Residual formaldehyde after low-temperature steam and formaldehyde sterilization

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SYNOPSIS The levels of formaldehyde remaining in various articles have been estimated immediately after a low-temperature steam and formaldehyde sterilizing process and after various periods of aeration. These levels have been compared with the levels of ethylene oxide remaining after exposure to an ethylene oxide sterilizing process.

In rubber and polythene and a plastic, formaldehyde levels are low and slowly fall even further. Ethylene oxide levels are relatively much higher even after seven days' aeration.

It is not considered that the residual levels of formaldehyde in rubber, polythene, and a plastic should constitute a danger. Residual levels of formaldehyde in fabrics and paper are higher but this may be of value by giving a self-disinfecting action on storage.

The use of low-temperature steam and formaldehyde for the disinfection and sterilization of heat-labile materials was first put on a practical basis in 1966 by Alder, Brown, and Gillespie working in Bristol. Since then it has been adopted in other centres and has been found to be a useful and acceptable method for processing a wide variety of objects and materials (Gibson and Johnston, 1967).

It is inevitable that any consideration of formaldehyde as a sterilizing agent must take into account its advantages and its disadvantages, particularly in comparison with ethylene oxide, the only practical alternative at this time.

The advantages of formaldehyde lie in its much lower toxicity, its ease of control, and the complete absence of danger from fire and explosion. This allows formaldehyde and steam to be used in a large simple chamber without any special precautions. A disadvantage lies in the need to maintain a temperature of the nature of 80°C which may still be damaging to a few objects and materials, although, apart from pacemakers as presently constituted, we know of few objects which can be processed by ethylene oxide which cannot also be processed by low-temperature steam and formaldehyde. Early reports indicated that penetration of narrow-bore tubing was poor (Alder *et al*, 1966) but a later development has disposed of this problem (Alder, 1968). The new method consists of saturating the chamber with formaldehyde vapour

before the admission of steam by injecting formalin at 2 ml per cubic foot of autoclave space into the chamber after the initial preevacuation stage and reevacuating the vaporized formalin after a minute. This procedure is carried out three times and is then followed by the normal cycle previously detailed (Alder *et al*, 1966). This modification, however, has not proved to be necessary with our apparatus to penetrate narrow tubing adequately using the cycle we have described (Gibson and Johnston, 1967).

The great practical disadvantage of the toxicity of ethylene oxide really lies in the long 'airing off' period required to ensure that processed articles do not cause poisonous effects when used in tissues or on skin or in contact with fluids entering the body. It has not appeared to us that this type of toxicity should constitute a major problem with formaldehyde and steam sterilization, and indeed preliminary tests (Gibson and Johnston, 1967) appeared to bear this out. However, in view of the importance of freedom from toxicity in articles likely to be associated closely with body fluids for long periods it was decided to investigate this further and to compare in various representative articles the residual levels of formaldehyde after exposure to that process with those of ethylene oxide after exposure to that gas.

Aside from its actual toxic effects, formaldehyde may also be a contact allergen in some people. It has been shown that finishing processes using for-

maldehyde amine resins applied to textiles may result in allergic contact dermatitis (Marcussen, 1959; Blohm, 1959; Wereide, 1964). The increasing use of the steam and formaldehyde sterilization must therefore raise the question of the risk of such reactions, and must be related to the residual levels of formaldehyde in fabrics treated by this method and comparisons made with 'normal levels' found in paper, cotton, and other textiles finished by formaldehyde amine resins.

MATERIALS AND METHODS

The apparatus used for formaldehyde and low-temperature steam sterilization is a 21 cu ft dressings autoclave modified by the manufacturer to provide this additional cycle. A prevacuum with steam pulsing is followed by a steam injection period during which the steam also introduces formaldehyde vapour over four minutes to a total of 180 ml of formaldehyde solution B.P. There is then a sterilizing period of two hours at 80°C while a balance is maintained between steam pressure and vacuum, and finally a post-vacuum period, including further steam pulsing, which effectively dries the contents and extracts the formaldehyde.

The ethylene oxide apparatus is a commercial machine with a 0.8 cu ft chamber. A prevacuum period is followed by exposure to pure ethylene oxide gas (always maintained for safety at subatmospheric pressure) for one hour at 55°C. A post-vacuum period extracts the ethylene oxide.

Plastic cardiac catheters, polythene tubing, rubber gloves, and rubber catheters were chosen for test exposure to steam and formaldehyde, as they were thought to represent the types of material, though not necessarily the actual articles, which would probably be processed in this way. In addition a few powder sachets were processed and tested to observe the effect on a very absorptive material. Two types of polythene tube, one large-bore and one small-bore, were included. These, together with the rubber gloves and rubber catheter, provided some variation in the surface-area/weight ratio of different articles made of the same materials.

Twelve each of the articles were exposed to the full subatmospheric steam and formaldehyde cycle while double wrapped in Kraft paper. Six of each type were then immersed within two hours in 250 ml of distilled water in a 1 litre conical flask, the flask was sealed, and the contents were agitated and allowed to stand overnight. The remaining six specimens were allowed to 'air off' in their paper wraps on the bench at room temperature for 48 hours. They were then immersed in distilled water and treated as described above. Two samples of each article were not exposed to formaldehyde and were used as blank controls.

Formaldehyde in the distilled water after overnight extraction was estimated as follows. Duplicate 1.0 ml samples of the water were pipetted into 20 ml test tubes and 5.0 ml of chromotropic acid solution was added. The tubes were heated for 15 minutes in a boiling water bath in the absence of excess light. After cooling the purple colour produced was measured spectrophotometrically at 580 m μ .

Standard solutions prepared from Analar formaldehyde solution analysed iodometrically were introduced and were treated in the same way as the test solutions. Distilled water was used in the blank tube.

Chromotropic acid was prepared by dissolving 2 g of 1, 8 dihydroxy-naphthalene 3, 6 disulphonic acid in 200 ml distilled water. Separately 600 ml of concentrated sulphuric acid was added to 300 ml of distilled water cooled in ice. When cool the diluted sulphuric acid was added to the chromotropic acid with mixing.

The mixture should be stored in a brown bottle and prepared freshly every two or three weeks.

RESULTS

The results of the estimations carried out can be seen in Table I expressed as total formaldehyde extracted from each article and as parts per million of formaldehyde extracted from each article. In each case the results can be seen as estimated immediately after the sterilizing process and after an 'airing off' period of 48 hours.

These results show that the residual formalde-

TABLE I
FORMALDEHYDE ESTIMATIONS

Article	Average HCHO per Article (μ g)	Average Weight of Article (g)	Average HCHO (ppm)	Unexposed Control
Polythene large bore	62.6	6.260	10.0	0
Polythene large bore after 48-hr airing	41.4	6.179	6.7	0
Polythene small bore	91.5	5.865	15.6	0
Polythene small bore after 48-hr airing	45.0	6.522	6.9	0
Rubber gloves	269.0	12.283	21.9	0
Rubber gloves after 48-hr airing	279.4	12.700	22.0	0
Rubber catheter	103.7	12.646	8.2	0
Rubber catheter after 48-hr airing	65.0	13.265	4.9	0
Cardiac catheter	32.0	4.156	7.7	0
Cardiac catheter after 48-hr airing	47.0	4.312	10.9	0
Powder sachet (in 100 ml water)	402.0	1.446	278.0	0
Powder sachet after 48-hr airing (in 100 ml water)	393.0	1.455	270.0	0

TABLE II
FORMALDEHYDE ESTIMATION (PPM)

Article	HCHO (ppm) in Article					
	Day 0	Day 1	Day 2	Day 3	Day 7	Control
Rubber tube large bore	4.05	4.05	0	1.35	1.35	0
Rubber tube small bore	1.92	7.69	0	0	1.92	0
Polythene tube large bore	4.86	2.08	2.08	4.17	0.69	0
Polythene tube small bore	8.33	8.33	0	12.5	0	0
Cotton cloth	2170.0	1500.0	433.0	1060.0	1480.0	4.16
Paper	1470.0	1580.0	500.0	580.0	1870.0	4.44
Cotton cellular blanket	270.0	214.0	92.0	158.0	114.0	0
Woollen blanket	580.0	445.0	176.0	320.0	640.0	8.0

hyde is very low in all the articles processed although it reaches higher levels in the powder sachets which were included to represent a particularly absorbent material.

The reduction in formaldehyde levels after 48 hours' aeration is not dramatic at the low levels present but is significant in the case of the small-bore polythene tube at 51% ($0.005 > p > 0.001$) and the rubber tubing at 40.2% ($0.01 > p > 0.005$).

The low levels obtained would bear out the subjective impression following the formaldehyde process that there is no smell of formaldehyde or other evidence of heavy concentrations of it on rubber and plastic articles. There is, however, a certain tendency for the smell of formaldehyde to cling to fabric and paper.

In order further to investigate the drop in formaldehyde levels over a longer period of aeration, six samples of each of the materials described in Table II were exposed to the process and the levels of formaldehyde after aqueous extraction were estimated as before over seven days' aeration. The results in Table II again show that the levels are very low, and, while there is considerable variation, there is a general downward trend except in cloth and paper. These gave high levels and no evidence of appreciable dispersion within the time of the test.

To provide a comparison of the levels obtained after exposure to formaldehyde and steam with those obtained after exposure to ethylene oxide, similar articles to those in Table II were put through a cycle of sterilization by this method, including one hour's exposure to the gas and post-vacuum dispersal period.

METHOD

Aqueous extraction was used in the same manner as that described above and the ethylene oxide level determined by conversion to formaldehyde and colorimetric estimation. The ethylene oxide (at an elevated temperature) is hydrolysed by sulphuric acid to ethylene glycol which in turn is oxidized by periodic acid to yield 2 molecules

of formaldehyde. This conversion is quantitative under these conditions (Critchfield and Johnson, 1957).

Of the aqueous extracts, 25.0 ml was pipetted into airtight bottles and 0.5 ml of 0.5 NH_4SO_4 added to each. The bottles were placed in a water bath at $98^\circ\text{C} \pm 2^\circ\text{C}$ for 60 minutes. After cooling the contents were transferred with washing by distilled water to a 50-ml stoppered cylinder, 1.0 ml of 0.1N sodium periodate was added, mixed, and left for 15 minutes at room temperature. Then 1.0 ml of 5.5% sodium sulphite was added and the volume made up to 50 ml with distilled water and mixed. Samples of this solution, each of 1.0 ml, were used for the estimation of formaldehyde as described above.

RESULTS

The levels of residual ethylene oxide as estimated in this way are shown in Table III. They are very considerably higher than the formaldehyde levels in the previous tests for the polythene and rubber articles. They show a steady decline over the seven-day period of aeration but the levels then obtained are still many times larger than those found after formaldehyde sterilization even when this is estimated immediately after exposure. Levels in cotton, wool, and paper are more comparable but ethylene oxide levels are still higher.

These methods of exposure and subsequent aqueous extraction cannot of course be said to measure the total content of formaldehyde or ethylene oxide in the article concerned. They were adopted as giving a reasonable approximation to the situation *in vivo* when the agent absorbed into the substance of the article might be extracted into an ambient aqueous solution either in the patient himself or by some solution passing through the article and into the patient's body. Preliminary estimations showed that in fact the greater proportion of formaldehyde or ethylene oxide released from the article into the water is during the first few hours of extraction and that no significant quantity is released after 24 hours.

One cannot, except by methods such as steam

TABLE III
ETHYLENE OXIDE ESTIMATION (EXPRESSED AS HCHO PPM)

Article	HCHO (ppm) in Article					
	Day 0	Day 1	Day 2	Day 3	Day 7	Control
Rubber tube large bore	1770.0	530.0	216.0	91.9	47.3	4.05
Rubber tube small bore	1940.0	338.0	108.0	84.6	42.3	0
Polythene tube large bore	1670.0	861.0	3610.0	400.0	128.0	0
Polythene tube small bore	1800.0	700.0	230.0	75.0	75.0	16.7
Cotton cloth	3130.0	3230.0	2730.0	3170.0	2070.0	0
Paper	8890.9	3560.0	1780.0	1440.0	1510.0	33.3
Cotton cellular blanket	7420.0	1000.0	960.0	940.0	940.0	0
Woollen blanket	7450.0	1260.0	1300.0	1360.0	1550.0	0

distillation, extract from any article all of these chemicals absorbed into or combined with it. To attempt an assessment of the efficiency of cold water extraction, rubber and polythene articles exposed to formaldehyde or ethylene oxide by the standard methods described were extracted under reflux into 250 ml of boiling water in each case. The amounts of formaldehyde or ethylene oxide released were then determined.

The results showed that soaking overnight in 250 ml of distilled water extracts 92% of the ethylene oxide from polythene and 75.4% from rubber and 75% of formaldehyde from polythene and 34% from rubber when the reflux extraction is regarded as yielding 100% in each case.

DISCUSSION

As long as it is still necessary to sterilize heat-labile materials at hospital level, any decision on the method to be used means taking into account the advantages and disadvantages of ethylene oxide and low-temperature steam and formaldehyde.

It is recognized that a major disadvantage of ethylene oxide is the high residuum of the toxic chemical which remains absorbed into many articles after the sterilization period is over. This has necessitated the acceptance of an 'airing off' period of at least 24 hours before use. We have shown that in fact even this length of time is not sufficient to disperse the ethylene oxide which remains and can be released by aqueous extraction from materials such as rubber and polythene as well as paper and cloth. Similar tests carried out to estimate the residual formaldehyde after exposure to low-temperature steam and formaldehyde have shown that this is of a significantly lower level and is reduced in most cases even further with aeration. These low formaldehyde residual levels, using the low-temperature steam and formaldehyde process, may be a result of the prevention of polymerization of the chemical on fabric and material by the pre-

sence of steam acting as a vehicle for it, and by the maintenance of the temperature at 80°C while pressure is subatmospheric. The powerful post-vacuum period must also contribute to the removal of the residual traces of the formaldehyde. The low-temperature steam and formaldehyde process seems thus to have removed what was regarded as a major disadvantage of formalin sterilization only a few years ago (Kelsey, 1963). Indeed we would suggest that the low residual levels of formaldehyde in rubber and plastic when compared with the relatively high levels of ethylene oxide following the use of that gas could be regarded as a major advantage of the low-temperature steam and formaldehyde process.

The levels of residual formaldehyde following the processing of cotton, paper, and wool are particularly interesting. The occurrence of cases of contact dermatitis resulting apparently from residual formaldehyde in textiles is well established. The levels we obtained in control samples of cotton, paper, and wool were very much lower than those in the processed samples, even after up to seven days' airing off, but the use of formaldehyde amine resins in the manufacture of the materials could not be established. As the figures obtained, for example, by Blohm (1959) in his estimations on various textiles, cannot be compared with ours, further work would have to be done to establish comparisons for levels between residual formaldehyde in 'finished' textiles and residual formaldehyde in material exposed to the steam/formaldehyde process. Alder and Gillespie, however, have used formaldehyde-treated blankets for years with no reports of contact dermatitis (Gillespie and Alder, 1957; Gillespie, Alder, Ayliffe, Bradbeer, and Wypkema, 1959).

The persistent levels of formaldehyde in fabric and paper following the steam/formaldehyde process may of course have advantages. Dickinson and Wagg (1967) have recently shown that a formaldehyde rinse in the laundering of blankets gives them strong self-disinfecting powers. The same effect

occurs in blankets treated with steam and formaldehyde and then stored (Alder, 1968). The residual levels in paper might be used in the storage of goods from central sterilization departments, for example, to play a similar self-disinfecting role in paper bags and wraps. A small injection of formaldehyde vapour into the chamber during autoclaving might be of value in this respect.

The method used to recover the chemicals by aqueous extraction does, we feel, give meaningful results but we cannot, except by a method such as steam distillation or reflux, claim to have extracted from any article all of such chemicals absorbed into or combined with it. However, the results obtained by aqueous extraction represent a major proportion of the total which could be extracted by refluxing. In any case the level of recovered formaldehyde or ethylene oxide obtained by aqueous extraction may well bear a closer relation to the levels likely to be found in use *in vivo* when aqueous solutions are passed through tubing into a patient, or the tubing or other article is inserted into a patient and surrounded by aqueous solutions. The solubility of both ethylene oxide and formaldehyde in water is

high compared with the levels obtained in the aqueous extractions and enables us to discount this factor in comparisons.

We consider that in rubber and plastic articles, residual levels of formaldehyde following the low-temperature steam and formaldehyde sterilization process are not likely to be toxic to patients. They are certainly much lower than the ethylene oxide levels we obtained following sterilization by that gas. In fabric and paper the higher formaldehyde levels may in fact be of value as they may give a continuing self-disinfecting effect during storage.

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