THE EFFECT OF VOLATILE ANAESTHETICS ON BACTERIAL GROWTH

S. MEHTA, M.B., B.S., F.F.A., R.C.S. (ENG.), * G. BEHR, M.R.C.P., F.R.C.PATH., † AND
D. KENYON, F.I.M.L.T. ‡

THERE HAS BEEN little investigation of the effect on bacterial growth of volatile anaesthetics in concentrations used in clinical anaesthesia. Waterhouse,¹ a Surgeon from Charing Cross Hospital, was the first person to mention the clinical results obtained by the use of di-ethyl ether in cases of various pyogenic infections. Topley² a Bacteriologist from the same hospital, showed that ether vapour exerts a definite bactericidal action on certain micro-organisms. John Bray³ described a method of suppressing proteus and coliform bacteria on routine blood agar plates and came to the conclusion that ether vapour had a weak antiseptic effect. Hunter *et al.*⁴ used chloroform vapour as a preservative for culture media. Barry, Paiement and Dubeau⁵ were the first to claim that halothane and methoxyflurane vapour in clinical concentrations had no antibacterial activity against *Staphylococcus aureus* and *E. coli*. More recently Wardley-Smith and Nunn⁶ confirmed the findings of Barry *et al.* and showed that for certain species of non-pathogenic bacteria in broth, the effect on bacterial growth rate was negligible at clinical concentrations of halothane. On the other hand, Horton, Sussman and Mushin⁷ exposed *E. coli* spread on cellulose acetate membranes to the vapours of volatile anaesthetic agents and showed that halothane, chloroform, trichloroethylene and methoxyflurane reduce the number of surviving organisms and that the reduction in survival was proportional to the concentration of the agent used.

There are three main reasons why this knowledge of the effect of anaesthetics in clinical concentration on bacteria is useful. Firstly, it has clinical relevance to anaesthesia in patients with severe infection; secondly, it appeared likely that it might add to our scanty knowledge of the effect of anaesthetics at the molecular and cellular level and might reveal the possible mechanism of narcosis (Wardley-Smith *et al.* 1971⁶ and White *et al.* 1970⁸); and thirdly, it might throw light on the antibacterial action of anaesthetic vapours on organisms present on the surface of anaesthetic equipment, a factor which might be responsible for an incidence of post-operative pulmonary infection which is much lower than might be expected (Horton *et al.*⁷ 1970). This paper describes studies of the effect of clinical concentrations of halothane, methoxyflurane and trichloroethylene on the growth of two species of bacteria grown on blood agar plates and cellulose acetate membranes.

*S. Mehta, M.B., B.S., F.F.A., R.C.S. (England), Consultant Anaesthetist, Burnley Group of Hospitals, General Hospital, Casterton Avenue, Burnley.

†G. Behr, M.R.C.P., F.R.C.PATH., Consultant Pathologist, Burnley Group of Hospitals, General Hospital, Casterton Avenue, Burnley.

‡D. Kenyon, F.I.M.L.T., Chief Technician, Bacteriology Department, Pathological Laboratory, Burnley Group of Hospitals, General Hospital, Casterton Avenue, Burnley.

METHODS

In the present investigation two species of bacteria, *Escherichia coli* and *Staphylococcus aureus* were selected for ease of growth and because they can be readily handled with simple laboratory equipment. In fact they are among the easiest of micro-organisms to cultivate *in vitro*.

Preparation of bacterial suspension

Two tubes containing 5 ml of nutrient broth were inoculated with a strain of *Staphylococcus aureus* and *E. coli* respectively. They were then incubated overnight at 37° C. Half a millilitre of these overnight cultures was added to 4.5 ml of nutrient broth which were then incubated at 37° C for 90 minutes. This technique has been found by Horton, Sussman and Mushin⁷ to give a reasonably reproducible suspension of organisms. Further dilutions of each suspension were made in sterile physiological saline to give approximately 1×10^3 organisms per ml.

Inoculation of organisms on the surface of blood agar and cellulose acetate membrane

We endeavoured to simulate the environment of the body tissues by spreading the bacteria on the surface of blood agar and that of anaesthetic equipment, i.e. moist inert substrate in the absence of nutrient, by spreading the organisms on cellulose acetate membrane, before exposing them to vapours of various anaesthetic agents. Thus two methods were used.

The first method consisted of spreading 0.1 ml of the bacterial suspension evenly with a sterile glass rod over the surface of blood agar. These inoculated blood agar plates were then exposed to the vapours of the anaesthetic agents in a desiccator for periods of 2, 3 and 4 hours.

In the second method 0.1 ml of the stock bacterial suspension was placed on the sterile cellulose membrane (HAWG 047 50 with grid) resting on dried sterile 2 per cent agar in petri dishes. The inoculum was spread evenly over the surface of the membrane with a sterile glass rod to ensure an even distribution of organisms. Care was taken to exclude all bubbles of air between the agar and the membrane. The saline was drawn through the membrane by imbibition by the agar and completion of this process was indicated by a return to the original matt surface of the membrane.

Exposure to anaesthetic vapours

Two inoculated membranes, one containing *Staph. aureus* and the other *E. coli* were suspended from sterile clips attached to a hook on the stopcock of the desiccator. Two blood agar plates inoculated with *Staph. aureus* and *E. coli* were also placed in each desiccator. An atmosphere of high relative humidity was obtained by maintaining a layer of distilled water at the base of each desiccator. Air from the desiccator was evacuated by creating a vacuum of 55 mm of mercury. The anaesthetic vapours were simply drawn in by attaching the desiccator to the reservoir bag. This process was repeated twice to ensure accuracy of concentration of anaesthetic vapour inside the desiccator.

TABLE I
REPRODUCIBILITY OF TECHNIQUES OF DILUTION, INOCULATION OF ORGANISMS
ON MEMBRANES AND SUBSEQUENT INCUBATION ON NUTRIENT AGAR

Experiment No.	Organism	Mean number of Colonies	S.D.	Number of Membranes
1	<i>Staph. aureus</i>	379.67	16.80	3
	<i>E. coli</i>	402.00	26.91	3
2	<i>Staph. aureus</i>	510.33	27.93	3
	<i>E. coli</i>	540.33	22.81	3
3	<i>Staph. aureus</i>	320.67	21.23	3
	<i>E. coli</i>	410.33	27.39	3

TABLE II
REPRODUCIBILITY OF TECHNIQUES OF DILUTION, INOCULATION OF ORGANISMS
ON BLOOD AGAR PLATE AND SUBSEQUENT INCUBATION

Experiment No.	Organism	Mean number of Colonies	S.D.	Number of Plates
1	<i>Staph. aureus</i>	445.00	22.91	3
	<i>E. coli</i>	439.00	22.65	3
2	<i>Staph. aureus</i>	503.33	29.28	3
	<i>E. coli</i>	581.33	18.04	3
3	<i>Staph. aureus</i>	341.67	24.58	3
	<i>E. coli</i>	436.67	25.01	3

Air was used as a carrier gas. The required concentration of anaesthetic vapour was obtained by allowing a flow of 5 l/min of air through thermo-compensated vapourizers. Halothane concentrations of 2, 3 and 5 per cent were provided by a Fluotec Mark III vapourizer. Trichloroethylene concentrations of 0.5, 1 and 1.5 per cent were delivered by means of a Tritec vapourizer and 0.5, 1 and 2 per cent methoxyflurane vapour was obtained by using a Pentec Mark II vapourizer. As a control in each experiment the inoculated membranes and blood agar plates were simply exposed to air.

After exposure for periods of 2, 3 and 4 hours the inoculated membranes were placed on Diagnostic Sensitivity Test agar (Oxoid). Then both the membranes and blood agar plates were incubated at 37° C overnight. Surviving organisms produced colonies which were counted the next day.

RESULTS

Reproducibility of the technique

The reproducibility of the diluting and inoculating technique was determined by inoculating several membranes and blood agar plates. The inoculating membranes were then placed on nutrient agar. Later both the membranes on nutrient agar and blood agar plates were incubated overnight at 37° C and the colonies counted. The standard deviation of the number of colonies cultured in the experiments was determined, and it was found that the counts are subject to a standard deviation of not more than 7.5 per cent of the mean count (Table I and Table II).

Effect of anaesthetic agents

Tables III, IV and V show growth of *Staph. aureus* and *E. coli* expressed as per

TABLE III
GROWTH OF ORGANISMS EXPRESSED AS PER CENT OF CONTROL AFTER EXPOSURE TO HALOTHANE

Organism	Inoculated Surface	2-hour exposure		3-hour exposure		4-hour exposure	
		Vapour concentration 2% 3% 5%		Vapour concentration 2% 3% 5%		Vapour concentration 2% 3% 5%	
<i>Staph. aureus</i>	Cellulose acetate membrane	37	35	30	33	35	28
<i>E. coli</i>	Cellulose acetate membrane	16	13	13	16	13	11
<i>Staph. aureus</i>	Blood agar plate	90	88	85	97	95	91
<i>E. coli</i>	Blood agar plate	93	90	89	88	88	86

TABLE IV
GROWTH OF ORGANISMS EXPRESSED AS PER CENT OF CONTROL AFTER EXPOSURE TO METHOXYFLURANE

Organism	Inoculated surface	2-hour exposure		3-hour exposure		4-hour exposure	
		Vapour concentration 0.5% 1% 2%		Vapour concentration 0.5% 1% 2%		Vapour concentration 0.5% 1% 2%	
<i>Staph. aureus</i>	Cellulose acetate membrane	29	32	28	34	32	33
<i>E. coli</i>	Cellulose acetate membrane	11	9	8	8	8	7
<i>Staph. aureus</i>	Blood agar plate	91	82	78	87	84	81
<i>E. coli</i>	Blood agar plate	90	87	83	91	91	89

TABLE V
GROWTH OF ORGANISMS EXPOSED AS PER CENT OF CONTROL AFTER EXPOSURE TO TRICHLOROETHYLENE

Organisms	Inoculated surface	2-hour exposure			3-hour exposure			4-hour exposure		
		Vapour concentration 0.5% 1% 1.5%			Vapour concentration 0.5% 1% 1.5%			Vapour concentration 0.5% 1% 1.5%		
<i>Staph. aureus</i>	Cellulose acetate membrane	39	33	25	39	31	30	39	30	27
<i>E. coli</i>	Cellulose acetate membrane	50	28	27	48	28	27	40	26	23
<i>Staph. aureus</i>	Blood agar plate	91	88	84	87	83	78	88	81	78
<i>E. coli</i>	Blood agar plate	94	94	92	92	91	87	91	88	85

TABLE VI
THE DIFFERENCE BETWEEN NUMBER OF CONTROL ORGANISMS AND MEAN
NUMBER OF SURVIVING ORGANISMS EXPOSED TO VARIOUS ANAESTHETICS
ON THE SURFACE OF MEMBRANE AND THEIR SIGNIFICANT LEVELS

Anaesthetic	Duration of Exposure	Organisms	Difference	Significant level P
Halothane	2 hours	<i>S.A.</i>	214	0.001
		<i>E.C.</i>	335	0.001
	3 hours	<i>S.A.</i>	239	0.001
		<i>E.C.</i>	377	0.001
	4 hours	<i>S.A.</i>	217	0.001
		<i>E.C.</i>	336	0.001
Methoxyflurane	2 hours	<i>S.A.</i>	238	0.001
		<i>E.C.</i>	236	0.001
	3 hours	<i>S.A.</i>	274	0.001
		<i>E.C.</i>	452	0.001
	4 hours	<i>S.A.</i>	327	0.001
		<i>E.C.</i>	512	0.001
Trichloroethylene	2 hours	<i>S.A.</i>	326	0.001
		<i>E.C.</i>	368	0.001
	3 hours	<i>S.A.</i>	271	0.001
		<i>E.C.</i>	317	0.001
	4 hours	<i>S.A.</i>	265	0.001
		<i>E.C.</i>	300	0.001

S.A. - *Staph. aureus*, *E.C.* - *E. coli*.

cent of control after exposure to various vapour concentrations of halothane, methoxyflurane and trichloroethylene respectively for periods of 2, 3 and 4 hours. Growth of both species of bacteria on blood agar plates appeared to be unaffected by the vapour concentrations of these volatile anaesthetics used in the clinical range. The number of colonies were not appreciably different on test and control plates. On the other hand, when these bacteria were exposed to anaesthetics on the surface of membranes, the growth expressed as per cent of control is considerably reduced. To further assess whether the differences between the number of surviving organisms and the number of control organisms were statistically significant it was decided to evaluate the mean number of surviving organisms for three concentrations and test if this was different from the number of control organisms. The difference between the number of surviving organisms and the number of control organisms in each case was highly significant (Table VI). Figures 1, 2 and 3 show dose/response curves for inhibition of growth of two species of bacteria on membrane and blood agar plates by vapours of the various anaesthetics.

Although the inhibition of growth was proportional to the concentration of agent used, the differences between the number of survivors at three concentrations of each anaesthetic agent were not always significant. Only in one case did a significant difference emerge. This was for a 2-hour exposure of *E. coli* on membrane to trichloroethylene ($P < 0.025$).

Effect of duration of exposure

Figures 4, 5 and 6 show time course of inhibition of growth of *E. coli* and *Staph. aureus*. Maximum inhibition of growth occurred during exposure to anaesthetic

FIGURE 1. Dose/response curves for inhibition of growth of *Staph. aureus* and *E. coli* exposed to halothane for 2 hours.

FIGURE 2. Dose/response curves for inhibition of growth of *Staph. aureus* and *E. coli* exposed to methoxyflurane for two hours.

agents for a period of 2 hours and further exposure up to 4 hours did not make any significant difference.

Individual bacterial resistance to anaesthetics

Tables III and IV show that *Staph. aureus* on the surface of membrane has higher resistance to halothane and methoxyflurane than *E. coli* at all concentrations. Thus there was much greater inhibition of growth of *E. coli* with these two agents. When

FIGURE 3. Dose/response curves for inhibition of growth of *Staph. aureus* and *E. coli* exposed to trichloroethylene.

FIGURE 4. Time course of inhibition of growth of *Staph. aureus* and *E. coli* by two per cent halothane.

these bacteria were exposed to 0.5 per cent trichloroethylene on the surface of membrane, *E. coli* showed higher resistance than *Staph. aureus* (Table V).

DISCUSSION

Our results confirm the observations of Barry, Paiement and Dubeau⁵ (1964) who concluded that in usual clinical concentrations halothane and methoxyflurane vapours have hardly any antibacterial activity. Wardley-Smith and Nunn⁶ (1971)

FIGURE 5. Time course of inhibition of growth of *Staph. aureus* and *E. coli* by one per cent methoxyflurane.

FIGURE 6. Time course of inhibition of growth of *Staph. aureus* and *E. coli* by one per cent trichloroethylene.

arrived at a similar conclusion and stated that growth of bacteria was virtually unaffected by concentrations of halothane in the range used during clinical anaesthesia. Barry *et al.*⁵ in their experiments exposed the organisms to the anaesthetic vapours on the surface of blood agar, while Wardley-Smith *et al.*⁶ assessed bacterial growth rate either on nutrient agar plate or in nutrient broth. The authors exposed the organisms to the anaesthetic vapours on the surface of blood agar. It seems certain that the culture medium in some way protects these organisms against the anaesthetic vapours. Wilson and Miles⁹ (1964) consider that

serum proteins present in blood agar medium have a protective effect. It seems likely that growth of bacteria in host tissues will also be unaffected by concentrations of anaesthetic agents in range used during clinical anaesthesia since these micro-organisms in the body tissues will be protected in the same way as are bacteria on the surface of blood agar, nutrient agar or in nutrient broth.

An attempt has been made to evaluate the antibacterial activity of various anaesthetic agents whilst the organisms were exposed on the surface of cellulose acetate membrane, simulating circumstances of contamination of anaesthetic equipment. Such activity would indeed offer definite advantage in the prevention of cross infection through anaesthetic equipment and post-operative pulmonary complications. From the results it appears that there is a significant reduction in growth and indeed survival of the bacteria studied when exposed to the anaesthetic vapours in clinical concentrations on the surface of the membrane. Our results confirm the observation of Horton, Sussman and Mushin.⁷ The cellulose acetate membrane provides a convenient model of the state of affairs in the anaesthetic equipment during use.

Bacterial contamination of anaesthetic equipment is common (Jenkins and Edgar¹⁰ 1964). Pandit, Mehta and Agarwal¹¹ (1967) have shown that different items of inhalation anaesthetic equipment may be heavily contaminated both with bacteria and fungi under normal conditions. They also pointed out that the parts of the anaesthetic equipment nearest to the mouth, such as face mask, angle piece, catheter mount and endotracheal connections are prone to be heavily contaminated, as might be anticipated. Although this bacterial contamination of anaesthetic equipment is mainly by commensals and non-pathogenic organisms and the usual respiratory pathogens have only infrequently been isolated, it is remarkable that severe post-operative chest infection is not more common than it is despite coincidental depression of ciliary activity by the anaesthetic agents. These observations would suggest, therefore, that the vapours of anaesthetic agents in clinical concentrations do provide a certain protection against cross infection, possibly by inhibiting the growth of bacteria responsible for post-operative chest infection.

Our study shows the same slowing of the death rate towards the end of the experiment as is seen as a result of exposure to disinfectants. This is thought by Wilson and Miles⁹ (1964) to be due to development of adaptive changes by bacteria during exposure. This may explain the finding that maximum inhibition of growth occurred at two-hour exposure to vapours of anaesthetic agents. Exposure for longer periods, up to four hours, did not result in further inhibition of growth of these two species of bacteria. However, *E. coli* and *Staph. aureus* are not respiratory pathogens and were selected for ease of growth and handling. Work is already in progress to investigate the effect of anaesthetic vapours on common respiratory pathogens.

SUMMARY

The effect of the vapours of volatile anaesthetic agents on *Staphylococcus aureus* and *Escherichia coli* has been investigated. Known numbers of organisms on the surface of blood agar and cellulose acetate membrane were exposed to clinical concentrations of halothane, methoxyflurane and trichloroethylene under con-

trolled conditions of humidity. The growth of bacteria when exposed on the surface of blood agar was unaffected by the anaesthetic agents. But there was a significant reduction in growth when these organisms were exposed to anaesthetic vapours on the surface of membrane. The implications of these findings are discussed.

RÉSUMÉ

Nous avons fait des recherches sur l'effet des vapeurs d'agents anesthésiques volatils sur le staphylococque doré et sur l'Escherichia coli. Sur des membranes de sang agar et d'acétate de cellulose, nous avons exposé des nombres connus de microbes à des concentrations cliniques d'halothane, de méthoxyflurane, de trichloréthylène à des taux connus d'humidité. La croissance des bactéries, si exposée sur la surface de sang agar, n'était pas modifiée par les agents anesthésiques. Toutefois, lorsqu'exposée sur la membrane de cellulose, la croissance de ces organismes était réduite de façon significative en présence de vapeurs d'agents anesthésiques. Nous discutons les implications de ces résultats.

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REFERENCES

1. WATERHOUSE, H.F. A report on the employment of ether in surgical therapeutics. *Brit. Med. J.* 1: 233 (1915).
2. TOPLEY, W.C.W. The action of ether on certain micro-organisms. *Brit. Med. J.* 1: 237 (1915).
3. BRAY, J. A method of suppressing proteus and coliform bacteria on routine blood agar plates. *J. Path. Bact.* 57: 395 (1945).
4. HUNTER, S.H. & BJERKNES, C.K. Volatile preservatives for culture media. *Proc. Soc. Exp. Biol. Med.* 67: 393 (1948).
5. BARRY, P.P., PAIEMENT, B., & DUBEAU, M. Recherches sur l'activité antibactérienne de certains agents anesthésiques. *Canad. Anaesth. Soc. J.* 11: 640 (1964).
6. WARDLEY-SMITH, B. & NUNN, J.F. The effect of halothane on bacterial growth rate. *Brit. J. Anaesth.* 43: 919 (1971).
7. HORTON, J.N., SUSSMAN, M., & MUSHIN, W.W. The antibacterial action of anaesthetic vapours. *Brit. J. Anaesth.* 42: 483 (1970).
8. WHITE, D.C. & DUNDAS, C.R. Effect of anaesthetics on emission of light by luminous bacteria. *Nature* 226: 458 (1970).
9. WILSON, C.S. & MILES, A.A. Principles of bacteriology and immunity. 5th ed. London, Arnold, pp. 156-166 (1964).
10. JENKINS, J.R.E. & EDGAR, W.M. Sterilization of anaesthetic equipment. *Anaesthesia* 19: 177 (1964).
11. PANDIT, S.K., MEHTA, S., & AGARWAL, S.C. Risk of cross infection from inhalation anaesthetic equipment. *Brit. J. Anaesth.* 39: 838 (1967).