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Editorial

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Mini Review segment: Unsafe water is an important contributor to other potentially waterborne diseases, including typhoid, hepatitis A and E, polio and cholera. Improving the microbiological quality of drinking water, is effective in preventing diarrhoea in settings where it is endemic. The WHO is promoting the treatment of water to provide a means of accelerating the health gains associated with safe drinking water. One possible alternative is dichloroisocyanurate (NaDCC), also known as sodium dichloro-s triazinetrione. Widely used for the emergency treatment of water, NaDCC has recently been approved by the United States Environmental Protection Agency and the WHO for the routine treatment of drinking water. NaDCC is the sodium salt of a chlorinated hydroxytriazine and is used as a source of free available chlorine (FAC), in the form of hypochlorous acid (HOCl), for the disinfection of water.

Our Current Trends segment: Humans are very susceptible to the Tuberculosis infection but are remarkably resistant to the Tuberculosis disease; which is dependent largely on the state of the hosts immune system. Of all the Mycobacterial species Mycobacterium tuberculosis remains the most common cause of pulmonary tuberculosis and remains the most virulent of all the Mycobacterial species.

In Profile segment: Jules Bordet's pioneering research made clear the exact manner by which serums and antiserums act to destroy **bacteria** and foreign blood cells in the body, thus explaining how human and animal bodies defend themselves against the invasion of foreign elements. Bordet was also responsible for developing **complement** fixation tests, which made possible the early detection of many disease-causing bacteria in human and animal blood. For his various discoveries in the field of **immunology**, Bordet was awarded the Nobel Prize for medicine or physiology in 1919.

Bug of the month segment: Borrelia burgdorferi is a bacterial species of the spirochete class of the genus *Borrelia*. *B. burgdorferi* exists in North America and Europe and is the predominant causative agent of Lyme disease. B. burgdorferi sl (a total of 12 species) belongs to the genus Borrelia, family Spirochaetaceae. According to current knowledge, four species are pathogenic to humans: B. burgdorferi sensu stricto (ss), B.garinii, and B.afzelii B.spielmanii. These are gram-negative spiral bacteria, a culture of bacteria is possible in special media. Presumably, the transition takes place in round body for spirochetal survival in the tissue has a significant role.

Best Practices Segment: Antiseptics are agents that destroy or inhibit the growth and development of microorganisms in or on living tissue. Unlike antibiotics that act selectively on a specific target, antiseptics have multiple targets and a broader spectrum of activity, which include bacteria, fungi, viruses, protozoa, and even prions. We had already seen a few in the last issue, here are some more…….

Did You Know segment: Marijuana refers to the dried leaves, flowers, stems, and seeds from the hemp plant *Cannabis sativa*, which contains the psychoactive (mind-altering) chemical delta-9-tetrahydrocannabinol (THC), as well as other related compounds. This plant material can also be concentrated in a resin called hashish or a sticky black liquid called *hash oil*. Marijuana is the most common illicit drug used in the United States.

All work & no play makes Jack a dull boy! We don't forget that ever. Each issue comes with its own bouquet of jokes, so enjoy……

Feedback & suggestions are always welcomed.

Sodium dichloroisocyanurate (NaDCC),

An effective alternative to sodium hypochlorite for water treatment

General description

Molecular formula: anhydrous NaC3N3O3Cl2, dihydrate NaC3N3O3Cl2·2H2O

The IUPAC name for sodium dichloroisocyanurate, or NaDCC, is 1,3-dichloro-1,3,5- triazinane-2,4,6-trione. It is also known as sodium dichloro-s-triazine trione and sodium troclosene.

Physicochemical properties (IPCS, 2004)

Relative molecular mass 220.96

Melting point Decomposes below melting point at 230 °C

Relative density >1

Water solubility (20 °C) 25 g/100 ml (dihydrate 28 g/100 ml)

Background

Contaminated drinking water, along with inadequate supplies of water for personal hygiene and poor sanitation, are the main contributors to an estimated 4 billion cases of diarrhoea each year causing 2.2 million deaths, mostly among children under the age of five in developing countries. Unsafe water is also an important contributor to other potentially waterborne diseases, including typhoid, hepatitis A and E, polio and cholera.

An estimated 1.1 billion people lack access to improved water supplies; many more are forced to rely on supplies that are microbiologically unsafe (World Health Organization (WHO), 2004). While universal access to safe, piped-in water is an important long-term goal, this is likely to be elusive for many years to come due to the costs of building and maintaining such systems. Improving the microbiological quality of drinking water, is effective in preventing diarrhoea in settings where it is endemic. The WHO is promoting the treatment of water to provide a means of accelerating the health gains associated with safe drinking water.

One possible alternative is dichloroisocyanurate (NaDCC), also known as sodium dichloro-s triazinetrione. Widely used for the emergency treatment of water, NaDCC has recently been approved by the United States Environmental Protection Agency and the WHO for the routine treatment of drinking water. Like other forms of chlorine, NaDCC produces hypochlorous acid, a well-known oxidizing agent. Bound with cyanuric acid, however, the compound presents certain advantages over NaOCl as a water disinfectant. It may also offer other advantages in terms of stability, safety, up-front cost and convenience.

NaDCC is the sodium salt of a chlorinated hydroxytriazine and is used as a source of free available chlorine (FAC), in the form of hypochlorous acid (HOCl), for the disinfection of water. It is widely used as a stable source of chlorine for the disinfection of swimming pools and in the food industry, since it is more stable in sunlight than most other sources of chlorine. It is also used as a means of disinfecting drinking-water, primarily in emergencies, when it provides an easy-to-use source of free chlorine, and, more recently, as the form of chlorine for household point-of-use water treatment.

Basic chemistry and potential advantages of NaDCC over NaOCl

Chlorine has been used as a disinfectant for the treatment of drinking water for more than 100 years. It is by far the most commonly used means of disinfecting water, and its effectiveness as a microbicide has been widely assessed. While most conventional systems in developed countries treat water with chlorine gas (delivered as a liquid in pressurized systems), other common alternatives include calcium hypochlorite, sodium hypochlorite, lithium hypochlorite and chloroisocyanurates (sodium dichloroisocyanurate or trichloroisocyanuric acid). Until recently, the isocyanurates were used chiefly in the disinfection of water for swimming pools and industrial cooling towers. They are also a common microbial agent in cleaning and sanitizing applications, including baby bottles and contact lens. All of these compounds disinfect water by releasing free available chlorine (FAC) in the form of hypochlorous acid (HOCl).

For example, $NaOCl + H_2O \longrightarrow HOCl + NaOH$

Sodium hypochlorite dispersion in water

$NaCl_2(NCO)_3 + 2H_2O \longleftrightarrow 2HOCl + NaH_2(NCO)_3$

NaDCC dissolution in water

FAC (chlorine in the +1 oxidation state) is an effective biocide against a wide range of bacteria, fungi, algae, and viruses (White, 1998). Regardless of the original source of the available chlorine, the active microbicidal agent is hypochlorous acid. This also means that the most common method used in the field to assess the safety of drinking water—measuring FAC using the DPD reagent—is equally applicable with respect to water treated with NaDCC.

The FAC content of pure NaDCC is 64.5% and of the dihydrate is 55.5%; the FAC of elemental chlorine is 100% by definition. Thus, to produce 1 mg of available chlorine per litre requires 100/64.5 = 1.55 mg of NaDCC per litre.

While both NaOCl and NaDCC rely on HOCl as the active agent, there are important differences in the performance of the two compounds. Unlike NaOCl which releases all of its chlorine as FAC, NaDCC releases only approximately 50% of the chlorine as FAC, the balance remaining as "reservoir chlorine" (bound) in the form of chlorinated isocyanurates. When the FAC is used up, the equilibrium is disturbed, immediately releasing further FAC from the "reservoir" until the total available is used up.

This "reservoir" of FAC also enhances the biocidal protection over NaOCl when water is subject to high or variable organic loads. Such conditions are common in some remote settings, forcing the use of more costly point-of-use water treatments.

NaDCC also presents certain advantages over NaOCl in those settings where the pH is high or variable. Hypochlorous acid is a weak acid, which tends to dissociate in water at increasing pH:

$HOCl \longleftrightarrow H^+ + OCl^-$.

It is well known that chlorine loses its effectiveness to disinfect water at higher levels of pH, due to the dissociation of HOCl. While 78% of chlorine exists in the active HOCl at neutral pH 7, at pH 8 the level drops to 26%. The capacity of NaDCC to continue to release significant amounts of HOCl allows it to operate over a wider pH range.

Moreover, insofar as NaDCC tablets are acidic in solution, (the effervescent base contributes to their acidity), they tend to reduce

the pH of water favouring the formation of undissociated HOCl; hypochlorites, being alkaline, tend to disadvantageously increase the pH and, therefore, the dissociation of HOCl. This is another parameter that is difficult to control or adjust for.

Even in a tightly closed opaque bottle, NaOCl has a recommended life of only 6 months after opening.

Decomposition produces undesirable by-products (chlorite or chlorate ions). Internal testing under industry standards has shown that tabulated and strip packaged NaDCC, on the other hand, has a shelf life of 5 years in temperate and tropical climates. The stability and retention of chlorine activity has been cited as an advantage of NaDCC not only over NaOCl but also over other donors of free chlorine.

Finally, the different presentation of the chlorine sources makes effervescent (self-dissolving) NaDCC tablets/powder considerably more convenient to use than NaOCl. Bleach, though less hazardous than elemental chlorine, is a corrosive liquid subject to spillage. For water treatment, users typically measure out the recommended dose using the bottle cap. NaDCC, on the other hand, is delivered as a solid tablet/powder specifically sized to treat a given volume of wate. While liquid NaOCl (bleach) contains approximately 5% available chlorine, anhydrous NaDCC contains about 62%, roughly the equivalent of calcium hypochlorite. The potential for mis-dosing is minimized with the use of tablets/powder, whereas the use of a bottle cap can lead to over or underdosing. Excess dosing would lead to an unpalatable level of residual chlorine and higher concentrations of potentially toxic chlorinated aromatic compounds. Investigators have found NaDCC to be advantageous to NaOCl in the production of trihalomethanes.

Toxicity and regulatory review

All chlorine products have some level of toxicity; this is what renders them such effective microbicides. When chlorinated water is ingested, however, the available chlorine is rapidly reduced by saliva and stomach fluid to harmless chloride ions salts.

This is true for all sources of chlorine, including both NaOCl and NaDCC. The unique characteristic of the isocyanurates is cyanuric acid, the carrier that allows the chlorine to be contained in a solid, stable and dry form. It is the potential toxicity of such cyanuric acid, therefore, that required review by regulatory agencies prior to the approval of NaDCC for the routine treatment of drinking water. Cyanuric acid (H3C3N3O3), while confusingly similar in name, is not chemically related to cyanide. The toxicity of NaDCC and cyanuric acid have been extensively studied and documented in support of the registration of isocyanurates with the US EPA. These have been summarized (US Environmental Protection Agency (US EPA), 1992).

Studies performed on acute toxicity and irritancy were intended to assess the safety of handling the dry product. These studies found chlorinated isocyanurates no more than slightly toxic and not corrosive. Chronic and sub-chronic toxicity studies also found no toxicity. Developmental toxicity studies have also established that the compound is not fetotoxic, teratogenic (causing birth defects), mutagenic or carcinogenic. Chlorinated isocyanurates are not metabolized in the body and do not bioaccumulate.

In 2002, the WHO requested a review of the use of NaDCC as a disinfectant for drinking water as part of the rolling revisions of its Guidelines for Drinking Water Quality. The review was conducted by the Joint Food and Agriculture Organization/WHO Expert Committee on Food Additives (JECFA) and, like the EPA review, required the submission of detailed toxicological data. In June, 2003, JECFA recommended that the tolerable daily intake (TDI) for anhydrous NaDCC from treated drinking water be set at 0–2.0 mg per kg of body weight per day (WHO, 2004). Using standard methods (WHO, 1993) guideline values (GVs) for NaDCC can be derived from the TDI. This translates into a GV for adults (60 kg, with a daily drinking water consumption of 2 l) of 60 mg/l NaDCC; a GV for children (10 kg, with a daily consumption of 1 l) of 20 mg/l NaDCC; and a GV for infants (5 kg, with a daily consumption of 0.75 l) of 13 mg/l.

Microbial effectiveness

As noted above, NaDCC is an alternative source of FAC (HOCl). Accordingly, the significant body of evidence on the antimicrobial action of chlorine is as relevant to NaDCC as it is to NaOCl and other sources of chlorine. While certain bacterial spores have shown greater resistance to NaDCC, thus at least suggesting the potential for differences in activity based on the chlorine donor, no differences have been reported in respect to waterborne pathogens. Susceptibility to hypochlorous acid has been established with respect to a wide variety of bacteria, including Escherichia coli, Salmonella dysenteriae, Shigella sonnei, Campylobacter jejuni, Yersinia enterocolitica; viruses, including hepatitis A, poliovirus (type 1), rotavirus, adenovirus and calicivirus; helminthes; and protozoa, including cysts of Entamoeba histolytica and Giardia lamblia.

Microbicidal activity is a function of chlorine concentration and contact time. At doses of a few mg/l and contact time of about 30 min, free chlorine inactivates more than 4 logs of most waterborne pathogens. Cryptosporidium has demonstrated considerable resistance to chlorination and Mycobacterium has also been reported as resistant. It should also be noted that in some cases, certain viruses have also exhibited greater resistance to chlorine and chlorine compounds than common bacterial indicators of faecal contamination. This may have implications for determining the required concentration and contact time required to kill or deactivate potential pathogens in the untreated water collected for use in emergency and development settings.

A number of studies have compared the biocidal effectiveness of NaDCC with NaOCl and other disinfectants against a variety of microbes. D'Auria et al. (1989) assessed the antimicrobial activity of NaDCC among 29 Gram-positive and 29 Gram-negative bacteria, as well as 66 fungi. They reported good activity and, significantly, no adverse influence by temperature and pH. Nascimento et al. (2003) found that at concentrations of 200 ppm, NaDCC yielded superior results compared to NaOCl and certain other agents used to sanitize fresh vegetables against aerobic mesophiles, molds and yeasts, total coliforms, E. coli and Salmonella sp. In another study at concentrations of 100 ppm, NaDCC was more effective than NaOCl against Vibrio cholerae. NaDCC has also been reported effective against encysted forms of Acanthamoeba castellanii. Mazzola et al. (2003) compared the efficacy of NaDCC/sodium salt tablets with various chemical disinfectants, including a 10% solution of NaOCl on a variety of bacteria relevant to hospital settings. They recommended NaDCC over NaOCl for certain hospital applications due to its biocidal effectiveness, its slow decomposition and liberation of HOCl, its capacity to maintain an appropriate level of available chlorine without affecting the pH of the water, its low level of toxicity and its lower corrosivity against metal, plastic and rubber.

While NaDCC was shown to be comparable or superior to NaOCl in these studies of non-water treatment applications, It is that

found few studies that compared the microbiological performance of NaDCC with other agents in respect of the treatment of drinking water.

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Mycobacterium Tuberculosis

(...continued from the previous issue)

AFB Culture and Isolation

The modern bacteriology has many mycobacteriological media available to it. An ideal medium should be able to produce rapid and abundant growth, enhance phenotype characteristics, inhibit the growth of contaminants and should be usable for antimicrobial techniques. However despite advances, the isolation of *Mycobacterium tuberculosis* is still a slow process ranging from 10 days to 8 weeks.

Solid media: L. J. medium produces a slightly higher rate of TB isolation however it is prone to slant contamination. A good L. J. medium is non-selective, light green in colour, smooth slant without bubble formation so as to view Mycobacterial growth easily. The concentration of Malachite green is critical for achieving a good color contrast for visualisation of Mycobacterial colonies. Sub optimal concentration of Malachite green in the medium produces higher contamination rates where as excessive Malachite green can suppress and delay the Mycobacterium growth itself.

Agar based medium such as Middlebrook are transparent, allow quicker examination of colony morphology. Middlebrook is more resistant to contamination and produces growth of *Mycobacterium tuberculosis* faster than L.J. medium. Some commercially available 7H11 medium have been modified to increase the amount of Malachite green. Laboratory workers should be careful to determine this, for while the increase content of aniline dye retards growth of contaminating bacteria, it can also inhibit the growth of Mycobacterium.

Exposure of 7H10 to strong light or storage of the media at 2-8^{0}C for more than four weeks can be associated with deterioration and release of formaldehyde. The presence of formaldehyde results in a very inhibitory media with little or no growth of Mycobacteria.

Both Middlebrook 7H10 and 7H11 can be used for Mycobacterial drug susceptibility testing, although 7H11 is preferred.

When laboratories rely primarily on solid medium it will take a minimum of 3 weeks to produce colonies of *Mycobacterium tuberculosis*.

Liquid media: Such as Middlebrook 7H9, Dubos Tween albumin broth and Kirchner medium have been developed for the enrichment of growth of small number of Mycobacteria. They are valuable in isolating bacteria from uncontaminated specimen such as CSF, pleura and peritoneal fluids. There is an increased growth rate of *Mycobacterium tuberculosis* in liquid medium. Inclusion of Antibiotic cocktails such as PACT (Polymyxin B, Amphotericin B, Carbenicillin, Trimethoprim) or PANTA (Polymyxin B, Amphotericin B, Nalidixic Acid, Trimethoprim, Azlocillin) is required to make the liquid media sufficiently inhibitory to the growth of other bacteria and fungi especially when sputum specimens are used.

It is recommended internationally that specimen for Mycobacterial culture should be inoculated in both types of media. According to DIN and DZK guidelines atleast 3 different media should be inoculated, and atleast one of them being a liquid medium.

The different composition of the media and combination of different media have an impact on the yield and positive cultures, thereby increasing sensitivity of culture & Mycobacterial isolation.

Recent Indian studies have also indicated that 'Lowenstein-Jensen' medium and 'Kirchner's' liquid medium are the best combination for the isolation of Mycobacteria from specimens other than sputum.

Ideally the cultures are incubated at 36±1°C; with an atmosphere of 5-10% of CO_2 being stimulating to the growth of Mycobacteria.

Radiometric media: Developed in 1970, represent a significant improvement in the rapid isolation of *Mycobacterium tuberculosis*. Detection time is directly proportional to the number of metabolically active bacteria present and the metabolic rate is influenced by the type of specimen, number of organisms, therapy status of patient, decontamination procedures and the incubation temperature.

The average time for reporting the isolation of *Mycobacterium tuberculosis* using radiometric technique is reportedly 22±9 days as compared to 31±9 days for solid media.

However the radiometric system is more labor intensive, requires disposal of radioactive material and still cannot detect some *Mycobacterium tuberculosis* isolates that can only be detected on agar slants. Some laboratories prefer to use L.J. slants as a backup to Radiometric media. Considering the cost aspects and the fact that *Mycobacterium tuberculosis* is largely a problem of the third world, use of radiometric media is still restricted and use of solid and liquid media is widely practiced.

Susceptibility Testing of Mycobacterium tuberculosis

Resistance to antitubercular agents was recognised soon after their introduction in early 1960s, and standardized methods for antimicrobial susceptibility have been developed. Routine laboratory susceptibility testing of primary TB isolates has not been generally suggested unless drug resistance in a particular community exceeds 5%. However with the resurgence of TB drug resistance, C.D.C. USA has recently recommended that susceptibility tests should be performed on all primary isolates.

In a recent Indian study, a total of 3181 samples were processed for isolation of tubercle bacilli; and 707 samples were culture positive. The pattern of drug resistance is shown in the following table:

Pattern of Drug Resistance for *Mycobacterium tuberculosis*

Drug	Per cent Resistance
Isoniazid	30.41
Rifampin	58.55
Streptomycin	46.95
Ethambutol	3.67
D Cycloserine	24.32
Kanamycin	14.42
Ethionamide	60.67
Amikacin	15.84
Ciprofloxacin	7.49

* Adapted from Bombay Hospital Journal; Drug Resistance in Tuberculosis; by Lina Deodhar et al. April 1999.

In India, it has been observed that private practitioners use different drug regimens to treat tuberculosis and very few regimens match with the standard (recommended by W.H.O.).

The problem of acquired drug resistance (ADR) is truly man made. Poor administered Tuberculosis control programme, inadequate dosages, monotheraphy, insufficient durations of treatment, irregularity in drug intake, frequent defaults are some of the common reasons for emergence of ADR. In addition, HIV is quickening the pace at which Tuberculosis is spreading. Therefore, Tuberculosis is becoming the leading killer disease of HIV-positive people.

Clinicians should ensure that *Mycobacterium tuberculosis* susceptibility tests are carried out for patients:

- Who fail to respond after 3 months of treatment;
- Who do not convert to having negative smears after 3 months of treatment; with regimens that included INH and Rifampin, and 5 months for treatment without INH and Rifampin.
- Whose smears demonstrate increasing number of AFB after an initial decrease;
- Patients whose cultures do not become negative after 4-6 months;
- Patients who relapse.

TB susceptibility testing has three main goals:

- It provides data as to what drug should be used for treatment;
- Screens for drug resistance;
- Measures incidence and prevalence of drug resistance within the community.

Susceptibility Testing Methodology

Susceptibility tests can be performed directly, from a smear positive specimen, or indirectly, from the growth of colonies from the specimens. The former has the advantage of measuring the sensitivity prior to cultivation on laboratory media. The direct method also produces results more rapidly but; because of uncertainty on the species of Mycobacterium, and due to less control of the viable inoculums size, the results require confirmation with an indirect test, the direct test is not generally utilized.

Three methods make use of critical concentrations to define drug resistance and can be performed directly or indirectly:
Absolute concentration method;

- Resistance ratio method;
- Proportion method.

The absolute concentration method determines if 1% or more of an inoculum will grow after being cultured on media containing critical concentrations of a drug on the plate. It requires growth of the patient strain on drug free medium to demonstrate the viability, but does not compare the colony numbers on drug free and drug containing media so that the inoculum must be carefully standardized.

The resistance ratio is similar to the absolute concentration method except that the patient strain is compared with the growth of a standard laboratory strain. Results are reported as the ratio of the MIC of the patient strain to that of the laboratory strain. A patient strain with a ratio of 8:1 is considered resistant, while 4:1 is suggestive of resistance. This method is more tolerant to variation in concentrations of drugs within different batches of media.

The proportion method compares the growth of a patient strain in the presence and absence of a drug. If 1% or more of the inoculum produces colonies on media that contains an agent at the critical concentration compared with controls, the isolate is considered to be resistant. This method is the most popular and is relatively simple to perform and interpret.

Susceptibility Testing of Mycobacteria

Eleven drugs are used in the treatment of Tuberculosis. Five are considered "primary" and include Streptomycin, Isoniazid, Rifampin, Pyrazinamide and Ethambutol, while the remaining six, Ethionamide, Ciprofloxacin, Kanamycin, D cycloserine, para-Aminosalicylic acid and Amikacin are considered "secondary" and used only when resistance develops to the primary drugs.

Although drugs have been incorporated in inspissated egg-based media for conducting susceptibility tests, many laboratories internationally now prefer using Middlebrook 7H11 or 7H10 as a base medium, adding the drugs after cooling the agar to 45^{0}C. Adding the drugs to the agar medium after autoclaving decreases the loss of activity that can occur in egg-based medium such L.J. during inspissation. An additional loss of drug activity may occur in egg-based media with binding of some agents to egg albumin and other proteins.

Drug Concentrations for Proportion Method Susceptibility Testing using various culture media*

Drug	Drug Concentrations (µg/ml)		
	7H10	7H11	Lowenstein-Jensen
Isoniazid	0.2, 1.0	0.2, 1.0	0.2, 1.0
p-Aminosalicylic Acid	2.0	8.0	0.5
Streptomycin	2.0	2.0	4.0
Rifampin	1.0	1.0	40.0
Ethambutol	2.0	7.5	2.0
Ethionamide	5.0	10.0	20.0
Kanamycin	5.0	6.0	20.0
Capreomycin #	10.0	10.0	20.0
D Cycloserine	20.0	30.0	30.0
Pyrazinamide	50.0	-	100.0

Amikacin & Ciprofloxacin are used in L.J. medium at 20µg/ml & 20µg/ml concentrations respectively, adapted from Bombay Hospital Journal; Drug Resistance in Tuberculosis; by Lina Deodhar et al. April 1999.

* Adapted from Clinical Diagnosis & Management by Laboratory Methods, Todd, Sanford & Davidsohn, 17th Edition 1998, Edited by J. B. Henry and Gradwohl's Clinical Laboratory Methods & Diagnosis; Edited by A. C. Sonnenwirth & L. Jarett. Vol.2, 8th Edition, 1982.

A simplified method for preparing drug susceptibility plates has also been developed. This method uses filter paper disks containing the primary antitubercular drugs, and the test for susceptibility is run in a similar fashion as the Kirby Bauer method for routine drug susceptibility tests.

As discussed, the direct Mycobacterial susceptibility test is inoculated from digested and concentrated sputum found to be positive for acid-fast bacilli. The indirect susceptibility test is inoculated from colonies isolated from a primary culture. The direct test will usually give good results only if large numbers of Mycobacteria are present in the specimen. The advantage of the direct susceptibility test is an earlier report (three to four weeks) in contrast to the indirect test, which may take up to six to eight weeks. The disadvantage of the direct susceptibility test is that it usually requires a large number of Mycobacteria for successful growth and is often overgrown by large numbers of contaminating bacteria.

Other novel methods of susceptibility testing have been developed based on the mycobacteriophage technique, using the luminescent luciferase activity. Other researchers have localized specific *Mycobacterium Tuberculosis* mutations responsible for drug resistance. These sites have been used as amplification targets and promise to provide a rapid method for testing the susceptibility of patient isolates to these drugs.

Other Markers

Adenosine deaminase, a surrogate marker, for the diagnosis of Tuberculosis has also shown promise. It is based on the measurement of activity of Adenosine deaminase, an enzyme produced by lymphocytes. The test has excellent sensitivity for TB meningitis and for examining pleural infections.The sensitivity and specificity is reported well above 90%, the test is easy to perform and relatively inexpensive.

To conclude, the objective of adapting different types of technology and instruments is to shorten the times for isolation, identification and susceptibility testing of bacteria and other microorganisms has been particularly relevant for Mycobacteria. Hopefully alternative methods to the standard procedures now used, could be developed soon enough for routine use, to provide cultures and susceptibility information in a shorter time interval. Till such time the AFB staining, culture and sensitivity remain the Gold Standard for accurate and early diagnosis of Tuberculosis, improvements and standardisation of techniques for these classical methods is important for better laboratory diagnosis and clinician information support. Significant cost savings might be effected by a reduction in hospitalization and return of the patient to a productive career.

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Jules Bordet

Born:
13 June 1870, Soignies, Belgium
Died:
6 April 1961, Brussels, Belgium
Affiliation at the time of the award:
Brussels University, Brussels, Belgium
Prize motivation:
For his discoveries relating to immunity
Field:
Immunity

Jules Bordet's pioneering research made clear the exact manner by which serums and antiserums act to destroy **bacteria** and foreign blood cells in the body, thus explaining how human and animal bodies defend themselves against the invasion of foreign elements. Bordet was also responsible for developing **complement** fixation tests, which made possible the early detection of many disease-causing bacteria in human and animal blood. For his various discoveries in the field of **immunology**, Bordet was awarded the Nobel Prize for medicine or physiology in 1919.

Jules Jean Baptiste Vincent Bordet was born in Soignies, Belgium, a small town situated twenty-three miles southwest of Brussels. He was the second son of Charles Bordet, a schoolteacher, and Célestine Vandenabeele Bordet. The family moved to Brussels in 1874, when his father received an appointment to the École Moyenne, a primary school. Jules and his older brother Charles attended this school and then received their secondary education at the Athéné Royal of Brussels. It was at this time that Bordet became interested in chemistry and began working in a small laboratory that he constructed at home. He entered the medical program at the Free University of Brussels at the age of sixteen, receiving his doctorate of medicine in 1892. Bordet began his research career while still in medical school, and in 1892 published a paper on the adaptation of **viruses** to vaccinated organisms in the *Annales de l'Institut Pasteur* of Paris. For this work, the Belgian government awarded him a scholarship to the Pasteur Institute, and from 1894 to 1901, In 1899, Bordet married Marthe Levoz; they eventually had two daughters, and a son who also became a medical scientist.

During his seven years at the Pasteur Institute, Bordet made most of the basic discoveries that led to his Nobel Prize of 1919. Soon after his arrival at the Institute, he began work on a problem in immunology. In 1894, Richard Pfeiffer, a German scientist, had discovered that when cholera bacteria was injected into the peritoneum of a guinea pig immunized against the infection, the pig would rapidly die. This bacteriolysis, Bordet discovered, did not occur when the bacteria was injected into a non-immunized guinea pig, but did so when the same animal received the **antiserum** from an immunized animal. Moreover, the bacteriolysis did not take place when the bacteria and the antiserum were mixed in a test tube unless fresh antiserum was used. However, when Bordet heated the antiserum to 55 degrees centigrade, it lost its power to kill bacteria. Finding that he could restore the bacteriolytic power of the antiserum if he added a little fresh serum from a nonimmunized animal, Bordet concluded that the bacteria-killing phenomenon was due to the combined action of two distinct substances: an **antibody** in the antiserum, which specifically acted against a particular kind of bacterium; and a non-specific substance, sensitive to heat, found in all animal serums, which Bordet called "alexine" (later named "complement").

In a series of experiments conducted later, Bordet also learned that injecting red blood cells from one animal species (rabbit cells in the initial experiments) into another species (guinea pigs) caused the serum of the second species to quickly destroy the red cells of the first. And although the serum lost its power to kill the red cells when heated to 55 degrees centigrade, its potency was restored when alexine (or complement) was added. It became apparent to Bordet that hemolytic (red cell destroying) serums acted exactly as bacteriolytic serums; thus, he had uncovered the basic mechanism by which animal bodies defend or immunize themselves against the invasion of foreign elements. Eventually, Bordet and his colleagues found a way to implement their discoveries. They determined that alexine was bound or fixed to red blood cells or to bacteria during the immunizing process. When red cells were added to a normal serum mixed with a specific form of bacteria in a test tube, the bacteria remained active while the red cells were destroyed through the fixation of alexine. However, when serum containing the antibody specific to the bacteria was destroyed, the alexine and the solution separated into a layer of clear serum overlaying the intact red cells. Hence, it was possible to visually determine the presence of bacteria in a patient's blood serum. This process became known as a complement fixation test. Bordet and his associates applied these findings to various other infections, like **typhoid fever**, carbuncle, and hog cholera. **August von Wasserman** eventually used a form of the test (later known as the **Wasserman test**) to determine the presence of **syphilis** bacteria in the human blood.

Already famous by the age of thirty-one, Bordet accepted the directorship of the newly created Anti-rabies and Bacteriological Institute in Brussels in 1901; two years later, the organization was renamed the Pasteur Institute of Brussels. In 1907, he also began teaching following his appointment as professor of bacteriology in the faculty of medicine at the Free University of Brussels, a position that he held until 1935. Despite his other activities, he continued his research in immunology and bacteriology. In 1906, Bordet and Octave Gengou succeeded in isolating the bacillus that causes **pertussis** (whooping cough) in children and later developed a **vaccine** against the disease. Between 1901 and 1920, Bordet conducted important studies on the coagulation of blood. He was in the United States to raise money for new medical facilities for the wardamaged Free University of Brussels when he received word that he had been awarded the Nobel Prize. After 1920, he became interested in **bacteriophage** , the family of viruses that kill many types of bacteria, publishing several articles on the subject. In 1940, Bordet retired from the directorship of the Pasteur Institute of Brussels and was succeeded by his son, Paul. Bordet himself continued to take an active interest in the work of the Institute despite his failing eyesight and a second German occupation of Belgium during World War II. Many scientists, friends, and former students gathered in a celebration of his eightieth birthday at the great hall of the Free University of Brussels in 1950. He died in Brussels in 1961.

In bio practical:
Examiner: Tell me the name of this bird by seeing its legs only?
Sardar: I don't know.
Examiner: You have failed, what's your name?
Sardar: See my legs & tell my name.

Guide: I welcome you all to Niagara Falls. They are the world's largest waterfalls and the sound intensity of the waterfall is so high, even 20 supersonic planes passing by can't be heard.
Now I request the ladies to keep quite so that we can hear the Niagara Falls.

Wife: "I look fat. Can you give me a compliment?"
Husband: "You have perfect eyesight."

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."

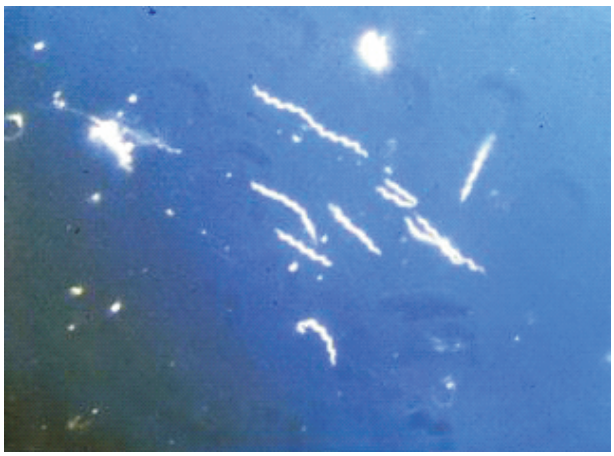
Phool wala: Sahab yeh phool apni girlfriend ke liye lelo.
Santa:Meri koi girlfriend nahi hai.
Phool wala: To phir apni mangetar ke liye hi lelo.
Santa: Woh bhi nahi hai.
Phool wala: To phir apni biwi ke liye hi lelo.
Santa: Meri biwi bhi nahi hai.
Phool wala: To phir aye duniya ke sabse khush-kismat insaan, meri tafar se yeh phool tere liye.

Santa: Hurry-up, hurry-up, give me a drink. Fight is about to start.
Bartender gives him a drink.
Santa again says: Hurry-up, hurry-up, give me drink. Fight is about to start.
Bartender again gives him a drink.
Santa again asks for a drink as the fight is about to start.
Bartender: When on earth the fight will start?
Naughty Santa: When you will ask for money.

Great Quotes on Sales

1. "A good listener is not only popular everywhere, but after a while he knows something." – Wilson Mizner
2. "Lack of direction, not lack of time, is the problem. We all have twenty-four hour days." – Zig Ziglar
3. "Excellence is not a skill. It's an attitude." – Ralph Marston
4. "Your competition is EVERYTHING else your prospect could conceivably spend their money on." – Don Coope
5. "You will get all you want in life if you help enough other people get what they want." – Zig Ziglar
6. "Prospecting – Find the man with the problem." – Ben Friedman
7. "Stop selling. Start helping." – Zig Ziglar
8. "Money is good for bribing yourself through the inconveniences of life." – Gottfried Reinhardt
9. "You will never find time for anything. If you want time you must make it." – Charles Robert Buxton
10. "Every sale has five basic obstacles: no need, no money, no hurry, no desire, no trust." – Zig Ziglar
11. "If opportunity doesn't knock, build a door." – Milton Berle
12. "Business is like riding a bicycle. Either you keep moving or you fall down." – Frank Lloyd Wright
13. "Some men see things as they are and ask why…I dream of things that never were and ask why not?" – Robert Kennedy
14. "People with goals succeed because they know where they're going." – Earl Nightingale
15. "It is hard to fail, but it is worse never to have tried to succeed." – Theodore Roosevelt
16. "You can have anything in this world you want, if you want it badly enough and you're willing to pay the price." – Mary Kay Ash
17. "There are no gains without pains." – Benjamin Franklin
18. "It is not your customer's job to remember you. It is your obligation and responsibility to make sure they don't have the chance to forget you." – Patricia Fripp
19. "The end result of kindness is that it draws people to you." – Anita Roddick
20. "To speak and to speak well are two things. A fool may talk, but a wise man speaks." – Ben Jonson
21. "Live out of your imagination, not your history." – Stephen Covey
22. "Winning is a habit. Unfortunately, so is losing." – Vince Lombardi
23. "Confidence and enthusiasm are the greatest sales producers in any kind of economy." – O. B. Smith
24. "Even if you are on the right track, you'll get run over if you just sit there." – Will Rogers
25. "Quality begins on the inside… and then works its way out." – Bob Moawad
26. "Never, never, never quit." – Winston Churchill

Borrelia burgdorferi



Scientific classification

Domain : Bacteria
Phylum : Spirochaetes
Order : Spirochaetales
Family : Spirochaetaceae
Genus : *Borrelia*
Species : ***B. burgdorferi***

Borrelia burgdorferi is a bacterial species of the spirochete class of the genus *Borrelia*. *B. burgdorferi* exists in North America and Europe and is the predominant causative agent of Lyme disease. *Borrelia* species are considered diderm (double-membrane) bacteria rather than gram positive or negative.
B. burgdorferi sl (a total of 12 species) belongs to the genus Borrelia, family Spirochaetaceae. According to current knowledge, four species are pathogenic to humans: *B. burgdorferi* sensu stricto (ss), B.garinii, and B.afzelii B.spielmanii. These are gram-negative spiral bacteria, a culture of bacteria is possible in special media. Presumably, the transition takes place in round body for spirochetal survival in the tissue has a significant role.

Borrelia Burgdorferi epidemiology

Lyme disease occur in the northern hemisphere and are widely used in all German regions. The transmission of pathogens carried by a tick bite (Ixodes ricinus, Ixodes vulgo). Pathogen reservoirs are small rodents, birds and deer. Adult ticks are about 20% infected nymphs and larvae of 10 to 20% to about 1%. The period of tick activity is dependent on the weather from about March to October. About 1.5 to 6% of people with tick bites show a seroconversion (infection), at 0.3 to 1.4% is expected to overt disease. After contact with infected ticks, a seroconversion developed in 20 to 30% of cases. In the eastern states, including Berlin, the number of reported infections has risen to 2006 with 6241 infections in the ensuing years has declined moderately. Transmission from human to human is not known, not even during pregnancy or through breast milk.

Borrelia Burgdorferi pathogenesis

The transmission of the pathogen occurs in the late act of sucking for 24 to 48h. After penetrating into the bloodstream pathogens show a tropism, are primarily affected skin, myocardium, synovial fluid and nerve tissue. The name derives from the Lyme disease Lyme, Connecticut (USA). Similar to syphilis, the disease course in three will be divided overlapping stages, with a remission is possible in each of the stages.

Stage I (after a few weeks and months): erythema (chronicum) migrans skin. Beginning z.T. with an initial papule, followed by a sharply demarcated, painless, non-itching erythema which spreads centrifugally and fades away in the middle. Partly uncharacteristic symptoms such as fever, headache (meningism), myalgia, arthralgia, and conjunctivitis Lympknotenschwellungen. Antibodies are detectable at 20 to 50% of patients. Early neuroborreliosis with facial palsy, lymphocytic meningitis (mainly as a manifestation of head pain, and photophobia, nausea, vomiting and mortality was 0.6%, mainly in children between 5 and 15 years), encephalitis, radiculitis.

Stage II (after weeks and months): Garin Bujadoux-Bannwarth syndrome (lymphocytic meningoradiculitis) with burning radicular pain, asymmetric flaccid paralysis and unsystematically distributed, often combined with sensory deficits, which can last for weeks or months. Cranial nerves are affected, mainly consist-or bilateral facial paralysis. In rare cases, with a participation of the Optic nerve and blindness results. On the skin it comes in rare cases as a manifestation of Borrelia Lymphozytom (Lymphocytoma cutis benigna other types) in the form of a livid reddish tumor (earlobe, nipple or scrotum). Manifestations may occur as the heart myocardium, and peri-carditis, atrioventricular conduction disturbances are possible up to a complete block and ST-T changes, atrial fibrillation, ventricular extrasystoles, tachycardia, impaired ventricular function through to heart failure. Antibodies are detectable at 70 to 90% of patients.

Stage III (months to years): Acrodermatitis chronica atrophicans (ACA) Herxheimer with atrophy of the skin ("cigarette paper-thin"), antibodies are not detectable in all patients. Lyme arthritis is a relapsing or chronic mono-or oligoarticular disease, are affected ankles, elbows, fingers, toes and wrist joints as well as TMJ. In rare cases, the infection manifests as a chronic encephalomyelitis with para-and tetra paresis. Antibodies are detectable at 90 to 100% of those affected, usually only IgG. Involvement of the eyes in all three stages in the form of uveitis or optic neuritis described. Hotly debated the existence of a so-called "post-Lyme syndrome" is.

Borrelia Burgdorferi diagnosis

In typical manifestations (skin, neurological symptoms, heart, joints) is primarily driven by the differential diagnosis of Lyme disease, which is backed by one antibody detection (serum, CSF or serum-CSF pairs to determine the indigenous production of specific antibodies in CSF). It should be noted, however, that a negative serology does not always rule out Lyme disease. As an ELISA screening test is recommended despite lower sensitivity of immunoblot as confirmatory test. The cultivation of the pathogen (CSF, skin biopsy) is possible but time-consuming and subject to special laboratories. An alternative is Nukleinsaureamplifikationsmethoden (eg PCR) is particularly suitable is the investigation of joint aspirates or synovial, since

there obviously cannot be cultured Borrelia survive even after therapy. The same applies to endomyocardial biopsies in patients with new onset dilated cardiomyopathy.

Borrelia Burgdorferi prevention and treatment

Prevention by exposure prophylaxis, after appropriate exposure, the body should be examined for ticks, depending removed earlier, the lower the risk of transmission. A vaccine is not available. Treatment in the early phase is an easy mission by amoxicillin or doxycycline, orally for few weeks. However, the recommended regimen in late stages include parenteral ceftriaxone, analgesics to control the severe pain, and anti-inflammatory drugs, usually required for months. It is crucial for early diagnosis and subsequent treatment with doxycycline (DOXYHEXAL etc.) over 10 to 21 days. Alternatively, can be administered amoxicillin (amoxicillin-ratiopharm, etc.). In later stages of the disease, with cardiac involvement in long-standing arthritis and neurological symptoms is a treatment with ceftriaxone (Rocephin, etc.) displayed for three weeks, an additional subsequent therapy with oral amoxicillin for another 100 days does not lead to better treatment success. As an alternative to doxycycline could in refractory cases, administration of tigecycline (Tygacil) are trying to effect on the spirochetal round body. In refractory arthritis and negative PCR, the administration of nonsteroidal anti-inflammatory drugs, possibly in combination with hydroxychloroquine (QUENSYL) is recommended. In endemic areas, the use of prophylactic antibiotics after tick bite may be useful. An obligation exists only in some states.

Application to Biotechnology

Borrelia burgdorferi is well studied pathogen that causes Lyme disease. Most of the research on this bacteria is focused on understanding the vector and host transmission as well as its pathogenic activity in order to develop better methods to prevent infection. It does not have a current, widespread use in biotechnology.

Current Research

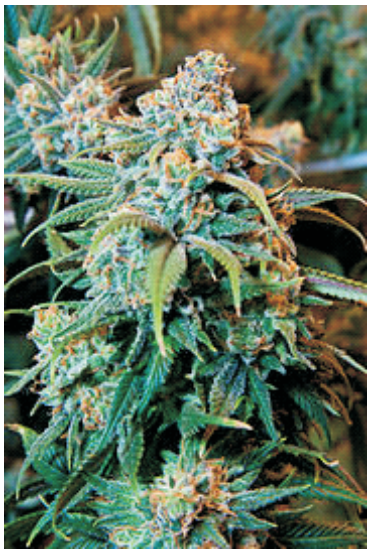
Much of the current research surrounding *B. burgdorferi* concentrates on discovering the mechanisms through which the bacteria invades the human body in order to produce more effective treatments against it. As a member of the Spirocheate phylum, *B. burgdorferi* is quite distinct from many other bacterial groups and therefore many of the common genetic tools do not work on the bacteria. It is also very difficult to transform in the laboratory.

The most current research of *B. burgdorferi* surrounds the discovery of the immunosuppressive tick salivary protein, Salp15. This protein is believed to be involved in the transmission of the bacteria from the vector to the host. It is believed that the tick produces Salp15, which binds to *B. burgdorferi* and protects it from the host antibody response (thus earning its immunosuppressive title). This makes Salp15 an attractive target for research and possible use in vaccines ("Transmission").

In April 2007, Juncadella and colleagues determined that Salp15 inhibited T-cell activation by interfering with the binding of CD4 receptors o nT-cells and disrupting their signalling cascade. The researchers were able to identify the C-terminal peptide of Salp15 as responsible for its immunosuppressive properties. They confirmed that the Salp15-CD4 interactions caused premature activation and a defective immune response. These results can help future researchers uncover methods to block the activity of Salp15 and possibly develop an effective vaccine.

Current research is also investigating other mechanisms in which *Borrelia burgdorferi* is able to evade the host organisms immune response. One theory is that the bacterial outer surface proteins are able to bind to host factor H, a regulator in the complement activation pathway. By binding to this regulator, the bacteria may be able to protect themselves from complement killing. Researchers tested this theory by developing strains of mice that were deficient in host factor H in order to see whether *B. burgdorferi* binding was necessary for infection. Their results showed that the deficient mice were infected at similar levels to wild-type mice. In addition, control experiments showed that many of the bacteria did not bind to host factor H even when they had outer surface proteins present capable of doing so. They also showed that some mice deficient in complement activating pathways were able to contain the *Borrelia* infection. These results indicate that *B. burgdorferi* must have other mechanisms to prevent complement activation in the host, and that complement mediated killing may not be the only way for the host to combat the bacteria. The results of these experiments help us better understand the mechanisms involved in *B. burgdorferi* infections (Woodman).

Interesting research was also performed concerning the sensitivity of certain individuals to tick bites and whether this sensitivity could limit the ability of *B. burgdorferi* to infect that person. The researchers sampled a population in Rhode Island and determined that people who had more exposure to tick bites had stronger and faster reactions to the bites and also had less risk of developing Lyme disease. Their results showed that people who were frequently bitten by ticks developed an immunity against them, possibly against tick salivary antigens. Therefore, the new hypersensitivity may be able to protect this individuals from *Borrelia* infection and help them recognize tick bites and remove them before the bacteria can be transmitted. These results lend support to the idea that immunity against tick salivary proteins may help prevent infection by *B. burgdorferi* and therefore the symptoms of Lyme disease (Burke).



Marijuana

Botanical : Cannabis
Source plant(s) : Cannabis sativa, Cannabis sativa forma indica, Cannabis ruderalis
Part(s) of plant : flower
Geographic origin : Central and South Asia.
Active ingredients : Tetrahydrocannabinol, cannabidiol, cannabinol, tetrahydrocannabivarin

Marijuana refers to the dried leaves, flowers, stems, and seeds from the hemp plant Cannabis sativa, which contains the psychoactive (mind-altering) chemical delta-9-tetrahydrocannabinol (THC), as well as other related compounds. This plant material can also be concentrated in a resin called hashish or a sticky black liquid called hash oil. Marijuana is the most common illicit drug used in the United States.

How is Marijuana Used?

Marijuana is usually smoked in hand-rolled cigarettes (joints) or in pipes or water pipes (bongs). It is also smoked in blunts—cigars that have been emptied of tobacco and refilled with a mixture of marijuana and tobacco. Marijuana smoke has a pungent and distinctive, usually sweet-and-sour, odor. Marijuana can also be mixed in food or brewed as a tea.

How Does Marijuana Affect the Brain?

When marijuana is smoked, THC rapidly passes from the lungs into the bloodstream, which carries the chemical to the brain and other organs throughout the body. It is absorbed more slowly when ingested in food or drink.

However it is ingested, THC acts on specific molecular targets on brain cells, called cannabinoid receptors. These receptors are ordinarily activated by chemicals similar to THC that naturally occur in the body and are part of a neural communication network called the endocannabinoid system. This system plays an important role in normal brain development and function.

The highest density of cannabinoid receptors is found in parts of the brain that influence pleasure, memory, thinking, concentration, sensory and time perception, and coordinated movement. Marijuana overactivates the endocannabinoid system, causing the "high" and other effects that users experience. These effects include altered perceptions and mood, impaired coordination, difficulty with thinking and problem solving, and disrupted learning and memory.

Marijuana also affects brain development, and when it is used heavily by young people, its effects on thinking and memory may last a long time or even be permanent. A recent study of marijuana users who began using in adolescence revealed substantially reduced connectivity among brain areas responsible for learning and memory. And a large long-term study in New Zealand showed that people who began smoking marijuana heavily in their teens lost an average of 8 points in IQ between age 13 and age 38. Importantly, the lost cognitive abilities were not fully restored in those who quit smoking marijuana as adults. Those who started smoking marijuana in adulthood did not show significant IQ declines.

What Are the Other Health Effects of Marijuana?

Marijuana use may have a wide range of effects, particularly on cardiopulmonary and mental health.

Marijuana smoke is an irritant to the lungs, and frequent marijuana smokers can have many of the same respiratory problems experienced by tobacco smokers, such as daily cough and phlegm production, more frequent acute chest illness, and a heightened risk of lung infections. One study found that people who smoke marijuana frequently but do not smoke tobacco have more health problems and miss more days of work than those who don't smoke marijuana, mainly because of respiratory illnesses. It is not yet known whether marijuana smoking contributes to risk for lung cancer.

Marijuana also raises heart rate by 20-100 percent shortly after smoking; this effect can last up to 3 hours. In one study, it was estimated that marijuana users have a 4.8-fold increase in the risk of heart attack in the first hour after smoking the drug. This risk may be greater in older individuals or in those with cardiac vulnerabilities.

A number of studies have linked chronic marijuana use and mental illness. High doses of marijuana can produce a temporary psychotic reaction (involving hallucinations and paranoia) in some users, and using marijuana can worsen the course of illness in patients with schizophrenia. A series of large studies following users across time also showed a link between marijuana use and later development of psychosis. This relationship was influenced by genetic variables as well as the amount of drug used, drug potency, and the age at which it was first taken—those who start young are at increased risk for later problems.

Associations have also been found between marijuana use and other mental health problems, such as depression, anxiety, suicidal thoughts among adolescents, and personality disturbances, including a lack of motivation to engage in typically rewarding activities. More research is still needed to confirm and better understand these linkages.

Marijuana use during pregnancy is associated with increased risk

of neurobehavioral problems in babies. Because THC and other compounds in marijuana mimic the body's own endocannabinoid chemicals, marijuana use by pregnant mothers may alter the developing endocannabinoid system in the brain of the fetus. Consequences for the child may include problems with attention, memory, and problem solving.

Additionally, because it seriously impairs judgment and motor coordination, marijuana contributes to risk of injury or death while driving a car. A recent analysis of data from several studies found that marijuana use more than doubles a driver's risk of being in an accident. The combination of marijuana and alcohol is worse than either substance alone with respect to driving impairment.

Is Marijuana Medicine?

Many have called for the legalization of marijuana to treat conditions including pain and nausea caused by HIV/AIDS, cancer, and other conditions, but clinical evidence has not shown that the therapeutic benefits of the marijuana plant outweigh its health risks. To be considered a legitimate medicine by the FDA, a substance must have well-defined and measurable ingredients that are consistent from one unit (such as a pill or injection) to the next. As the marijuana plant contains hundreds of chemical compounds that may have different effects and that vary from plant to plant, and because the plant is typically ingested via smoking, its use as a medicine is difficult to evaluate.

However, THC-based drugs to treat pain and nausea are already FDA approved and prescribed, and scientists continue to investigate the medicinal properties of other chemicals found in the cannabis plant—such as cannabidiol, a non-psychoactive cannabinoid compound that is being studied for its effects at treating pain, pediatric epilepsy, and other disorders.

Industrial Hemp

Hemp is a distinct variety of the plant species cannabis sativa L. that contains minimal (less than 1%) amounts of tetrahydrocannabinol (THC), the primary psychoactive ingredient in marijuana. It is a tall, slender, fibrous plant similar to flax or kenaf. Various parts of the plant can be utilized in the making of textiles, paper, paints, clothing, plastics, cosmetics, foodstuffs, insulation, animal feed and other products.

Hemp produces a much higher yield per acre than do common substitutes such as cotton and requires few pesticides. In addition, hemp has an average growing cycle of only 100 days and leaves the soil virtually weed-free for the next planting.

The hemp plant is currently harvested for commercial purposes in over 30 nations, including Canada, Japan and the European Union. Although it grows wild across much of America and presents no public health or safety threat, hemp is nevertheless routinely uprooted and destroyed by law enforcement. Each year, approximately 98% of all the marijuana eliminated by the DEA's "Domestic Cannabis Eradication/Suppression Program" is actually hemp.

Antiseptics and Areas of concern (Part-2)

Polymeric biguanide (PHMB)

Polymeric biguanide is a hetero disperse mixture of polyhexamethylene biguanides (PHMB) with a molecular weight of approximately 3,000. Earlier Polymeric biguanides have found use as general disinfecting agents in the food industry and, very successfully, for the disinfection of swimming pools. But presently it is found to be very effective antiseptic for acute as well as chronic wounds. This is active against gram-positive and gram-negative bacteria and also sporicidal. PHMB is a membrane-active agent that also impairs the integrity of the outer membrane of gramnegative bacteria, although the membrane may also act as a permeability barrier. Activity of PHMB increases on a weight basis with increasing levels of polymerization, which has been linked to enhanced inner membrane perturbation. Unlike chlorhexidine but similar to alexidine, PHMB causes domain formation of the acidic phospholipids of the cytoplasmic membrane. Permeability changes ensue, and there is believed to be an altered function of some membrane-associated enzymes. The proposed sequence of events during its interaction with the cell envelope of E. coli is as follows: (i) there is rapid attraction with strong and specific adsorption to phosphate-containing compounds; (ii) the integrity of the outer membrane is impaired, and PHMB is attracted to the inner membrane; (iii) binding of PHMB to phospholipids occurs, with an increase in inner membrane permeability (K1 loss) accompanied by bacteriostasis; and (iv) complete loss of membrane function follows, with precipitation of intracellular constituents and a bactericidal effect.

Iodine and iodophors

Although less reactive than chlorine, iodine is rapidly bactericidal, fungicidal, tuberculocidal and virucidal. Although aqueous or alcoholic (tincture) solutions of iodine have been used for 150 years as antiseptics, they are associated with irritation and excessive staining. In addition, aqueous solutions are generally unstable; in solution, at least seven iodine species are present in a complex equilibrium, with molecular iodine (I2) being primarily responsible for antimictrobial efficacy. These problems were overcome by the development of iodophors ("iodine carriers" or "iodine-releasing agents"); the most widely used are povidoneiodine and poloxamer-iodine in both antiseptics and disinfectants. Iodophors are complexes of iodine and a solubilizing agent or carrier, which acts as a reservoir of the active "free" iodine. Although germicidal activity is maintained, iodophors are considered less active against certain fungi and spores than are tinctures. Similar to chlorine, the antimicrobial action of iodine is rapid, even at low concentrations, but the exact mode of action is unknown. Iodine rapidly penetrates into microorganisms and attacks key groups of proteins (in particular the freesulfur amino acids cysteine and methionine, nucleotides, and fatty acids, which culminates in cell death. Less is known about the antiviral action of iodine, but nonlipid viruses and parvoviruses are less sensitive than lipid enveloped viruses. Similarly to bacteria, it is likely that iodine attacks the surface proteins of enveloped viruses, but they may also destabilize membrane fatty acids by reacting with unsaturated carbon bonds.

Silver Compounds

In one form or another, silver and its compounds have long been used as antimicrobial agents. The most important silver compound currently in use is silver sulfadiazine (AgSD), although silver metal, silver acetate, silver nitrate, and silver protein, all of which have antimicrobial properties, are listed in Martindale, The Extra Pharmacopoeia. In recent years, silver compounds have been used to prevent the infection of burns and some eye infections and to destroy warts.

Silver nitrate

The mechanism of the antimicrobial action of silver ions is closelymrelated to their interaction with thiol (sulfydryl,™SH) groups, although other target sites remain a possibility. Liau et al demonstrated that amino acids such as cysteine and other compounds such as sodium thioglycolate containing thiol groups neutralized the activity of silver nitrate against P. aeruginosa. By contrast, amino acids containing disulfide (SS) bonds, nonsulfurcontaining amino acids, and sulfur-containing compounds such as cystathione, cysteic acid, L-methionine, taurine, sodium bisulfite, and sodium thiosulfate were all unable to neutralize Ag1 activity. These and other findings imply that interaction of Ag1 with thiol groups in enzymes and proteins plays an essential role in bacterial inactivation, although other cellular components may be involved. Hydrogen bonding, the effects of hydrogen bondbreaking agents, and the specificity of Ag1 for thiol groups were discussed in greater detail by Russell and Hugo. Virucidal properties might also be explained by binding to ™SH groups. Lukens proposed that silver salts and other heavy metals such as copper act by binding to key functional groups of fungal enzymes. Ag1 causes the release of K1 ions from microorganisms; the microbial plasma or cytoplasmic membrane, with which is associated many important enzymes, is an important target site form Ag1 activity. In addition to its effects on enzymes, Ag1 produces other changes in microorganisms. Silver nitrate causes marked inhibition of growth of Cryptococcus neoformans and is deposited in the vacuole and cell wall as granules. Ag1 inhibits cell division and damages the cell envelope and contents of P. aeruginosa. Bacterial cells increase in size, and the cytoplasmic membrane, cytoplasmic contents, and outer cell layers all exhibit structural abnormalities, although without any blebs (protuberances). Finally, the Ag1 ion interacts with nucleic acids; it interacts preferentially with the bases in DNA rather than with the phosphate groups, although the significance of this in terms of its lethal action is unclear.

Silver sulfadiazine

AgSD is essentially a combination of two antibacterial agents, Ag1 and sulfadiazine (SD). The question whether the antibacterial effect of AgSD arises predominantly from only one of the compounds or via a synergistic interaction has been posed repeatedly. AgSD has a broad spectrum of activity and, unlike silver nitrate, produces surface and membrane blebs in susceptible (but not resistant) bacteria. AgSD binds to cell components, including DNA. Based on a chemical analysis, Fox

proposed a polymeric structure of AgSD composed of six silver atoms bonding to six SD molecules by linkage of the silver atoms to the nitrogens of the SD pyrimidine ring. Bacterial inhibition would then presumably be achieved when silver binds to sufficient base pairs in the DNA helix, thereby inhibiting transcription. Similarly, its antiphage properties have been ascribed to the fact that AgSD binds to phage DNA. Clearly, the precise mechanism of action of AgSD has yet to be solved.

Bis-Phenols

The bis-phenols are hydroxy-halogenated derivatives of two phenolic groups connected by various bridges. In general, they exhibit broad-spectrum efficacy but have little activity against P. aeruginosa and molds and are sporostatic toward bacterial spores. Triclosan and hexachlorophane are the most widely used biocides in this group, especially in antiseptic soaps and hand rinses. Both compounds have been shown to have cumulative and persistent effects on the skin.

Hexachlorophene

Hexachlorophene (hexachlorophane; 2,29-dihydroxy-3, 5, 6, 39, 59, 69-hexachlorodiphenylmethane) is another bis-phenol whose mode of action has been extensively studied. The primary action of hexachlorophene, based on studies with Bacillus megatherium, is to inhibit the membranebound part of the electron transport chain, and the other effects noted above are secondary ones that occur only at high concentrations. It induces leakage, causes protoplast lysis, and inhibits respiration. The threshold concentration for the bactericidal activity of hexachlorphene is 10 mg/ml (dry weight), but peak leakage occurs at concentrations higher than 50 mg/ml and cytological changes occur above 30 mg/ml. Furthermore, hexachlorophene is bactericidal at 0°C despite causing little leakage at this temperature. Despite the broad-spectrum efficacy of hexachlorophene, concerns about toxicity, in particular in neonates, have meant that its use in antiseptic products has been limited.

Halophenols

Chloroxylenol (4-chloro-3,5-dimethylphenol; p-chloro-mxylenol) is the key halophenol used in antiseptic or disinfectant formulations. Chloroxylenol is bactericidal, but P. aeruginosa and many molds are highly resistant. Surprisingly, its mechanism of action has been little studied despite its widespread use over many years. Because of its phenolic nature, it would be expected to have an effect on microbial membranes.

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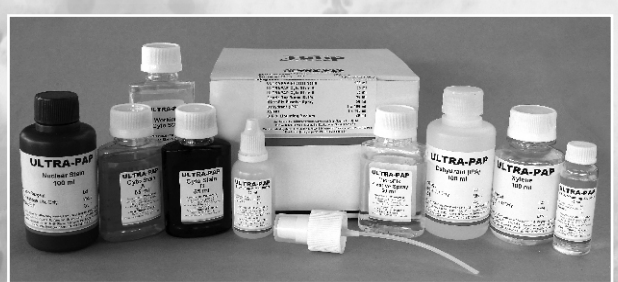

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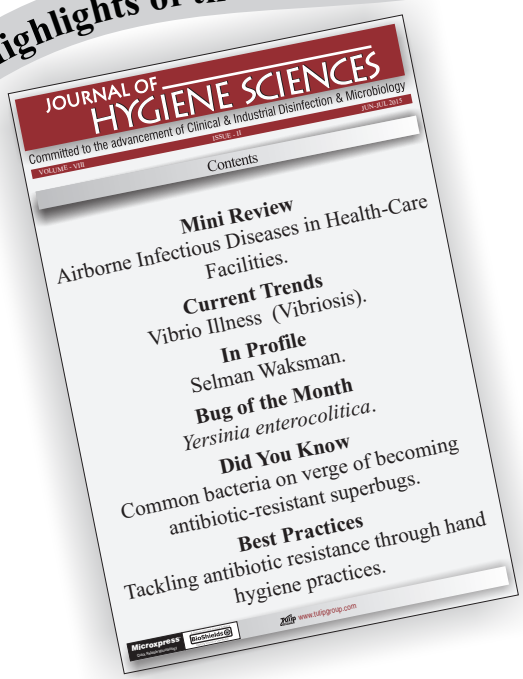
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