SUMMARY

Chloral alcoholate will separate from the N. F. prescription when alcohol is added in excess of 10%. In an alcoholic prescription, increasing the sugar beyond 21%, or the KBr beyond 25%, or the chloral hydrate beyond 29% will result in separation. In the absence of sugar the alcoholate will separate when 23% of KBr is dissolved. In the absence of both sugar and KBr, no separation occurs. In the absence of KBr, sugar alone, even if added to the point of saturation, will not cause separation of the alcoholate. The addition of an alcohol-soluble dye to the prescription promptly delineates any separation.

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A Fast Method of Dry, Low-Heat Sterilization*

By P. Goedrich and W. Schmidl

Sterilization, as we all know, is destruction of every form of life, harmful or innocuous. Bacteriologists now agree that any distinction between disease-producing and other microörganisms is vague, and that apparently there is no organism which might not cause disease (1). Even the ordinarily harmless *Bacillus subtilis* (hay bacillus) has been known to cause serious infection in the human eye (2) and occasionally to invade the blood stream (3). It is therefore essential in sterilization to destroy not only all vegetative forms of bacilli, but also all spores. Unfortunately, there are wide discrepancies in the results reported concerning the thermal resistance of spores. But all authorities agree that no period of exposure to boiling water has been found to be completely adequate. In the opinion of leading bacteriologists, boiling water never constitutes adequate sterilization for surgical instruments (4).

Robert Koch (5), who established that all vegetative forms of bacteria were killed by a temperature just over 100° C. in 1½ hrs., overestimated the value of boiling water against spores. Falcioni (6) found spores of tetanus to resist destruction for 2½ hrs. in live steam. Theobald Smith (7) showed that tetanus spores occasionally were able to survive 70 min. of steaming. Von Hibler (8) studied some strains of *Clostridium tetani* that required 3 hrs. of boiling to destroy them. Bigelow and Esty (9) found some microörganisms remaining virulent for 22 hrs. at 100° C., while at 110° C. nearly 4 hrs. was necessary to destroy them. Becker (10) reported the death point of two strains of tetanus as 2 hrs. and 3 hrs., respectively, in boiling water. Murray and Headler (11) found strains of *Clostr. welchii* to be resistant to boiling for 90 min. Esty and Meyer (12) found the heat resistance of tetanus spores to vary at 100° C. from 15 to 90 min., the average survival time being 25 min. Dried spores of *B. anthracis* were found to withstand boiling temperature for hours (13).

In general it is recognized that a temperature of 150° C. continued for at least an hour will destroy any bacterial spores. Nevertheless, glassware in the bacteriological laboratory is usually sterilized at 160° C. for 2 to 3 hrs. (14). By using steam

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under pressure, 30 to 40 min., with a pressure of 15 lbs. (121.5° C. at sea level), provide a satisfactory margin of safety (15). In order to employ steam without pressure, one submits the material to intermittent heating on three consecutive days, using apparatus of the usual "steam sterilizer" type. But according to Theobald Smith (16, 17) even this time-consuming procedure is not reliable. Abbott (18) also states that, in general, dry objects like instruments, bandages, etc., do not offer the conditions for the operation of the principles underlying the intermittent method of sterilization. It must also be considered that the boiling point of water is lower at higher altitudes, but the death of the microörganisms depends on the temperature and not on the boiling point. Thus steam sterilization, already doubtful at sea level, becomes worthless at mountainous elevations.

From the foregoing it appears that there is need for a new inexpensive way of sterilization for instruments, syringes, hypodermic needles, etc., in physicians' and dentists' offices and everywhere that a complete sterilization is wanted without the use of autoclaves or dry heat at 160° C.

As early as 1902, Esmarck (19) had shown that the action of live steam is markedly increased by addition of small quantities of volatile chemical disinfectants like formaldehyde, chlorine, etc. Spores of potato bacillus which were killed in live steam at 100° C. in 2 hrs., were killed in 2 min. by steam to which 1% of formaldehyde was added. Clemmensen (20) found that materials would be sterilized at a temperature from 68° to 70° C. when exposed to formaldehyde–water vapors. Likewise Delepine found that anthrax spores may be easily killed by exposure to warm formaldehyde vapors (21). But this kind of sterilization was qualified as "possible but not practical" (22), the apparatus necessary being even more complicated and more expensive than autoclaves.

The new method, reported in this paper, uses the vapors of certain dry germicides in closed containers and effects complete sterilization in a relatively short time and at low heat. For practical reasons paraformaldehyde (trioxymethylene) appears to be the most promising one among many theoretically possible substances, such as thymol, chlorthymol, chloramine, etc.

EXPERIMENTAL

The method consists of evaporation of paraformaldehyde by comparatively moderate heat in a closed chamber. The materials to be sterilized are contained in individual receptacles contained in this master-chamber. These receptacles can be closed by revolving shutters or by other means after the sterilization period has elapsed. After the receptacles are taken out, the instruments, etc., contained in them, will stay sterile for a prolonged time.

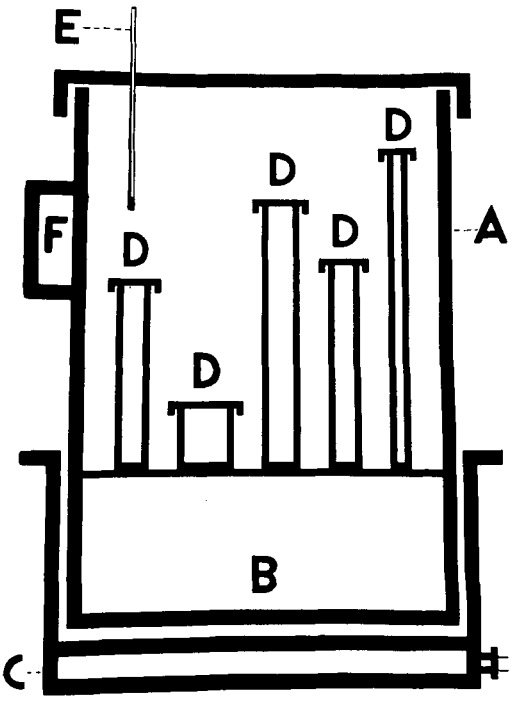

Figure 1.

Figure 1 illustrates the principles of the new method. In the master-chamber (*A*), hermetically closed, paraformaldehyde spread over the bottom (*B*) is evaporated by means of the heating element (*C*). This master-chamber encloses several individual receptacles (*D*) containing the instruments and the other articles to be sterilized. The receptacles are provided with revolving shutters, the shutters being opened before sterilization. A thermometer (*E*) registers the temperature. After the sterilization time has elapsed, the master-chamber may be taken away with the handle (*F*), cooled and opened. The revolving shutter of each individual container is closed and the instruments, etc., remain in it in sterile condition until they are wanted for use. It has been found, moreover, that the disinfectant vapors penetrate even closed glassine paper envelopes, and

thus make their opening before sterilization and closing thereafter unnecessary.

The experiments conducted with paraformaldehyde demonstrated that the liberation of formaldehyde depends on (*a*) the temperature, (*b*) the time element and (*c*) the exposed surface. Table I demonstrates the liberation of formaldehyde, at a constant temperature in closed containers of constant capacity, from various amounts of paraformaldehyde.

TABLE I.—EVAPORATION OF VARIOUS QUANTITIES OF PARAFORMALDEHYDE IN 1000-CC. CLOSED CONTAINERS AT 70° C.

Quantity, Gm.	Loss of Weight after One Hour	
	Gm.	Per Cent
0.014	0.0038	27
0.05	0.0155	31
0.5	0.091	18
1.0	0.139	14
2.0	0.180	9

Table II shows the liberation of formaldehyde in closed containers of various capacities from various quantities of paraformaldehyde at a constant temperature.

The most important question was, of course, the investigation of the sterilizing effect of this method. Formaldehyde is known to have one of the greatest temperature coefficients of any disinfectant (23). Holm and Gardener (24), investigating formaldehyde sterilization, recognized that it is the coöperation of four elements, (*a*) concentration of the fumigant, (*b*) temperature, (*c*) time of exposure and (*d*) moisture, that determines sterilization.

Realizing that very small amounts of moisture also improve the killing action of heat (25), it is possible to comply with the requirement of moisture and nevertheless have the material virtually dry, as 1000 cc. of steam at 100° C. under atmospheric pressure has a weight of only 0.6 Gm. It is obvious that a few drops of water per L. volume may suffice without injuring even sensitive objects. The investigation was conducted at various temperatures, at various times of exposure and at various concentrations of the vapors of paraformaldehyde.

Table III shows the results of the new method, when using an F. D. A. culture of *Staphylococcus aureus*.

It can be seen on this table that, while it was impossible to achieve sterilization at 20° C. in three days, the bacilli were killed in 45 min. at 70° C., and in 10 min. at 90° C. Dry heat of 100° C. did not kill the same strain of bacteria in 1 hr. If one accepts the very much disputed theory that there is generally little likelihood that instruments may be infected with organisms of the very resistant type, this short-time "sterilization" might suffice.

Ten minutes at 90° C. with the new method produces at least the same results as short-time boiling or steaming in the usual office sterilizers, and whoever has to rely on this kind of sterilization may use short-time exposure to the vapors of paraformaldehyde and enjoy the advantage of having dry instruments, in individual containers, ready for immediate or later use. But for higher requirements, for instance, the destroying of all spores, the new method assures a complete sterilization, which boiling or steaming at 100° C. never can; by simply prolonging the time of exposure to the vapors of paraformaldehyde this is effected.

In order to determine this effectiveness against spore-bearing organisms, instruments were artificially infected with such organisms, previous to the sterilization. Saline suspensions of strains of *B. subtilis*, which were grown on slants of F. D. A. solid medium and which had survived more than 2½ hrs.' exposure to 7% U. S. P. Tincture of Iodine, were used (½-cc. saline suspensions in 10 cc. of tincture). The saline suspensions were made of 10 cc. of saline solution per one 10-cc. slant of F. D. A. solid medium. The same strains were found to be resistant to dry heat for 1½ hrs. at

TABLE II.—EVAPORATION OF VARIOUS QUANTITIES OF PARAFORMALDEHYDE IN CLOSED CONTAINERS OF VARYING CAPACITY AT 70° C.

Quantity, Gm.	Loss of Weight in Containers of—							
	500-Cc. Capacity		1000-Cc. Capacity		2500-Cc. Capacity		5000-Cc. Capacity	
	Gm.	Per Cent	Gm.	Per Cent	Gm.	Per cent	Gm.	Per Cent
0.1	0.017	17	0.028	28	0.04	40	0.05	50
0.5	0.05	10	0.091	18	0.11	22	0.14	28
1.0	0.057	5.7	0.139	14	0.15	15	0.20	20

TABLE III.—KILLING OF BACTERIA WITH VAPORS OF PARAFORMALDEHYDE (TEST ORGANISM, *Staph. Aureus*, F. D. A. CULTURE)

Temperature, °C.	Amount of Paraformaldehyde per 1000 Cc.	Time of Exposure—									
		10 Min.		20 Min.		30 Min.		45 Min.		3 Days	
		Broth Culture	Sub-culture	Broth Culture	Sub-culture	Broth Culture	Sub-culture	Broth Culture	Sub-culture	Broth Culture	Sub-culture
20	Excess	..	..	..	..	..	..	..	..	−	+
70	0.6 Gm.	..	..	..	..	−	+	−	−	..	..
80	0.6 Gm.	..	..	+	+	−	−	..	..	..	..
90	0.6 Gm.	−	−	..	..	..	..	..	..	..	..

TABLE IV.—KILLING OF SPORES WITH VAPORS OF PARAFORMALDEHYDE (TEST ORGANISM, *Bacillus subtilis*)

Temperature, °C.	Amount of Paraformaldehyde per 1000 Cc.	Time of Exposure: 45 Min. Broth Culture	45 Min. Subculture	60 Min. Broth Culture	60 Min. Subculture	90 Min. Broth Culture	90 Min. Subculture	1 Day Broth Culture	1 Day Subculture	3 Days Broth Culture	3 Days Subculture	8 Days Broth Culture	8 Days Subculture
20	Large excess	..	..	..	..	..	..	+	+	+	+	+	+
37	Large excess	..	..	..	..	..	..	+	+	−	−	..	..
60	1 Gm.	..	..	+	+	+	+	..	..	..	..	..	..
70	1 Gm.	..	..	+	+	+	+	..	..	..	..	..	..
80	0.4 Gm.	..	..	+	+	+	+	..	..	..	..	..	..
80	0.6 Gm.	..	..	−	+	−	+	..	..	..	..	..	..
90	0.2 Gm.	+	+	+	+	..	..	..	..	..	..	..	..
90	0.4 Gm.	+	+	−	+	..	..	..	..	..	..	..	..
90	0.6 Gm.	−	+	−	−	..	..	..	..	..	..	..	..

130° C., and to live steam under atmospheric pressure for one hour at least. A wide variety of objects, such as glass tubes, glass rods, strings, wood, nails, needles, metal instruments, empty ampuls, etc., were infected and dried after infection at 37° C., then put into individual containers and exposed to the vapors in the closed chamber. To ascertain the resistance of the strains of *B. subtilis*, appropriate controls without formaldehyde were always conducted.

As Table IV indicates, the spores of *B. subtilis* were not killed even after 8 days of exposure at 20° C, but 3 days at 37° C. and 60 min. at 90° C. were sufficient for complete sterilization.

As it is a well-established fact that organic matter reduces the effect of all germicides, and as it might happen that instruments are not free of blood, serum, pus, etc., before sterilization, two series of experiments were conducted, using (*a*) instruments which were immersed in a mixture of equal amounts of horse serum and saline suspensions of *B. subtilis* and (*b*) instruments which were immersed in horse serum, dried at 37° C., and then infected with *B. subtilis*. Even under these rigid conditions the results were the same as indicated in Table IV—complete sterilization, with destruction of all spores, could be achieved in the same period of time.

It has already been mentioned that the sterilized articles, contained in their individual receptacles, remained sterile, after they had been taken out of the master-chamber, for a prolonged time. When sterility tests were made, after the sterilized articles in their individual containers had been kept without special precautions for one, two and even three months, there was not a single case of subsequent infection.

The heat necessary for the experiments with this new method was provided by a thermostat-controlled electric oven. In practice, suitable means for heating and controlling the predetermined temperature will easily be provided. But as the temperature required for complete sterilization never exceeds 90° C., simple immersion of the closed master-chamber into boiling water for the time indicated (see Table IV) will be sufficient where means of electric or gas heating are not available.

Formaldehyde fumes are known to be irritant to the respiratory tract, but it was found that this can be practically eliminated by letting the master-chamber cool to room temperature, or, even better, by cooling it with tap water. This will polymerize formaldehyde again to the solid form of paraformaldehyde.

Quantitative determination of the deposit of paraformaldehyde on the surface of the sterilized instruments showed that it is in the average 0.00008 Gm./sq. cm. immediately after cooling, and 0.000015 Gm./sq. cm. (less than $^1/_{4000}$ gr.) after 24 hrs., an undoubtedly negligible amount.

SUMMARY

1. Neither boiling water nor live steam constitutes a reliable means of sterilization.

2. The routine "sterilization" of surgical instruments by short-time boiling or exposing to live steam at 100° C. may therefore have dangerous consequences.

3. A new method of sterilization of surgical instruments and other articles has been found, by which the same results are obtained as with a steam pressure autoclave.

4. The method consists of exposing the instruments, etc., to the vapors of formaldehyde, developed in a master-chamber, from paraformaldehyde under virtually dry conditions.

5. The method enables sterilization of instruments, etc., in individual containers, which, being properly closed after finished sterilization, will keep the instruments sterile for a prolonged time and ready for their use at the doctor's office, as well as for emergency calls.

6. Complete sterilization of heat-sensitive articles may be achieved at temperatures as low as 37° C., if the time element is not of major significance. At 90° C. one hour of

exposure is sufficient to kill spores of even extraordinary resistance.

7. The new method requires only small quantities of an inexpensive chemical and can be used in locations and under conditions where the bulky and heavy autoclave cannot be considered. The apparatus needed for this method is very simple, easy and safe to handle, and requires little attendance.

8. This new method may be of special importance for the armed forces, when their mobile hospitals are unable to use autoclave sterilization.

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A Histological Study of *Eriodictyon californicum**

By L. David Hiner and Kenneth J. Merrill†

Criticism of the United States Pharmacopœial description of *Eriodictyon californicum* (*Hydrophyllaceæ*) has led the Committee on Botany and Pharmacognosy to request that a further study of the drug be made. In accordance with this suggestion, an investigation of some of the histological features of the leaves was undertaken by Mr. Merrill during the past year. It was deemed advisable, however, to continue the study this summer, so additional samples were obtained for corroborative work as well as for checking additional points which were still in question. The study was based chiefly on a review of the older descriptions, and also on the newly proposed monograph resulting from the work of Drs. Youngken, Wirth and Goodrich of the present Committee.

Since the first histological studies of this drug which were made by Ritter (1), it appears that very little interest has been shown in the leaves until this Committee expressed its disapproval of the existing monograph. Some definite changes are suggested herewith as a result of their proposal to revive the study of *Eriodictyon californicum*, and ultimately to revise the monograph.

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