VIRICIDAL ACTION OF ETHYLENE OXIDE GAS

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Our interest in gaseous disinfection was stimulated recently when we were asked for information concerning the sterilization of plastic tubes, which are damaged by the more common techniques. The need arose with the substitution of plastic tubing for rubber in blood transfusion apparatus. Particular attention was paid to the conditions of exposure time, concentration and temperature, in which ethylene oxide is viricidal and effective sterilization achieved.

The practical question was whether the virus of serum hepatitis is killed by ethylene oxide vapour, and if so, can it be done economically? Since this virus could only be demonstrated by inoculating human volunteers a direct answer to the first part of the question was not possible and therefore it was suggested that the viricidal action of gaseous ethylene oxide on the viruses of vaccinia and Columbia SK encephalomyelitis should be studied. These two viruses were not only representative of 'large' and 'small' viruses, but, in addition, each had a different tropism. Although these results cannot be applied to any viruses other than those studied, it seemed likely that the investigation would open new aspects on the sterilization of certain materials infected with viruses in general.

PROPERTIES OF ETHYLENE OXIDE

It has been known since 1928 that this compound has a powerful insecticidal, fungicidal and bactericidal action, and it has been used for sterilizing storage space for food and other materials liable to spoilage. Patents have been granted for most of these methods of sterilization. The gas not only sterilizes the air, but penetrates many types of containers, provided they are not hermetically sealed, so that even foodstuffs packed in paper, cellophane or similar materials can be sterilized in this way. The fumigant can be removed easily by aeration following treatment, without damaging either taste or colour (Phillips & Kaye, 1949; Phillips, 1949; Kaye, 1949; Kaye & Phillips, 1949).

Ethylene oxide is a clear, colourless, watery liquid below its boiling-point at 10·7° C. The liquid is highly inflammable and the vapour forms an explosive mixture with air in all proportions from 3 % to 80 % by volume. The explosion hazard can be eliminated when the vapour is mixed with more than 7·15 times its volume of carbon dioxide. It is commercially available in cylinders containing 10 % ethylene oxide and 90 % carbon dioxide, in which mixture it can be added to air in any proportion without the risk of explosion. The gas is slightly toxic; its limits of toxicity are stated to correspond to those of ammonia. A search of the literature revealed no investigations into the viricidal properties of gaseous ethylene oxide, although Ginsberg & Wilson (1950) have investigated liquid ethylene oxide.

EQUIPMENT AND MATERIALS

The apparatus consisted of a metal cylinder containing liquid ethylene oxide (compressed) and carbon dioxide in the ratio of 1 to 9 to which was attached an apparatus which measured out that amount of fluid required to fill a metal cylinder of about 10 l. capacity with the fumigant.

The transparent plastic tubes, used by the Blood Transfusion Service, had a diameter of $\frac{1}{5}$ in. and were about 8 in. long. Both ends of these tubes could be closed by conical glass stoppers, which were slightly warmed before insertion to obtain good sealing. Since preliminary experiments had proved that ethylene oxide gas diffuses through plastic tubes, control tubes were placed inside glass cylinders which were closed at both ends to prevent contact of the gas with the plastic. In this way the control tubes could be exposed to the same conditions of time and temperature, but not to the gas.

The Columbia SK encephalomyelitis virus consisted of a 10% mouse brain suspension in saline from animals which had died after intracerebral inoculation with this virus. The suspension was centrifuged for 15 min. at 3000 r.p.m. and the supernatant was used as virus suspension. The LD_{50} of the supernatant varied from 10^{-9} to 10^{-10}.

Vaccinia virus was the commercially available vaccine lymph diluted 1 in 4 with saline. The titre of this virus, determined by both the rabbit skin test and on the chorioallantoic membrane of the developing chick embryo, varied from $10^{-5 \cdot 5}$ to $10^{-6 \cdot 5}$.

METHODS

Quantities of 0·1 ml. virus suspension were pipetted into the open plastic tubes. The entire inside surface of the wall was moistened by rotating the tube. Larger quantities of suspension formed a drop which was liable to leak from the tube when the metal cylinder was evacuated, with risk of contaminating jar and contents with virus. The plastic tubes with the upper ends open and all control tubes, hermetically sealed, were then placed vertically in the metal cylinder. After this cylinder had been made air-tight by means of a lid, rubber ring and clamps, it was partly evacuated before the ethylene oxide + CO_2 mixture was admitted in order to obtain better volatilization of the liquid. The tubes were then exposed to the action of the gas in the enclosed chamber at room temperature (18–20° C.) over a wide range of times. Each time the cylinder was opened to take out one or more of the tubes, it was evacuated by means of a water pump, the gas thus being dissolved in the water and carried away.

The contents of the exposed plastic tubes were then thoroughly rinsed out a few times with 0·2–0·3 ml. of saline. In the initial stage a drop of penicillin (10·000 u./ml.) and streptomycin (25 mg./ml.) was added to the suspension; 0·02 ml. of the brain suspension was then inoculated intracerebrally into mice; the vaccine lymph, further diluted to 1/50, was inoculated on to the chorioallantoic membrane of the developing chick embryo. The eggs were harvested after 3 days' further incubation and searched for typical vaccinia lesions.

RESULTS

The results of the tests made with the Columbia SK virus suspensions exposed to ethylene oxide are presented in Table 1.

Table 1. *Effect of exposure to ethylene oxide: Columbia SK virus*

Length of exposure to ethylene oxide (hr.)	Days after inoculation					
	1	2	3	4	5	6
4	0/4	0/4	1/4	4/4	—	—
6	0/4	0/4	1/4	4/4	—	—
8	0/4	0/4	0/4	0/4	0/4	0/4
10	0/4	0/4	0/4	0/4	0/4	0/4
12	0/4	0/4	0/4	0/4	1/4	1/4
14	0/4	0/4	0/4	0/4	0/4	0/4
16	0/4	0/4	0/4	0/4	0/4	0/4
24	0/4	0/4	0/4	0/4	0/4	0/4
No contact (controls)						
6	0/4	0/4	3/4	4/4	—	—
12	0/4	0/4	0/4	4/4	—	—
24	0/4	0/4	0/4	1/4	2/4	4/4

The denominator indicates number of mice inoculated, the numerator the number of deaths in each group.

The experiments with the vaccinia virus are summarized in Table 2.

Table 2. *Effect of exposure to ethylene oxide: vaccinia virus*

No. of eggs	Length of exposure to ethylene oxide (hr.)	Changes on the chorioallantoic membrane
4	4	4 positive
3	6	2 positive, 1 negative
4	8	3 negative, 1 non-specific lesion*
4	10	4 negative
4	12	4 negative
4	14	4 negative
3	16	3 negative
3	24	3 negative
No contact (controls)		
4	6	all positive
3	12	all positive
3	24	all positive

* In the smear stained according to Gispen (1952) no elementary bodies were observed, and on further egg passage no vaccinia lesions were seen.

All these experiments have been repeated several times, each time with similar results. It is obvious from the data obtained that ethylene oxide exerts a viricidal effect on both viruses, and that the action of the fumigant on both viruses is very much the same.

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Contact for 6 hr. appeared to be insufficient to sterilize either virus; after 8 hr., however, viable virus could no longer be detected.

Since it is known that ethylene oxide also acts as a strong germicidal fumigant, its use may be seriously considered in laboratories and hospitals as a sterilizing agent for many materials, such as rubber, plastics, cork, leather, woollen blankets or clothing, without the risk of damaging objects treated. The technique of sterilization is easy, quick and cheap. Other desirable features, such as rapid action, low toxicity to human beings, ease of storage, ready availability and the ease of removal by aeration following treatment, enhance its utility.

SUMMARY

Investigation of the viricidal effect of a mixture of ethylene oxide and carbon dioxide on two viruses, Columbia SK and vaccinia, yielded favourable results. Since ethylene oxide also exerts a germicidal effect, it offers simple and inexpensive disinfection of certain materials, especially those of low thermostability.

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