microbial synergy, the host immune response and the quality of tissue must be considered collectively in assessing the probability of infection. Whatever the outcome of these processes, wound microbiota are considered to be polymicrobial. The polymicrobial ecosystem of the wound is composed of a vast array of microorganisms which can be classified according to their nutritional and environmental requirements. One fundamental factor significant to wounds is the availability of oxygen which dictates which types of microbes can proliferate (Table 1). With acute and chronic wound infections, mixed populations of both aerobic and anaerobic microorganisms are commonly found. When anaerobes are evident, this is indicative of a more complex microenvironment in the wound. The existence of anaerobic bacteria in wounds may be significant but their presence is often overlooked as many standard laboratories do not routinely screen for them. Examples of common bacteria that have been isolated from chronic wounds may be seen in Table 2. However, the mere presence of these bacteria does not constitute an infected wound.

Table 1

Type Requirements	
Obligate aerobe	Must have access to oxygen
Obligate anaerobe	Will only grow in the absence of oxygen
Facultative aerobe	An anaerobic organism will grow in the presence of oxygen
Facultative anaerobe	An organism that can grow in the presence and absence of oxygen

The age of a wound influences microbial composition and diversity, and the development of the microbial ecosystem can be divided into 3 phases. Phase I is predominately described as an aerobic process and the organisms most representative are classified as Gram-positive obligate aerobic or facultative anaerobic. This is an acute process. Phase II is transitional, occurring as the levels of oxygen are reduced by obligate aerobes, e.g. in poorly perfused tissue. This environment will encourage growth of anaerobic microbes, specifically obligate anaerobes. If such an environment persists, phase III may develop, reflected by a change in the predominant microbiota to a mixed microbial community favouring organisms that persist over time with less standard pathogenicity; key pathogenic features include enzymes and toxin production.

Historically, most cultures isolated from chronic wounds are based on the traditional culture methodology, either aerobic or anaerobic and have relied upon traditional methods of sampling and laboratory detection. Advanced technology now utilizes molecular techniques that allow for the identification of viable but non-culturable (VBNC) bacteria, that otherwise would remain undetected by traditional methods. This is a significant advance in wound microbiology. The significance of these VBNC organisms requires clarification specifically related to the area of bacterial synergy, which is known to be important in bacterial pathogenicity and in biofilm formation.

Wound infection

The list of microbes associated with skin and soft tissue infections is growing. This list (Table 2) while not exhaustive, illustrates the complexity of the microbiology involved in wound management. Bacteria, specifically staphylococci, almost never appear as a single isolate in infected wounds as they are most often found in synergistic relationships with other bacteria.

In many wounds, when using culture techniques, the number of aerobic isolates recovered range from 1-8 with an average of 2.7 organisms per wound. However, when molecular techniques are used, significantly more bacteria are found to be present. Infected chronic wounds are biochemically and microbiologically complex with many deep wounds frequently hypoxic as a consequence of poor blood perfusion. This creates an ideal growth environment for microbes, including fastidious anaerobes that will proliferate as residual oxygen is consumed by obligate, facultative aerobic and anaerobic bacteria.

Table 2

Aerobes	Anaerobes
Acinetobacter baumanii	*Bacteroides* spp
Coliforms	*Fusobacterium* spp
Enterococcus faecalis	*Peptostreptococcus* spp
MRSA	*Porphyromonas* spp
Pseudomonas aeruginosa	*Prevotella* spp
Staphylococcus aureus	*Veilonella* spp
Staphylococcus epidermidis	
Streptococcus pyogenes	

Bacterial species rarely exist in pure culture in wounds and as such, within a wound, the microbiology exists within a community structure. The complexes that occur within wounds are not clearly understood. A better understanding and knowledge base regarding bacterial interactions will be important in managing polymicrobial infected wounds. An example of a polymicrobial infected wound is considered to be a biofilm

community. Biofilms which are considered by some to be associated with delayed wound healing are by definition sessile, and this stationary mode of growth will reduce the hazards which bacteria are accustomed to within the free floating or planktonic state.

Sampling infected wounds

It is important to remember that the quality of the laboratory report is dependent on the quality of the specimen and that simple cultures provide limited information. Additionally, if unrepresentative samples are obtained, unrepresentative reports will be generated. If a swab is taken, the specimen must be accompanied with significant clinical information, including specific anatomic site, classification of wound and prior or ongoing antibiotic therapy, and transported in appropriate media and processed within the recommended time frame. Recovery of true wound bacteria when bordered by skin flora is difficult as these are often classed as contaminates. Consequently, assessing the true microbiology of a wound infection does not have the same clarity as a sample recovered from sterile fluid such as blood or cerebrospinal fluid. Ideally, wound microbiology should only be interpreted in combination with the clinical diagnosis.

Biofilm overview

Biofilms are found widely in nature and have been rigorously studied for many years. However, the study of biofilms in relation to health and in particular wounds is a relatively recent development. The National Institutes of Health (NIH) suggest that 80% of human infectious disease is caused by biofilm, usually manifesting as chronic infection.These chronic infections often viewed as benign are in fact insidious and progressive in nature and produce death tolls each year rivaling that of heart disease or cancer, yet clinicians appear to have developed an extremely passive relationship with biofilm disease including those implicated in wound infection. Most clinicians are familiar with planktonic bacteria as they are routinely cultured in the laboratory, challenged by antibiotics with sensitivity or resistance recorded and a treatment recommendation made. The problem with this approach is that chronic wound bacteria are quite different from their laboratory planktonic counterparts.

A biofilm is a complex community comprising a mixed population of different microorganisms.

It is typified by the secretion of extracellular polymeric substance (EPS), a glue that protects the bacteria and holds the community together. The EPS matrix protects the individual bacteria from environmental stresses, scavenges nutrients from the environment and provides shelter for the unique heterogeneous micro-niches inside the biofilm. The biofilm microcolony achieves a critical density of bacteria (a quorum) through the release of signaling molecules and permits differentiation into a true biofilm society. This complex system of quorum-sensing molecules is tightly controlled and suggests that biofilm is most appropriately thought of as an organism composed of billions of individual cells and specialized structures.

Reproduction is carried out by the biofilm breaking down portions of itself and releasing fragments which contain cells incased in matrix material. These detachment fragments have the ability to attach to a suitable surface, become metabolically active, and reform a biofilm community. The biofilm community also forms secondary structures, including mushroom-type projections off the surface, water channels and extensions. These structures allow nutrient inflow and waste outflow throughout the biofilm.

Biofilm's defenses (resistance)

The survivability of biofilm is a result of adaptation strategies developed over millions of years. These strategies together with brief explanations of their mechanisms may be found in Table 1.

Strategies	Mechanisms
Extracellular polymeric substance (EPS)	Constructed by the bacteria of the biofilm to protect the community from desiccation, predators, immune cells, and toxins. The components of the EPS can include pathogen and host polysaccharides, proteins, and nucleic acids. The chemical structure of the EPS may also work to prevent some antimicrobials from entering the biofilm.
Enzymatic Protection	Metabolically active cells are able to produce enzymes such as catalase or beta lactamase that can neutralize biocides and antibiotics and shield the inner members of the community.
Altered microenvironments	By-products of the biofilm create acidic and hypoxic areas which produce slow growth and diversify the ecology of the biofilm.
Plastic phenotype	Biofilms have a dramatically different expression of proteins. Up to 50% of the outer membrane proteins are different from their planktonic counterparts, which demonstrates the phenotypic heterogeneity that can be found within a species.
Heterogeneity	When combined with slower growth, heterogeneity makes most antibiotics less effective.
Quorum sensing	Where groups of bacteria are present, cellto-cell signaling takes place. The bacterial pheromones facilitate cooperation or result in competitive antagonism, which work together to yield a climax biofilm community that is best suited for the stresses and nutrients of the wound environment.
Evasion of Host Defenses	Most chronic infections are firmly entrenched within the host. Complement pathways, antibodies and even white blood cells have been found to be very ineffective against biofilm.

Imaging studies, including light and electron microscopy of samples from 50 wounds, demonstrated that 60% of chronic wounds possess biofilm, whereas 16 acute wounds failed to show significant biofilm. The chronic wounds healed in over 3 months (a delayed wound healing trajectory), whereas all the acute

wounds healed within 3 weeks. This suggests that not only is biofilm present but it may impair healing. A biofilm model may explain many of the clinical challenges that can make wound care so intricate and complex. It has been established that chronic wounds become "stuck" in a chronic inflammatory state. This chronic inflammation is defined at a molecular level by increases in macrophagederived MMPs 2 and 9 and neutrophil-derived MMP 8 and elastase. At the cellular level, excessive neutrophils predominate within the wound bed. The presence of biofilm on the surface of the wound can explain the molecular and cellular findings in chronic wounds. Differences in opinion of the value of antibiotics in acute and chronic wound care may be found. When antibiotics are used as a single agent, they fail to "heal" a chronic wound the vast majority of times. Clinically, what is often seen following antibiotic administration is a short-term improvement in the wound, that is followed by a subsequent deterioration or recalcitrance. This is possibly due to failure of the antibiotic to reduce the bioburden to a level at which the host defenses can prevail, resulting in reconstruction of the biofilm and enhanced resistance. Clinical support for biofilm's role in impaired healing is demonstrated by a retrospective study which showed that wounds treated with anti-biofilm strategies were more likely to heal when compared to those treated by standard care methods. The results provide good working explanations for what is seen clinically in wound care.

Biofilm-based wound management

Suppressing wound biofilm while managing the other known barriers to wound healing (pressure, poor perfusion, poor nutrition, etc.) holds the potential to radically advance wound healing.

Chronic wounds are often managed using a single strategy (e.g. enzyme, topical antiseptic, or a specialty dressing) at a time. Early progress may be observed but often healing is stalled and another strategy is applied. Sequential strategies often result in failure to close the wound.

Using a biofilm model to explain the organization of wound bacteria, it becomes clear that a single strategy is unlikely to succeed. Biofilms are polymicrobial with important interspecies synergies along with the ability to control their environment through modifications of their protective matrix. This has led dentistry and many other industries to adopt a multiple concurrent strategy in managing biofilms. Dentistry has managed biofilm (dental plaque) successfully over several decades. This has resulted in the well-known daily regimen of: debridement (brushing) at the same time applying an anti-biofilm substance, namely toothpaste. These anti-biofilm agents block reattachment, impair EPS formation, or are biocidal, killing the community members of the plaque. For more recalcitrant plaques, harsher biocides are applied through oral rinses and aggressive debridement can be carried out through flossing, ultrasonic debridement, or professional cleaning. This process of suppression, which will continue throughout our lifetime, does not aim to *eradicate* the biofilm but to *suppress* it below a level that would cause periodontal disease. The same principles seem reasonable when applied to managing wound biofilm. It is important to note that as biofilm reconstitutes itself and before it has formed a stable climax community, it is much more susceptible to antimicrobials. Frequent debridement sets the stage for treating agents to be more effective.

Debridement provides a cornerstone in the management of chronic wounds and evidence demonstrates that frequent debridement improves wound healing. However, in most wounds, when slough or biofilm is removed from the surface, it rapidly reconstitutes itself on the surface within 24 hours. Clinically, what is seen is a cleanbleeding wound bed post-debridement one day but the next day the slough that was removed the day before debridement is seen on the wound bed. In the laboratory, it takes biofilm about 24 hours to re-establish the biomass of the community.

Topical antiseptics, such as silver and honey provide some evidence of their value in managing biofilm. Empirically, the authors have noted that iodine preparations, particularly cadexomer, also possess the capability to manage biofilm infection. The goal is not eradication but to get multiple different strategies producing significant stress to the biofilm at the same time.

It is recognized that biofilm demonstrates increased resistance to antibiotics, biocides and host defenses. However, when used concomitantly with frequent debridement and other topical agents that impair biofilm defenses, antibiotics can be more successful. Clinical medicine has found that for biofilm diseases such as osteomyelitis and endocarditis, higher doses of antibiotics for longer periods of time are more successful. In a chronic wound, use of antibiotics as a single agent struggles to suppress biofilm, but when used in conjunction with the other strategies indicated above, does show significant impact in healing wounds. Because wound biofilms are resistant to antibiotics and host defenses, clinicians struggle to manage successfully many chronic wounds. Aggressively targeting wound biofilm suppresses the bioburden over a period of time to a level at which the host immune response will prevail and resolve the chronic wound.

Silver

Silver-based products are extensively used in wound care(Klasen 2000a, 2000b; Demling and De Santi, 2001; Clarke, 2003), with skin discolouration (argyria) and irritation being the only visible side effects (White, 2002). It is thought that silver has a number of antimicrobial modes of action (Thurman and Gerba, 1989; Russell and Hugo, 1994). However, questions have been raised over the long-term use of these dressings, especially in infants (Denyer, 2009a; 2009b).

Recently, there have been concerns about silver toxicity (Parsons et al, 2005; Burd et al, 2007), and the systemic uptake and deposition of silver in organs have been noted in a number of studies (Wan et al, 1991; Denyer, 2009a; Wang et al, 2009).

To date, the pathological consequences of this are unknown. Added to this, there are fears about the emergence of silver resistance (Percival et al, 2005; Loh et al, 2009). It would seem that, in academic circles at least, questions exist over its continued widespread clinical use. This has been further enhanced by questions about its cost-effectiveness (Bergin and Wraight, 2006; Chaby et al, 2007; Michaels et al, 2009), which in some areas has led to product restrictions.

Iodine

Iodine-based products have been used in wound care for many years. Like all antiseptics, iodine simultaneously affects multiple sites in microbial cells, resulting in cell disruption and death (Cooper, 2007). However, not only have its antimicrobial efficacy and chemical stability been debated, but also its toxicity to host tissues and the ensuing effect on patient comfort (Kramer, 1999; Wilson et al, 2005). It has been found that providone-iodine is not as effective as some other biocides in eradicating Staphylococcus epidermis within in vitro biofilms (Presterl et al, 2007). Cadexomer iodine provides sufficient iodine for biofilm suppression without causing significant damage to the host

(Akiyama et al, 2004; Rhoads et al, 2008) but pain has been reported as a side effect of its use (Hansson, 1998).

Chlorhexidine

Chlorhexidine has been used clinically for about 50 years (Russell, 2002). It is active against gram-negative organisms such as Pseudomonas aeruginosa and gram-positive organisms such as Staphylococcus aureus and Escherichia coli, although meticillin-resistant Staphylococcus aureus (MRSA) resistance has been recorded (Cookson, 2000). Chlorhexidine appears to be relatively safe, with little effect on the healing process.

However, results from studies are insufficient to draw conclusions about its use on open wounds. In addition, there are concerns about the safety of additives frequently used in chlorhexidine-based preparations to modify their handling properties. More human trials need be performed to assess its efficacy and long-term safety (White et al, 2001; Main, 2008)

Honey

In recent years, there has been resurgence in interest in honey-based products for bioburden management (White, 2002). The exact mode of action of honey is not yet fully understood. However, it is hyperosmolar and, thus, restricts the availability of environmental water to bacteria and other organisms (Molan, 2001), leading to cell disruption and death. However, this effect is lessened as the honey becomes more diluted by wound exudate (Molan, 1999). A secondary action is the release of hydrogen peroxide as the honey is diluted by exudate (Molan and Betts, 2004). However, some honeys, particularly *Leptospermum* or manuka varieties, have been found to retain their bactericidal properties even without the presence of hydrogen peroxide (Cooper et al, 2002a; 2002b), which is thought to be associated with a phytochemical component (Karayil et al, 1998; Molan, 2002). The antibacterial properties of honey, therefore, vary according to its source.

The dilemma

Careful and objective review of the literature suggests that the use of many antiseptics in wound management must be subject to a risk-benefit assessment of possible local toxicity and beneficial antibacterial action (Brennan and Leaper, 1985). In short, it is advised that, before use, the beneficial antimicrobial effects and bioavailability should be weighed against any possible cellular toxicity (Wilson et al, 2005).

Given the widespread availability of antimicrobial products, factors likely to influence selection include:

- Clinician familiarity
- Availability, cost and reimbursement issues
- Ease of use and implications for pattern of care
- Efficacy and safety (WUWHS, 2008).

As there appears to be concern about the safety and efficacy of commonly used and familiar antimicrobial products, clinicians need to cast the net wider and search for alternative safe, effective and efficient products.

PHMB

The antiseptic agent polyhexamethylene biguanide (also known as polihexanide or PHMB) has been used for over 60 years in a wide range of applications from swimming pool sanitisers to preservatives in cosmetics and contact lens solutions. In Europe, it has been available as a wound irrigation fluid for some time.

PHMB is a fast-acting biguanide compound composed of a synthetic mixture of polymers. The compound is structurally similar to the antimicrobial peptides (AMPs) produced by many cells within the wound, such as keratinocytes and inflammatory neutrophils, where they are thought to help protect against infection (Sorensen et al, 2003; Ousey and McIntosh, 2009). AMPs have a broad spectrum of activity against bacteria, viruses and fungi, inducing cell death by disrupting cell membrane integrity (Ikeda et al, 1983; Ikeda et al, 1984; Moore and Gray, 2007;).

The structural similarities to AMP mean that PHMB can infiltrate bacterial cell membranes and kill bacteria in a similar way (Moore and Gray, 2007). However, PHMB does not interfere with the proteins that make up animal cell membranes. It, therefore, has a specific antimicrobial action that does not affect animal cell integrity. It is thought that, once it has adhered to the target cell membranes, PHMB causes them to leak potassium ions and other dissolved ions from the cytoplasm (Davies et al, 1968; Davies and Field, 1969; Broxton et al, 1984a; Yasuda et al, 2003; Gilbert, 2006), resulting in cell death. PHMB has an effect on both planktonic bacteria and those in biofilms (Seipp et al, 2005; Pietsch and Kraft, 2006; Harbs and Siebert, 2007). Its action on the bacterial cell membrane also means that the efflux pump (a mechanism used by many bacterial cells to remove toxins) is unable to remove the antiseptic, so intracellular bactericidal concentrations are maintained (Kingsley et al, 2009). Once inside the cell, there is evidence that PHMB binds to DNA and other nucleic acids, suggesting it may also damage or inactivate bacterial DNA (Allen et al, 2004).

Studies have shown that PHMB is effective in vitro, while clinical studies indicate it has a broad spectrum of activity, including against human immunodeficiency virus (HIV) (Wérthen et al, 2004; Krebs et al, 2005). Testing has demonstrated that exposure to PHMB causes viral cells to clump together, forming aggregates. This prevents invasion into the host cells, making PHMB a potent antiviral treatment in wound care (Pinto et al, 2009).

However, studies have shown that the product is safe in clinical use. Schnuch et al (2000; 2007) demonstrated that in trials including 3529 patients, skin sensitisation to PHMB is low (approximately 0.5%), even when the tested concentrations (2.5% and 5%) were 5–10 times that normally used in wound applications. Comparative tests of PHMB's biocompatibility (measurement of an antiseptic agent's activity in relation to its cytotoxicity) against other commonly used therapies have demonstrated its superiority to chlorhexidine, povidone-iodine, triclosan, silver and sulpadiazine (Müller and Kramer, 2008). In addition, no known resistance to PHMB has been reported, most likely owing to its rapid and non-specific bactericidal activity (Moore and Gray, 2007).

Wound care products incorporating PHMB have been shown to have positive effects on wound healing. In vitro and in vivo studies have shown that, in some of these products, the influence of PHMB:

- Reduces wound pain rapidly and effectively (Daeschlein et al, 2007; Galitz et al, 2009)
- Reduces wound malodour (Daeschlein et al, 2007)
- Increases formation of granulation tissue (Mueller and Krebsbach, 2008)
- Increases keratinocyte and fibroblast activity (Wiegand et al, 2008a)
- Reduces slough within the wound (Mueller and Krebsbach, 2008)
- Reduces MMP-induced periwound breakdown (Cazzaniga et al, 2002; Werthen et al, 2004)

● Helps remove non-viable tissue (Kaehn, 2009).

The success of PHMB has resulted in its recommendations as the primary antimicrobial in many European countries (Dissemond et al, 2010) and has prompted the publication of a UK consensus review (Wounds UK, 2010).

Bibliography

Allen MJ, Morby AP, White GF (2004) Cooperativity in the binding of the cationic biocide polyhexamethylene biguanide to nucleic acids. Biochem Biophys Res Commun **318**(2): 397–404

Ansorg RA, Azem T, Fabry WH, Rath PM (2002) Influence of mucin on the activity of the antiseptic Lavasept against Staphylococcus aureus. Chemotherapy **48**(3): 129–33

Bergin SM, Wraight P (2006) Silver based wound dressings and topical agents for treating diabetic foot ulcers. Cochrane Database Syst Rev Issue 1 Art. No.: CD005082. DOI: 10.1002/14651858.CD005082.pub2

Best Practice Statement (2011) The Use of Topical Antimicrobial Agents in Wound Management. Wounds UK, London. Available at: http://www. wounds-uk.com/best-practice-statements/best-practice-statement-the-use-of-topical-antisepticantimicrobial-agents-in-wound-management- 2nd-edition

Brennan SS, Leaper DJ (1985) The effects of antiseptics on the healing wound: a study using the rabbit ear chamber. Br J Surg **72**(10): 780–2

Broxton P, Woodcock PM, Heatley F, Gilbert P (1984a) Interaction of some polyhexamethylene biguanides and membrane phospholipids in Escherichia coli. J Appl Bacteriol **57**(1): 115–24

Burd A, Kwok CH, Hung SC, Chan HS, Gu H, Lam WK, Huang L (2007) A comparative study of the cytotoxicity of silver-based dressings in monolayer cell, tissue explant and animal models. Wound Repair Regen **15**(1): 94–104

Cazzaniga A, Serralta V, Davis S, Orr R, Eaglstein W, Mertz PM (2002) The effect of an antimicrobial gauze dressing impregnated with 0.2-percent polyhexamethylene biguanide as a barrier to prevent Pseudomonas aeruginosa wound invasion. Wounds **14**(5): 169–76

Chaby G, Senet P, Vaneau M, Martel P, Gauillaume JC et al (2007) Dressings for acute and chronic wounds: a systematic review. Arch Dermatol **143**(10): 1297–304

Clarke S (2003) The silver solution. Journal of Community Nursing **17**(1): 25–8

Cookson BD (2000) Methicillin-resistant Staphylococcus aureus in the community: new battlefronts, or are the battles lost? Infect Control Hosp Epidemiol **21**(6): 398–403

Cooper RA (2004) A review of the evidence for the use of topical antimicrobial agents in wound care. WorldWide Wounds. Available online at: http://tinyurl.com/76m6ctg

Cooper RA (2007) Iodine revisited. Int Wound J **4**(2): 124–37

Cooper RA, Halas E, Molan PC (2002b) The efficacy of honey in inhibiting strains of Pseudomonas aeruginosa from infected burns. J Burn Care Rehabil **23**(6): 366–70

Cooper RA, Molan PC, Harding KG (2002a) The sensitivity to honey of Gram-positive cocci of clinical significance isolated from wounds. J Appl Microbiol **93**(5): 857–63

Daeschlein G, Assadian O, Bruck JC, Meinl C, Kramer A et al (2007) Feasibility and clinical applicability of polihexanide for treatment of second-degree burn wounds. Skin Pharmacol Physiol **20**(6): 292–6

Davies A, Bentley M, Field BS (1968) Comparison of the action of vantocil, cetrimide and chlorhexidine on Escherichia coli and its spheroplasts and the protoplasts of Gram-positive bacteria. J Appl Bacteriol **31**(4): 448–61

Davies A, Field BS (1969) Action of biguanides, phenols and detergents on Escherichia coli and its sphereoplasts. J Appl Bacteriol **32**(2): 233–43

Demling R, DeSanti L (2001) The role of silver in wound healing: part 1. Effects of silver on wound management. Wounds: A Compendium of Clinical Research and Practice **13**(1 Suppl A): 4–15

Denyer J (2009a) Epidermal bullosa and silver absorption in paediatrics. Presentation at Wounds UK Conference, 11 November 2009, Harrogate

Denyer J (2009b) Management of the infant with epidermolysis bullosa. Infant **5**(6): 168–71

Dissemond J, Gerber V, Kramer A, Riepe G, Strohal R et al (2010) A practice-oriented expert recommendation for treatment of critically colonised and locally infected wounds using polihexanide. J Tissue Viability **19**(3): 106–15

Dow G (2001) Infection in chronic wounds. In: Krasner DL, Rodeheaver, GT, Sibbald RG eds. Chronic wound care: a clinical source book for healthcare professionals (3rd edn). HMP Communications Wayne, PA: 385–356

Dowsett C, Edwards-Jones V, Davies S (2004) Infection control for wound bed preparation. Br J Community Nurs **9**(9s): 12–17

Eberlein Th, Wild Th (2008) Approaches to therapy and an outlook on improving the clinical data situation, with a special focus on the polihexanide-containing Suprasorb X + PHMB Hydrobalance dressing. EWMA conference extended abstract. Available online at: http://tinyurl.com/cfs6rak

Edwards R, Harding KG (2004) Bacteria and wound healing. Curr Opin Infect Dis **17**(2): 91–6

European Wound Management Association (2006) Position Document: Management of wound infection. MEP Ltd, London

Galitz C, Hämmerle G, Signer M, Traber J, Eberlein T et al (2009) Polyhexanide versus silver wound dressings: first interim results of a controlled, randomized, prospective, multicentric study. Poster presentation. European Wound Management Association (EWMA) Conference, Helsinki. Available at: http://tinyurl.com/737n6go

Gilbert P (2006) Avoiding the resistance pitfall in infection control. Does the use of antiseptic products contribute to the spread of antibiotic resistance? Ostomy Wound Manage **52**(10A Suppl): 1S–3S

Harbs N, Siebert J (2007) In vitro efficacy of octenidine and polihexanide against biofilms composed of Pseudomonas aeruginosa. GMS Krankenhaushyg Interdiszpl **2**(2): Doc.45 (20071228)

Hansson C (1998) The effects of cadexomer iodine paste in the treatment of venous leg ulcers compared with hydrocolloid dressing and paraffin gauze dressing. Cadexomer Iodine Study Group. Int J Dermatol **37**(5): 390–6

Hunt TK, Hopf HW (1997) Wound healing and wound infection. What surgeons and anaesthesiologists can do. Surg Clin North Am **77**(3): 587–606

Ikeda T, Ledwith A, Bamford CH, Hann RA (1984) Interaction of a polymeric biguanide biocide with phospholipid membranes. Biochim Biophys Acta **769**(1): 57–66

Ikeda T, Tazuke S, Watanabe M (1983) Interaction of biologically active molecules with phospholipid membranes. I. Fluorescence depolarization studies on the effect of polymeric biocide bearing biguanide groups in the main chain. Biochim Biophys Acta **735**(3): 380–6

Karayil S, Deshpande SD, Koppikar GV (1998) Effect of honey on multidrug resistant organisms and its synergistic action with three common antibiotics. J Postgrad Med **44**(4): 93–6

PAP Stain - Papanicolaou Stain

Accurate cytological assessment may not be possible unless the smear is optimally fixed and stained. A well – stained Pap smear should demonstrate crisp blue/purple nuclei. Cytoplamic staining should show a broad spectrum of colours ranging from orange in highly keratinized cells through ranges of orange / pink in superficial cell and turquoise green/blue in intermediate and parabasal cells. Poor staining often results in nuclear abnormalities being misgraded or sometimes completely missed. Papanicolaou described a good staining method as one in which nuclear detail was well defined, transparency of cytoplasm was assured when cells overlapped, and cell types could be differentiated from one another.

Papanicolaou stain (also Papanicolaou's stain and Pap stain) was developed by Dr. Gerorge N. Papanicolaou in 1942, and subsequently modified by him in 1954 and 1960. It is polychrome staining reaction designed to demonstrate variation of cellular maturity and metabolic activity. Pap staining is used to differentiate cells in smear preparations of various bodily secretions; the specimens can be gynecological smears (Pap smears), sputum, brushings, washings, urine, cerebrospinal fluid, abdominal fluid, pleural fluid, synovial fluid, seminal fluid, fine needle aspiration material, tumor touch samples, or other materials containing cells. Pap staining is a very reliable technique. As such, it is used for cervical cancer screening in gynecology. The entire procedure is known as Pap smear.

The PAP stain is designed to meet 3 staining objectives:
1. Good nuclear detail,
2. Differential counterstaining,
3. Cytoplasmic transparency.

Cytoplasmic transparency is a function of high ethanol concentration of the stain. This is important in order to view multilayered cell aggregates.

There are four main steps in the staining procedure:
1. Fixation
2. Nuclear staining
3. Cytoplasmic staining
4. Clearing

The cell samples are smeared and must be immediately fixed in 95% ethanol or a spray fixative that contains polyethylene glycol.

The Importance of Fixation
Proper fixation is the most important step in Histotechnology, and the quality of fixation will affect every subsequent step. Fixation stabilizes the proteins throughout the specimen and prevents changes typically caused by postmortem activities such as mold or bacterial attack (putrefaction) and the continuation of enzyme metabolic processes (autolysis). Fixatives help maintain the relationship between cells and extracellular substances and change soluble substances within the cell to insoluble substances. They also provide contrast among different tissue constituents by enhancing the differences in their refractive indexes. One of the most important actions of a fixative is to protect the specimen from the denaturing effects of the dehydrating agents and the ravages of subsequent processing steps.

The vital components of the PAP stain are:
The important elements of this method are nuclear staining followed by orange and polychromic cytoplasmic staining.
Hematoxylin stain + Orange stain + Polychromic stain = Papanicolaou stain
Staining the cells is highly transparent, a feature which means diagnosis is possible even in areas of overlapping cells and when mucus and inflammatory cells are present.

1. **Harris's haematoxylin as a nuclear stain**
   Nuclear staining is accomplished using the natural dye, hematoxylin. Hematoxylin dye is mixed with a trivalent metal salt to produce a so-called hematoxylin lake, and it is this that is actually used to selectively stain the cell nuclei (DNA). The oxidized form, hematein, chelates with the trivalent metal ions (Al^{3+}, Fe^{3+}, Cr^{3+}) of certain alums. The chelated compound is used in an acid medium and, when rinsed with tap water, produces the characteristic blue color. This step also fixes the dye on the target structures. What makes the hematoxylin solutions special is the fact that, apart from the dye, all of the other components (an oxidizing agent, now iodate for safety reasons, and the metal ions), are present as salts. The constituents must be carefully matched so that oxidation occurs in a way that ensures there is always sufficient hematoxylin, i.e. hematein, present and that the oxidized dye is available in sufficient quantity throughout its entire declared useful life.

   Hematoxylin is used to demonstrate nuclear detail. The procedure can be performed using a progressive or a regressive method. In progressive staining, the reaction is stopped once the desired staining intensity has been achieved. In regressive staining, the smears are overstained and then differentiated in a dilute aqueous or ethanol and hydrochloric acid solution to achieve the desired results. The nuclear stain shifts from blue/purple to a pink/red. After differentiation the smears are rinsed in water and placed in an alkaline "bluing" solution to re-establish the insoluble blue colour dye lake. Bluing solutions include Scott's tap water, alcohol ammonia and lithium carbonate. A thorough rinse is necessary after this step. Many laboratories tend to use the progressive method of staining to avoid over/under differentiating and because it is easier to maintain standardization from one stain batch to another. The decision to use a progressive or regressive method lies solely with the personal preference of the user.

2. **Cytoplasmic staining with orange G staining solution**
   The first cytoplasmic stain is Orange G. It is prepared in an ethanol solution with phosphotungstic acid. It stains cytoplasm yellow to orange if keratin or prekeratin is present. The effect of the orange staining solution is particularly pronounced in smears with keratinized cells under acidic pH conditions, when the obviously orangeophilic cytoplasmic stain is recognizable and may point to the presence of hyperkeratosis, HPV infections or carcinoma cells. It is supposed that orange dyes have a ripening effect on the subsequent polychromic stain. The products used are Orange G, which stains the target structures yellowish-orange.

3. **Cytoplasmic staining with polychromic staining solutions**
   The second cytoplasmic stain is EA and it consists of a mixture of light green and eosin Y in an ethanol solution with

phosphotungstic acid. Eosin should stain superficial squamous cells, nucleoli, cilia and RSCs. Light green should stain the cytoplasm of metabolically active cells, such as metaplastic and intermediate squamous calls.

The effects of Orange G are only evident in smear when keratinised cells are present. However it is likely that it enhances red blood cell staining and acts as a mordant to the subsequent EA stain.

Dehydrating and clearing agents

Dehydration means the removal of water. The process is used in Histotechnology during both processing and staining techniques. Dehydration is normally accomplished using alcohol solutions; most commonly ethyl, denatured or isopropyl; occasionally methyl; or butyl for plant and animal tissue. Other solutions such as acetone and various universal solvents can also be used. If specimens are improperly dehydrated and water is left in the specimen, the clearant and infiltration medium will not penetrate the tissue and it will be soft and mushy. Excessive dehydration will remove the bound water, causing shrunken, hard, brittle specimens that require excessive soaking before sectioning.

- The most common dehydrating agent used in the cytology lab is **ethanol**. Ethanol is a clear colouless, flammable liquid. Ethanol is a rapid and efficient dehydrant.

Clearing, which is used in both processing and staining, originally received its name because many of the reagents used for this purpose have a high refractive index and will render the exposed specimen transparent. They are also referred to as "dealcoholization" agents since their primary purpose is to remove the alcohol used for dehydration and prepare the specimen for the infiltration medium.

- **Xylene** has been the most widely used clearing agent for many years. It is an aromatic hydrocarbon that rapidly replaces ethanol and has a refractive index capable of rendering the tissue transparent. It turns cloudy in the presence of water. It is a flammable reagent that should only be used with adequate ventilation, and skin contact should be avoided.

When performed properly, the stained specimen should display hues from the entire spectrum: red, orange, yellow, green, blue, and violet. The chromatin patterns are well visible, the cells from borderline lesions are easier to interpret, the photomicrographs are better, and the stained cells are pretty. The staining results in very transparent cells, so even thicker specimens with overlapping cells can be interpreted.

On a well prepared specimen, the cell nuclei are crisp blue to black. Cells with high content of keratin are yellow, glycogen stains yellow as well. Superficial cells are orange to pink, and intermediate and parabasal cells are turquoise green to blue. Metaplastic cells often stain both green and pink at once.

Sample material and preparation

- Gynaecological and non-gynaecological specimen as sputum, urine, FNAB, body effusions, lavages.
- Samples derived from the human body. The collected cells are smeared on a microscope slide and immediately wet fixed with a thin film to maximize cell preservation.

- In order to avoid errors, the staining process must be carried out by an expert.
- National guidelines for work safety and quality assurance must be followed.
- Microscopes equipped according to the standard must be used.
- If necessary use a centrifuge suitable for medical diagnostic laboratory.

Fixation

- Wet fixation immediately with Cytology spray fixative or wet fixation immediately in 95% ethanol for minimum 30 min. All samples must be clearly labelled. Suitable instruments must be used for taking samples and their preparation; manufacturer instructions for application /use must be followed.

PAPANICOLAOU METHOD

- Fix slides in acetic/alcohol (Ethanol) fixative for 15 minutes.
- Absolute alcohol for 2 minutes.
- 70% alcohol for 2 minutes.
- 50% alcohol for 2 minutes.
- Tap water for 2 minutes.
- Stain in haematoxylin for 4 minutes.
- Rinse in tap water briefly.
- Differentiate in acid alcohol for 5 seconds.
- Blue in tap water.
- Dehydrate in absolute alcohol x 2.
- Stain in orange G for 10 seconds.
- Rinse in absolute alcohol x 2.
- Stain in E.A. 50 for 2 minutes.
- Rinse in absolute alcohol x 2.
- Clear in xylene x 3.

Specimens for use in histology and cytology must be completely anhydrous prior to being mounted. Xylene should be added as a final stage in order to prevent turbidity brought about by solvents containing water.

To carry out the mounting process, drop approximately 0.5 ml mounting agent onto a horizontal slide using a glass rod. This fills the space between slide and cover glass. As soon as the specimen has been covered with a homogeneous solution, cover with a cover glass, taking care to avoid air bubbles.

Allow to harden over a period of 20-30 minutes in a horizontal position.

Result

The microscope used should meet the requirements of a medical diagnostic laboratory-

Cytoplasm Cyanophilic (basophilic)	Blue-green
Cytoplasm Eosinophilic (acidophilic)	Pink
Cytoplasm Keratinised	Pink-orange
Erythrocytes	Red
Nuclei	Blue, dark violet, black
Microorganisms	Grey-blue
Trichomonas	Grey-green

Troubleshooting the PAP Stain

Problem	Possible Reason	Remedy
Dark Nuclei	• Too much time in Harris' Hematoxylin. • Not enough time in HCl or HCl concentration less than recommended.	• Reduce time in Harris' Hematoxylin by 10, 15, 20, 30 sec intervals. • Increase time in acid by 5, 10 sec.
Pale Nuclei	• Polyethylene glycol coating not removed from cells prior to Hematoxyline. • Concentration of HCl greater than recommended or too much time in HCl. • Not enough time in Hematoxylin. • Hematoxylin diluted by water (if wter not properly drained from slides). • Stain not changed frequently enough resulting in Hematoxylin exhausted.	• Extend prestaining soak with aqueous ethanol • Reduce time in acid by 5, 10 sec and ensure correct amount of acid is added to the solution. • Increase time in Hematoxylin by 10, 15, 20, 30 sec intervals. • Ensure the arm of the staining machine is operating correctly. • Ensure a set amount of slides are stained and then stains are changed.
Cytoplasmic colour not consistent	• Air drying prior to fixation. • Polyethylene coating inadequately removed from cells. • Solutions not at proper level within staining dishes. • Excessive time in Hematoxylin or Hematoxylin not removed prior to OG and EA dyes. • Slides left too long in ethanol rinses or clearing solutions following OG and EA. • Inadequate rinsing of slides between solutions. • Insufficient rinsing following staining solutions. • pH of tap and distilled water not sufficiently alkaline. • pH of EA needs to be controlled (pH 4.5 to 5 achieves maximum results). • EA dye exhausted.	• Report the finding to the referring clinician. • Extend prestaining soak in aqueous ethanol. • Check staining solution level. • Reduces time in Hematoxylin by 10, 15, 20, 30 sec intervals. • Reduce ethanol rinse time. • Check if ethanol is changed regularly. • Increase ethanol rinse time. • Check pH • Check pH • Ensure a set amount of slides are stained and then stains are changed.
Macroscopically all slides are pink, orange or yellow	Slide drying oven temperature too high	If this happens there is nothing that can be done to obtain a well-stained sample.
Dull pink and degenerate appearance	This usually occurs to smears that accompany histology specimens. It is usually due to formalin fixation.	Ensure formalin pot and smear is transported in separate bags.
Dull grayish appearance of cells	• Water contamination of dehydrating and clearing solutions. • Polyethylene glycol coating not removed from cells prior to staining of filter.	• Ensure dehydrating and clearing solutions are changed regularly. • Extend the prestaining soak time.
Opaque/white colour on back of slides	Bluing agent not rinsed from slides.	Use teo separate but thorough water rinses following Scott's tap water substitute. (For progressive pap staining).
Stain deposit	Staining dyes not changed or filtered properly.	Ensure staining dyes changed or filtered regularly.
Fungal contamination	Slides contaminated by fungus during the staining process.	Change staining solutions regularly and ensure the staining containers are disinfected with a dilute bleach solution.

Reference:
(1) Akura & Takenaka (1991) Diagn Cytopathol 7(3):317. (2) Drijver & Boon (1983) Acta Cytolog 27(6):693-698. (3) Dighe SB, Dulhan A, Saleem P, Chinoy R. Papanicolaou Stain, Is it Economical to switch to rapid, economical,acetic acid, papanicolaou stain? Acta Cytologica 2006;50:643-646. (4) E.M. Burd, **Human Papilloma Virus and Cervical Cancer,** CMR, Vol. 16, No. 1, 2003. (5) Hess etal (1981) Acta Cytolog 25(2):111-133. (6) Koss L G: Diagnostic Cytology and Its Histopathologic Bases, Fourth edition. Second volume. Edited by, J B Lippincott,1992,pp1452-1509. (7) M. Corkill, D. Knapp, M.L. Hutchinson, **Improved accuracy for cervical cytology with the ThinPrep method and the endocervical brush-spatula collection procedure,** Journal of lower genital tract diseases, Vol. 2, No. 1, P. 12-16, 1998. (8) Schulte & Wittekind (1990) Analyt Quant Cytol Histol 12(3):149-160. (9) Schulte (1986) Diagn Cytopathol 2:160-167. (10) Turner & Collins (1987) Acta Cytol 9(6):521-530. (11) Yang GCH, Alvarez II: Ultrafast Papanicolaou stain: An alternative preparation for fine needle aspiration cytology. Acta Cytologica,1995;39:55-60

Margaret Pittman

Born	:	1902, Washington County, Arkansas, United States
Died	:	August 19, 1995
Education	:	Hendrix College, University of Chicago

Margaret Pittman was known worldwide for her pioneering research into the microbiology and immunology of infectious diseases. Her work in developing a vaccine for whooping cough remains the scientific basis (with later improvements) for protecting the children of Arkansas and the world from this potentially deadly disease.

Margaret Pittman was born near Prairie Grove (Washington County) on January 20, 1901, to James Pittman, a country doctor, and Virginia Alice McCormick. In 1909, the family moved to the village of Cincinnati (Washington County), where Margaret and her sister sometimes helped with administering anesthesia and vaccinating patients in their father's practice. After his early death, Virginia Pittman took her children—Margaret, Mary Helen, and James—to Conway (Faulkner County), where she did dressmaking and canned fruits and vegetables at home to help support their studies at Hendrix College.

Margaret Pittman graduated magna cum laude from Hendrix in 1923 with a BA in mathematics and biology. Afterwards, she taught at Galloway College in Searcy (White County), then went to study at the University of Chicago, where she earned a master's degree in bacteriology in 1926. She was offered a fellowship to pursue a Ph.D., "an opportunity undreamed of," she said. She left Chicago in 1928 to work as a research scientist at the Rockefeller Institute of Medical Research in New York. She obtained her Ph.D. degree from the University of Chicago in 1929.

At the time she graduated, bacteriology was a relatively new science. Rapid progress had been made in the discovery of the causes of infectious diseases, but even so, in the early twentieth century, many diseases—typhoid fever, diphtheria, pneumonia, whooping cough, scarlet fever, and others—could not be prevented. The year 1918 saw the worst outbreak of infectious disease in modern times, the "Spanish flu" pandemic in which some twenty to forty million people died worldwide, half a million of them in the United States. Pittman began her career in its aftermath, when society was dedicating great resources to the control of disease by public health measures.

At the Rockefeller Institute she studied the bacteria *Haemophilus influenzae,* which can cause a range of infections in humans, including bronchitis and sinusitis. She observed that some strains of these bacteria have a capsule (a special covering) that allows them to enter the blood stream readily and overcome natural defenses. In all, she identified six encapsulated strains, one of which causes a form of meningitis in children. These discoveries, along with co-authorship of several publications on pneumococcus pneumonia and other research papers, won her an international reputation before she was thirty years old.

In 1936, she joined the National Institute (later Institutes) of Health, where, for most of her career, she was involved in the production, testing, and standardization of vaccines to prevent typhoid, cholera, pertussis (whooping cough), and other diseases. She promoted the concept that the effectiveness of a vaccine is related to its potency, as determined by laboratory tests, and she became well known for developing methods for testing the potency of vaccines.

During World War II, Pittman worked on developing standards for blood plasma. Great amounts of plasma were used to treat the wounded, but blood transfusions sometimes caused severe fever and chills. She and colleague Thomas Probey developed means for assuring the safety of blood plasma.

In 1943, she began work on a vaccine for pertussis. Together with colleagues, she developed a technique for testing the safety and efficacy of the vaccine. Her work became the basis for an international potency requirement. Within five years of the establishment of the requirement, there was a dramatic drop in the death rate. She said that "despite the problems that have occurred with the pertussis vaccine… I consider this work one of my best accomplishments."

Pittman was chief of the Laboratory of Bacterial Products, Division of Biologics Standards, from 1957 until 1971, the first woman to head a major laboratory at the National Institutes of Health (NIH). She was known as a blunt, straight-to-the-point sort of person, systematic and energetic. Her colleague, Dr. Harry Meyer, said that she "was always seeking and embracing the new, and could recognize new opportunities in new technologies."

She was involved in research on cholera in Pakistan with the Southeast Asia Treaty Organization from 1960 to 1970, she served as NIH project director of cholera research for from 1965 to 1970, and she was a consultant to the World Health Organization (WHO) several times between 1959 and 1983. After her retirement in 1971, she continued by invitation as an unpaid guest worker at NIH. From time to time, she worked as a consultant for WHO and as a consultant or guest scientist in several countries, including Spain, Scotland, Egypt, and Iran.

In 1976, she was wrestling with the problem of why pertussis vaccine had a number of toxic side effects. As she paced the floor, an insight "suddenly… came to me that pertussis had a true exotoxin [a protein toxin excreted by bacteria] like diphtheria or cholera toxin, that caused the harmful effects." Her concept of pertussis as a toxin-mediated disease contributed to the development of a safer vaccine. It was "satisfying to have changed the direction of work on pertussis vaccine," she later said.

Among her honors was the Federal Women's Award in 1970, which stated that "her ability to identify problems, stimulate research and evaluate results have made her an unusually effective leader." In 1986, she was elected to Honorary Fellowship in the American Academy of Pediatrics for her "efforts in improving the health and welfare of children." The Margaret Pittman Lectureship at the National Institutes of Health was established in 1994 to honor her for her accomplishments there. As one in a series of lectures sponsored by NIH, it facilitates the interchange of information and recognizes outstanding scientific accomplishment.

Pittman loved to garden, enjoyed traveling, and was active in the Mount Vernon United Methodist Church of Washington DC. She died in Cheverly, Maryland, on August 19, 1995, and is buried in the Prairie Grove Cemetery.

References:

Dowling, Harry F. *Fighting Infection: Conquests of the Twentieth Century*. Cambridge, MA: Harvard University Press, 1977.

Harden, Victoria A. "Reflections of Fifty Years at NIH: An Interview with Margaret Pittman." *NIHAA Update*, Spring 1989, p. 1, 8–15.

McBride, Gail. "Margaret Pittman: Down to a Forty-Hour Work Week." *Medicine on the Midway: Bulletin of the Medical Alumni Association, University of Chicago*, Spring 1987.

Pittman, Margaret. "A Life with Biological Products." *Annual Review of Microbiology* 44 (1990): 1–25.

Funny Quotes

Fred is 32 years old and he is still single.
One day a friend asked, "Why aren't you married? Can't you find a woman who will be a good wife?"
Fred replied, "Actually, I've found many women that I have wanted to marry, but when I bring them home to meet my parents, my mother doesn't like them."
His friend thinks for a moment and says, "I've got the perfect solution, just find a girl who's just like your mother."
A few months later they meet again and his friend says, "Did you find the perfect girl? Did your mother like her?"
With a frown on his face, Fred answers, "Yes, I found the perfect girl. She was just like my mother. You were right, my mother liked her very much."
The friend said, "Then what's the problem?"
Fred replied, "My father doesn't like her."

☺   ☺   ☺

A: I have the perfect son.
B: Does he smoke?
A: No, he doesn't.
B: Does he drink whiskey?
A: No, he doesn't.
B: Does he ever come home late?
A: No, he doesn't.
B: I guess you really do have the perfect son. How old is he?
A: He will be six months old next Wednesday.

☺   ☺   ☺

A man goes to the doctor and says, "Doctor, wherever I touch, it hurts."
The doctor asks, "What do you mean?"
The man says, "When I touch my shoulder, it really hurts. When I touch my knee - OUCH! When I touch my forehead, it really, really hurts."
The doctor says, "I know what's wrong with you. You've broken your finger!"

☺   ☺   ☺

Two factory workers are talking.
The woman says, "I can make the boss give me the day off."
The man replies, "And how would you do that?"
The woman says, "Just wait and see." She then hangs upside-down from the ceiling.
The boss comes in and says, "What are you doing?"
The woman replies, "I'm a light bulb."
The boss then says, "You've been working so much that you've gone crazy. I think you need to take the day off."
The man starts to follow her and the boss says, "Where are you going?"
The man says, "I'm going home, too. I can't work in the dark."

☺   ☺   ☺

A man receives a phone call from his doctor.
The doctor says, "I have some good news and some bad news."
The man says, "OK, give me the good news first."
The doctor says, "The good news is, you have 24 hours to live."
The man replies, "Oh no! If that's the good news, then what's the bad news?"
The doctor says, "The bad news is, I forgot to call you yesterday."

☺   ☺   ☺

A guy says to his friend, "Guess how many coins I have in my pocket."
The friend says, "If I guess right, will you give me one of them?"
The first guy says, "If you guess right, I'll give you both of them."

☺   ☺   ☺

Student: "Would you punish me for something I didn`t do?"
Teacher:" Of course not."
Student: "Good, because I haven't done my homework."

☺   ☺   ☺

The teacher says: Today, we're going to talk about the tenses. Now, if I say "I am beautiful," which tense is it?
The student says: Obviously it's the past tense.

☺   ☺   ☺

A man was complaining to a railroad engineer.
What's the use of having a train schedule if the trains are always late.
The railroad engineer replied.
How would we know they were late, if we didn't have a schedule?

Toxoplasma gondii

Domain	: Eukaryota
Kingdom	: Chromalveolata
Superphylum	: Alveolata
Phylum	: Apicomplexa
Class	: Conoidasida
Order	: Eucoccidiorida
Family	: Sarcocystidae
Subfamily	: Toxoplasmatinae
Genus	: ***Toxoplasma***
Species	: ***T. gondii***

Toxoplasma gondii is an obligate, intracellular, parasitic protozoan that causes the disease toxoplasmosis.

Found worldwide, *T. gondii* is capable of infecting virtually all warm-blooded animals. In humans, it is one of the most common parasites; serological studies estimate that up to a third of the global population has been exposed to and may be chronically infected with *T. gondii*, although infection rates differ significantly from country to country. Although mild, flu-like symptoms occasionally occur during the first few weeks following exposure, infection with *T. gondii* generally produces no symptoms in healthy human adults. However, in infants, HIV/AIDS patients, and others with weakened immunity, infection can cause serious and occasionally fatal illness (toxoplasmosis).

Infection in humans and other warm-blooded animals can occur

1. by consuming raw or undercooked meat containing *T. gondii* tissue cysts
2. by ingesting water, soil, vegetables, or anything contaminated with oocysts shed in the feces of an infected animal.
3. from a blood transfusion or organ transplant
4. or transplacental transmission from mother to fetus, particularly when *T. gondii* is contracted during pregnancy

Signs and symptoms

Infection has three stages:

Acute toxoplasmosis

During acute toxoplasmosis, symptoms are often influenza-like: swollen lymph nodes, or muscle aches and pains that last for a month or more. Rarely will a human with a fully functioning immune system develop severe symptoms following infection. Young children and immunocompromised people, such as those with HIV/AIDS, those taking certain types of chemotherapy, or those who have recently received an organ transplant, may develop severe toxoplasmosis. This can cause damage to the brain (encephalitis) or the eyes (necrotizing retinochoroiditis). Infants infected via placental transmission may be born with either of these problems, or with nasal malformations, although these complications are rare in newborns. The toxoplasmic trophozoites causing acute toxoplasmosis are referred to as Tachyzoites, and are typically found in bodily fluids.

Swollen lymph nodes are commonly found in the neck or under the chin, followed by the axillae (armpits) and the groin. Swelling may occur at different times after the initial infection, persist, and recur for various times independently of antiparasitic treatment. It is usually found at single sites in adults, but in children, multiple sites may be more common. Enlarged lymph nodes will resolve within one to two months in 60% of cases. However, a quarter of those affected take two to four months to return to normal, and 8% take four to six months. A substantial number (6%) do not return to normal until much later.

Latent toxoplasmosis

It is easy for a host to become infected with *Toxoplasma gondii* and develop toxoplasmosis without knowing it. In most immunocompetent people, the infection enters a latent phase, during which only bradyzoites are present, forming cysts in nervous and muscle tissue. Most infants who are infected while in the womb have no symptoms at birth, but may develop symptoms later in life.

Cutaneous toxoplasmosis

While rare, skin lesions may occur in the acquired form of the disease, including roseola and erythema multiforme-like eruptions, prurigo-like nodules, urticaria, and maculopapular lesions. Newborns may have punctate macules, ecchymoses, or "blueberry muffin" lesions. Diagnosis of cutaneous toxoplasmosis is based on the tachyzoite form of *T. gondii* being found in the epidermis. It is found in all levels of the epidermis, is about 6 μm by 2 μm and bow-shaped, with the nucleus being one-third of its size. It can be identified by electron microscopy or by Giemsa staining tissue where the cytoplasm shows blue, the nucleus red.

Pregnancy precautions

Congenital toxoplasmosis is a special form in which an unborn fetus is infected via the placenta. A positive antibody titer indicates previous exposure and immunity, and largely ensures the unborn fetus' safety. A simple blood draw at the first prenatal doctor visit can determine whether or not a woman has had previous exposure and therefore whether or not she is at risk. If a woman receives her first exposure to *T. gondii* while pregnant, the fetus is at particular risk.

Pregnant women should avoid handling raw meat, drinking raw milk (especially goat milk) and be advised to not eat raw or undercooked meat regardless of type. Because of the obvious relationship between Toxoplasma and cats it is also often advised to avoid exposure to cat feces, and refrain from gardening (cat feces are common in garden soil) or at least wear gloves when so engaged. Most cats are not actively shedding oocysts since they get infected in the first 6 months of their life. They shed oocysts for only a short period of time (1–2 weeks.) However, these oocysts get buried in the soil, sporulate and remain infectious for periods ranging from several months to more than a year.[Numerous studies have shown living in a household with a cat is not a significant risk factor for *T. gondii* infection,[32][33][34] though living with several kittens has some significance.

For pregnant women with negative antibody titers, indicating no previous exposure to *T. gondii*, serology testing as frequent as monthly is advisable as treatment during pregnancy for those women exposed to *T. gondii* for the first time decreases dramatically the risk of passing the parasite to the fetus.

Treatment is very important for recently infected pregnant women, to prevent infection of the fetus. Since a baby's immune system does not develop fully for the first year of life, and the resilient cysts that form throughout the body are very difficult to eradicate with antiprotozoans, an infection can be very serious in the young.

In 2006, a Czech research team discovered women with high levels of toxoplasmosis antibodies were significantly more likely to have baby boys than baby girls. In most populations, the birth rate is around 51% boys, but women infected with *T. gondii* had up to a 72% chance of a boy. In mice, the sex ratio was higher in

early latent toxoplasmosis and lower in later latent toxoplasmosis.

Diagnosis

Diagnosis of toxoplasmosis in humans is made by biological, serological, histological, or molecular methods, or by some combination of the above. Toxoplasmosis can be difficult to distinguish from primary central nervous system lymphoma. It mimics several other infectious diseases so clinical signs are non-specific and are not sufficiently characteristic for a definite diagnosis. As a result, the diagnosis is made by a trial of therapy (pyrimethamine, sulfadiazine, and folinic acid (USAN: leucovorin)), followed by a brain biopsy if the drugs produce no effect clinically and no improvement on repeat imaging.

Detection of *T. gondii* in human blood samples may also be achieved by using the polymerase chain reaction. Inactive cysts may exist in a host that would evade detection.

Several serological procedures are available for the detection of T. gondii antibody in patients, which may aid diagnosis; these include the Sabin-Feldman dye test (DT), the indirect hemagglutination assay, the indirect fluorescent antibody assay (IFA), the direct agglutination test, the latex agglutination test (LAT), the enzyme-linked immunosorbent assay (ELISA), and the immunosorbent agglutination assay test (IAAT). The IFA, IAAT and ELISA have been modified to detect immunoglobulin M (IgM) antibodies.

IgG antibody

The most commonly used tests for the measurement of IgG antibody are the DT, the ELISA, the IFA, and the modified direct agglutination test. These tests reveal that IgG antibodies usually appear within 1–2 weeks of acquisition of the infection, peak within 1–2 months, decline at various rates, and usually persist for life.

Acute infections can be differentiated from chronic infections using the "differential" agglutination test (also known as the AC/HS test), which is best used in combination with a panel of other tests such as the TSP. The AC/HS test results when parasites are fixed for use in the agglutination test with two different compounds (i.e., acetone and formalin). The different antigenic preparations vary in their ability to recognize sera obtained during the acute and chronic stages of the infection.

IgM antibody

The most commonly used tests for the measurement of IgM antibody are double-sandwich or capture IgM-ELISA kits, the IFA test, and the immunosorbent agglutination assay (IgM-ISAGA). Commercial test kits often have low specificity, and the reported results are frequently misinterpreted. The IgM antibodies appear sooner after infection than the IgG antibodies and disappear faster than IgG antibodies after recovery. In most cases, T. gondii-specific IgM antibodies are detected initially in patients with recently acquired primary infection, but these titers become negative within a few months. However, in some patients, positive T. gondii-specific IgM titers can still be observed during the chronic phase of infection.

Congenital toxoplasmosis

A whole set of recommendations applies for the diagnosis of congenital toxoplasmosis: i) prenatal diagnosis based on molecular testing of amniotic fluid and ultrasound examinations; ii) molecular testing of placenta and cord blood, comparative mother-child serologic tests and a clinical examination at birth; iii) neurologic and ophthalmologic examinations and a serologic survey during the first year of life.

Even though diagnosis of toxoplasmosis heavily relies on serological detection of specific anti-Toxoplasma immunoglobulin, serological testing does have its limitations. For example, it may fail to detect the active phase of T.*gondii* infection because the specific anti-Toxoplasma IgG or IgM may not be produced until after several weeks of parasitemia. As a result, a pregnant mother might test negative during the active phase of T. *gondii* infection leading to the undetection of congenital toxoplasmosis of a fetus. Also, the test may fail to detect T. *gondii* infections in immunocompromised patients because the titers of specific anti-Toxoplasma IgG or IgM may fail to rise in this type of patient.

A lot of PCR-based techniques have been developed for the diagnosis of toxoplasmosis using various clinical specimens, including amniotic fluid, blood, cerebrospinal fluid, and tissue biopsy. The most sensitive method among the PCR-based techniques is nested PCR followed by hybridization of PCR products. The major downside to these techniques is that they are time consuming and do not provide quantitative data.

Real-time PCR is useful in pathogen detection, gene expression and regulation, and allelic discrimination. This PCR technique utilizes the 5' nuclease activity of Taq DNA polymerase to cleave a non extendible, fluorescence-labeled hybridization probe during the extension phase of PCR. A second fluorescent dye, e.g., 6-carboxy-tetramethyl-rhodamine, quenches the fluorescence of the intact probe. The nuclease cleavage of the hybridization probe during the PCR releases the effect of quenching resulting in an increase of fluorescence proportional to the amount of PCR product, which can be monitored by a sequence detector.

Toxoplasmosis cannot be detected with immunostaining. Lymph nodes affected by *Toxoplasma* have characteristic changes, including poorly demarcated reactivegerminal centers, clusters of monocytoid B cells, and scattered epithelioid histiocytes.

Treatment

Treatment is often only recommended for people with serious health problems, such as people with HIV whose CD4 counts are under 200, because the disease is most serious when one's immune system is weak. Trimethoprim/sulfamethoxazole is the drug of choice to prevent toxoplasmosis, but not for treating active disease. A new study (May 2012) shows a promising new way to treat the active and latent form of this Disease using two Endochin-like quinolones.

Acute

The medications prescribed for acute toxoplasmosis are:

Pyrimethamine — an antimalarial medication

Sulfadiazine — an antibiotic used in combination with pyrimethamine to treat toxoplasmosis

● Combination therapy is usually given with folic acid supplements to reduce incidence of thrombocytopaenia.

● Combination therapy is most useful in the setting of HIV.

Clindamycin

Spiramycin — an antibiotic used most often for pregnant women to prevent the infection of their children

(other antibiotics, such as minocycline, have seen some use as a salvage therapy).

Latent

In people with latent toxoplasmosis, the cysts are immune to these treatments, as the antibiotics do not reach the bradyzoites in sufficient concentration.

The medications prescribed for latent toxoplasmosis are:

Atovaquone — an antibiotic that has been used to kill *Toxoplasma* cysts inside AIDS patients

Clindamycin — an antibiotic that, in combination with atovaquone, seemed to optimally kill cysts in mice

Are You Killing Your Kidneys with Soda?

THE DANGERS OF DIET SODA

If you are still convinced that drinking a can of soda or two per day is not so bad, read on. Two new studies have linked soda consumption with deteriorative effects on the kidneys.

These studies add to the massive body of research that plainly shows why soda is absolutely awful for our health.

The first study, led by Dr. Ryohei Yamamoto of the Osaka University Graduate School of Medicine, linked soft drink consumption with proteinuria. Proteinuria is the increased excretion of protein in urine, and is seen as an early sign of kidney damage.

Dr. Yamamoto and his research team tested the urine of over 12,000 university employees for protein content during their annual health exams. In those that had normally functioning kidneys at the beginning of the study, 8.4 percent of the individuals that did not drink daily sodas developed proteinuria within a three-year median follow-up.

For those who drank one soda per day, the proteinuria development within this time frame was 8.9 percent, and for those who reported drinking two or more sodas per day, the proteinuria percentage increased to 10.7 percent.

Regarding this study, Dr. Orlando Gutierrez, a kidney specialist at the University of Alabama, states, "We can assume that this is a healthy population, so I think the results are relative to healthier people, not just those with kidney disease… We now understand that protein in the urine may be a really early marker for heart disease, stroke and heart failure."

The second study, led by Agustin Gonzalez-Vicente of Case Western Reserve University, found that fructose, even in moderate intake levels, can interfere with the kidneys' ability to regulate salt balance, leading to higher salt reabsorption in kidney cells.

The researchers have associated this finding with better

explaining how high fructose corn syrup, a sweetener used in many sodas, contributes to kidney failure, hypertension, diabetes and obesity.

Both of these studies were presented in early November at the American Society of Nephrology's Kidney Week 2013 event, held at the Georgia World Congress Center in Atlanta.

These two new studies are just the tip of the iceberg when it comes to documented research exploring the many dangers of soda and diet soda. As Dr. Anil Agarwal, a kidney specialist at Ohio State University, aptly concludes, "There is no safe amount of soda."

With so many delicious and healthy beverage options available (pure, distilled water being by far the best), soda is one drink that simply does not belong in our diets.

How Diet Soda Is Linked to Kidney Damage

Over the course of the last decade, doctors have been studying a controlled group of women to test out the theory that diet soda causes kidney damage. What they found is that if you drink two or more diet soda beverages per day, you are more likely to suffer from kidney damage than someone who drinks regular soda. The test group that included the women drinking diet soda did suffer from kidney damage over the years. One reason doctors believe this occurred is because diet soda contains high levels of sodium. They also believe that the artificial sweeteners that are used in all diet sodas are to blame for the kidney damage that is occurring.

Doctors are continuing to study the effects that artificial sweeteners have on the body. There's a strong possibility that the kidneys have trouble handling and processing these artificial sweeteners. There are a variety of sweeteners on the market now that may be used in diet sodas in the future to help limit the effects they have on your kidneys.

Allergic contact dermatitis caused by isopropyl alcohol: a missed allergen?

Isopropyl alcohol is a secondary alcohol, a structural isomer of propanol that is widely used as an industrial solvent and as a preservative and antiseptic in the clinical environment. It is known to be a mild irritant for the eyes and mucous membranes, but is considered to be a weak and infrequent sensitizer.

Since 2009, when the World Health Organization published its guidelines on hand hygiene for health care professionals, there has been a marked upswing in the distribution and use of alcohol-based products because of the numerous advantages they offer over traditional handwashing.

The hand rubs that have achieved the greatest commercial success are those that contain isopropyl alcohol.

Before these products were developed, isopropyl alcohol was rarely used in medical or cosmetic preparations. Type IV hypersensitivity reactions were, therefore, rare leading some authors to even doubt whether isopropyl alcohol was in fact an allergen in humans.

However, the marked increase in the use of products containing isopropyl alcohol has led to a substantial increase in exposure. At the same time, it has been found that isopropyl alcohol is potentially an important allergen, especially when used directly on the skin although also in the case of occupational exposure.

In Europe, there are already numerous reports of health professionals who have been diagnosed with contact allergy to this substance, especially nurses and nursing assistants working in highly specialized units where frequent hand sanitizing is required (An Goossens, personal communication).

It is important to remember that, in addition to isopropyl alcohol, commercial alcohol-based hand rubs may contain other ingredients, such as emulsifiers, additives (lanolin, propylene glycol, bisabolol), and perfumes, and that the allergenic potential of these components may be even greater than that of the alcohol.

What is Allergic Hypersensitivity?

Allergic contact dermatitis (ACD) is synonymous with "eczema", a term derived from the Greek word meaning "boiling out". It is characterized by redness, oozing, crusting, weeping, and chronic scaling. Contact dermatitis occurs when the skin or mucous membrane meets foreign chemicals. Contact dermatitis may be allergic (due to different mechanisms) or irritant; it is important to distinguish between the two to know what to avoid.

Allergic skin sensitization is due to special mechanisms of the immune system and is found in genetically susceptible persons. An allergic skin reaction requires re-exposure or continuous exposure to the allergen, the substance causing the allergy. The first exposure "sensitizes" the person and the later exposure "elicits" the reaction. The hypersensitivity to an allergen can be immediate (Type I) – a sometimes-hazardous response that occurs within minutes to an hour of contact with the allergen – or delayed (Type IV), appearing at 24-72 hrs.

Immediate (Type I) Reactions versus Allergic Contact Dermatitis (Type IV)

Allergic Contact Dermatitis (ACD) usually is due to delayed hypersensitivity, which has a mechanism something like a tuberculin skin test. Most contact allergens are small, simple, lipophilic chemicals like nickel; these haptens must combine with a skin protein before becoming allergenic. The allergenic chemical (called an antigen) penetrates the skin, reaching the living cells below. Antigen-presenting cells such as Langerhans Cells present the chemical to special sets of genetically pre-programmed T-lymphocyte cells that recognize the chemical. The complex goes into draining lymph nodes and circulates in the body. More T-cells become sensitized. Thus, the contact dermatitis can spread from the primary contact site to distant parts of the body not in direct contact with the allergen. ACD susceptibility varies with the allergen, e.g., almost everyone can become sensitive to poison ivy, but most contact allergens affect fewer than 1% of the population.

The immediate (Type I) allergic reaction occurs through a different mechanism from delayed hypersensitivity. In this reaction a certain serum antibody, immunoglobulin E, is produced by the B lymphocytes, and pharmacological mediators like histamine are released from special cells known as mast cells. Skin contact with antigens like latex protein or potatoes can cause the immediate skin condition called contact urticaria – characterized by hives, itching, tingling, burning, and an immediate "wheal and flare" skin reaction (having a raised area

and redness). Sometimes tiny vesicles appear, and sometimes it is followed by eczema. The reaction may disseminate to become a generalized urticaria. Common allergens causing urticaria by contact are found in medicines, foods such as flour, cosmetics, and industrial chemicals. Exposure to allergens systemically (by eating certain foods or injection) may also cause urticaria. Raw fruits and vegetables may elicit reactions in the mouth, lips, and tongue, and throat hoarseness or irritation may occur.

Urticaria may be mediated immunologically, non-immunologically, or through unknown mechanisms. Non-immunological contact reactions occur without previous sensitization. Among the causes of non-immunological reactions are cinnamic aldehyde (found in spices, flavors, and perfumes), benzoic acid and other preservatives. Acetylsalicylic acid or NSAIDS inhibits the reaction. Diagnosis is usually based on an open epicutaneous test. If negative, a prick test (antigen applied by puncture through the palm side of the forearm skin) or scratch test (antigen applied through a scratch in the skin) may be used. This identification is important for avoidance, since treatment is only palliative.

In the contact urticaria syndrome, immunologically-mediated systemic effects can appear in other organs, especially in atopic individuals (with a history of allergy). An additional immediate response is the severe body reaction known as anaphylaxis (a shock or drop in blood pressure). Anaphylaxis may occur in conjunction with urticaria, as with the serious allergic reactions to latex products. Other important immediate reactions may include rhinitis, bronchitis, and asthma. Certain people (atopics) have a family history of immediate allergy and are more prone to get Type I reactions; they constitute about 25% of the population.

The table below summarizes the classic types of hypersensitivity reactions that are involved in allergy. One chemical may elicit both immediate and delayed types of reactions.

Although allergic contact dermatitis is generally due to direct contact, the allergen can be transmitted through the air, as with dust, pollen, pesticides, or aerosols. Contact dermatitis can spread systemically from the primary contact site to distant parts of the body not in direct contact with the allergen.

In true respiratory hypersensitivity, characteristic changes occur in lung function tests; this does not usually occur with lung irritation and ill-defined conditions, such as sick building syndrome and multiple chemical sensitivity.

Long term exposure to Isopropyl alcohol

Drying and cracking of skin may results from prolong skin exposure. Epidemiological investigations have established that a carcinogenic substance is present in isopropyl manufacturing areas, but have not confirmed isopropyl alcohol as causative agent of a cancer.

"Forty-four patients showed an allergic response to isopropyl alcohol. Four cases presented as occupational hand eczema. Fourteen cases were seen in patients with leg ulcers. Twenty-six

patients presented with eczematous lesions following the use of products containing isopropyl alcohol to disinfect previous skin lesions. Eighty-four per cent of the patients showed sensitization to three or more allergens. Relevance was present in 84% of the patients."

The alcohols to be concerned about in skin-care products are ethanol, denatured alcohol, ethyl alcohol, methanol, benzyl alcohol (when it's one of the main ingredients), isopropyl alcohol, and SD alcohol, all of which can be extremely drying and irritating to skin, and are capable of generating free-radical damage and disrupting the skin's protective barrier.

Summary of toxicology

The most important toxic effect of isopropyl alcohol is necrosis, which occurs in mice at vapor concentrations of 3000 ppm, the effects increasing with duration of exposure. Exposure to higher concentrations results in ataxia, followed by deep necrosis and death. Reversible changes occurred in the liver fat of mice repeatedly exposed to high concentrations of vapor. Isopropyl alcohol is metabolized fairly rapidly, and acetone may be detected in urine following heavy exposures. Human volunteers reported mild irritation of eyes, nose and throat after 3-5 minutes exposure to vapor at 400 ppm; at 800 ppm the results were not severe, but most subjects found the atmosphere to be objectionable.

Accidental, extensive wetting of the skin could occur in industrial situations and as isopropyl alcohol is absorbed readily through the skin, the addictive effect of inhalation and skin absorption could have serious results.

Similarly, there is a risk of deliberate ingestion of isopropyl alcohol as a substitute for ethyl alcohol, which would add to the effect of inhalation. The defatting action of isopropyl alcohol can cause mild skin irritation, but a small percentage of workers may develop contact dermatitis of a serious nature. No chronic systemic effects have been reported.

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Highlights of the coming issue

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