1 W.R. Kellen, T.B. Clark, J.E. Lindegren, B.C. Ho, M.H. Rogoff and S. Singer, J. Invertebr. Path. *7*, 442 (1965).
2 S. Singer, Nature *244*, 110 (1973).
3 S. Singer, in: Developments in Industrial Microbiology, vol. 5, p. 187. Plenum Press, New York 1974.
4 E.W. Davidson, S. Singer and J.D. Briggs, J. Invertebr. Path. *25*, 179 (1975).
5 R.C. Hughes, Nature *282*, 526 (1979).
6 O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, J. biol. Chem. *183*, 265 (1951).
7 S. Singer, in: Biological Regulation of Vectors. A conference report. U.S. Department of Health, Education and Welfare, NIH 1975.

The effects of naloxone on the analgesic activities of general anaesthetics

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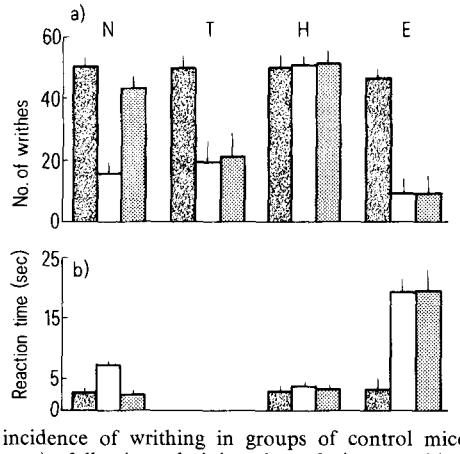
Summary. Inhaled concentrations of nitrous oxide (80%), halothane (0.5%), trichloroethylene (0.5%) and s.c. ethanol (1 ml/kg) caused similar degrees of excitation and ataxia in mice. Nitrous oxide, tricholoroethylene and ethanol caused analgesia (hot plate and writhing tests), but only that caused by nitrous oxide was antagonized by naloxone (20 mg/kg). Halothane lacked analgesic activity.

Anaesthetics differ in their analgesic potency at sub-anaesthetic concentrations. Whilst nitrous oxide and trichloroethylene are clinically useful analgesics, halothane has negligible acitivity[1], and barbiturates have been reported as being hyperalgesic[2]. The recent reports that the analgesic but not the anaesthetic action of nitrous oxide may be antagonised by naloxone implies that this drug releases endorphins[3,4]. The present experiments were designed to determine whether the analgesic actions of some other anaesthetics including trichloroethylene could be similarly explained.

Materials and methods. Groups of female mice (35–40 g) were used. Central depression was measured using an Animex meter set at 40 μA. Groups of 5 mice were placed on the counter in a Perspex equilibration chamber of floor area 580 cm^2. Activity was recorded at 1-min intervals for 15 min, then the gases were passed in at flow rates of 6 l/min (Rotameter). Activity was recorded for a further 35 min. Results are expressed as the difference from appropriate controls examined at the same time of day.

Mixtures of nitrous oxide in oxygen were delivered via Rotameters, those of halothane (Fluotec) and trichloroethylene (Tritec) using their respective vapourisers (Cyprane). Ethanol and naloxone were administered by s.c. injection.



a The incidence of writhing in groups of control mice (textured columns), following administration of nitrous oxide (80% N), trichloroethylene (0.5% T), halothane (0.5% H) and ethanol (1 ml/kg E) before (white columns) and after naloxone 20 mg/kg (shaded columns). *b.* Hot plate reaction times of groups of mice exposed to nitrous oxide, halothane and ethanol before and after naloxone (code as above). Limits are + SE.

Analgesia was assessed using 2 methods. Writhing was induced by the i.p. injection of 0.75% acetic acid (100 mg/kg). For the experiments using gases injection of acetic acid was immediately followed by naloxone or saline, and the mice placed in an equilibration chamber (9000 cm^3) through which gas passed at 6 l/min. The end point was taken as an extension of one or both hind legs accompanied by arching of the back and abdominal concavity. The number of such movements in the following 20 min was expressed as percentage inhibition from concurrent saline controls. The comparisons between naloxone and saline were performed 'blind'. The effect of naloxone was expressed as a percentage of the appropriate control. The hot plate was maintained electrically at 55 °C. Mice were placed in the same equilibration chamber through which passed the gas at a rate of 6 l/min. Following 10-min exposure a mouse was quickly transferred to an open-ended perspex box placed on the plate through which passed the same anaesthetic gas. The end point was taken as a sign of discomfort in a hind paw. A cut-off time of 45 sec was employed.

Each anaesthetic was examined using both tests except trichloroethylene which was not tested on the hot plate because of the drug's potential degradation on exposure to the heating element of the plate. Soda lime was used in the circuits in all experiments except those using trichloroethylene. Statistical comparisons were made using Student's t-test.

Results. The selection of doses of anaesthetics was made empirically based on the behavioural effects of a range of doses of anaesthetics. Nitrous oxide (100%), trichloroethylene and halothane (1%) and ethanol (2 ml/kg) caused rapid anaesthesia (< 3 min), followed by almost immediate recovery for the gases though ethanol had a longer duration of action.

The doses subsequently chosen were halothane and trichloroethylene (0.5%), nitrous oxide (80%) and ethanol (1 ml/kg). Doses of naloxone between 1 and 20 mg/kg were used. At these doses, halothane, trichloroethylene and ethanol all caused a similar increase in activity during the 15 min following administration. The increases (± SE) were respectively 257.7 (87), 258.1 (56.4) and 249.2 (145.7) Animex counts/15 min. These increases were significant ($p < 0.05$).

Following the 15-min period the 3 drugs had negligible effect on activity. Nitrous oxide caused far more hyperactivity during the 15-min period (847 ± 153.3), and thereafter the count remained about 60/min higher than concurrent controls for the remainder of the experiment. All 4 drugs caused ataxia.

Figure a compares the effects of the anaesthetics on writhing and the effect of naloxone on them. Halothane caused no significant inhibition of writhing ($p > 0.6$). Whilst nitrous oxide ($p \simeq 0$), trichloroethylene ($p < 0.005$) and ethanol ($p \simeq 0$) all significantly inhibited writhing, only that produced by nitrous oxide was antagonized by naloxone 20 mg/kg ($p < 0.001$). Thus there was no significant difference between the incidence of writhing on pretreatment with either nitrous oxide - naloxone combination or saline ($p > 0.2$). Trichloroethylene and ethanol were unaffected by naloxone ($p > 0.8$).

Figure b shows that whilst in the hot plate test both nitrous oxide and ethanol significantly increased reaction times ($p \simeq 0$), only nitrous oxide was antagonized by naloxone. In this way there was no significant difference between the effects of the nitrous oxide - naloxone mixture and saline ($p > 0.4$). Again halothane had no significant effect in this analgesic test ($p > 0.5$).

Discussion. The doses of anaesthetic chosen were such that the mice were in an excited state at the time of analgesic testing, i.e. the anaesthetics similarly depressed inhibitory systems. The excitation caused by 80% nitrous oxide was greater and more sustained than that caused by the other anaesthetics. Whilst sedation per se does not cause analgesia, we felt it necessary to ensure that at the analgesic doses chosen the mice were neither sedated nor motor co-ordination markedly impaired.

The results of the analgesic tests are compatible with those reported by others[3,4]. It is clear that naloxone only antagonized the analgesic action of nitrous oxide, that of ethanol and trichloroethylene were unaffected. Why only nitrous oxide amongst the anaesthetics used releases endorphins is unclear, though it is significant that nitrous oxide is the only inhalation anaesthetic to have psychotomimetic activity, and opioids are known to cause hallucinations. One other 'anaesthetic' whose analgesic action appears to be antagonized by naloxone is ketamine[5], and this drug also has acute psychotomimetic activity.

That analgesia is not a necessary consequence of anaesthetic application is apparent in the lack of analgesic activity of halothane. There is some controversy as to whether endorphins are involved in the phenomenon of anaesthesia[6–8]. If they are involved it seems a different phenomenon from the selective endorphin-mediated analgesic action of nitrous oxide.

Finally we would stress that the doses of naloxone are large - some 20 times greater than that necessary to antagonize exogenous opioids. This is presumably due to there being some differences between the receptors at which endogenous and exogenous opioids act[9]. Our own unpublished experiments showed that at therapeutic doses, naloxone (0.4 mg) has no effect on either the hallucinogenic or analgesic action of 40% nitrous oxide in human volunteers.

1 S.H. Ngai, in: Handb. exp. Pharm. vol. 30, Modern Inhalation Anaesthetics, p. 43. Ed. M.B. Chenoweth. Springer, Berlin 1972.
2 J.W. Dundee, Br. J. Anaesth. *32*, 407 (1960).
3 B.A. Berkowitz, A.D. Finck and S.H. Ngai, J. Pharmac. exp. Ther. *203*, 539 (1977).
4 B.A. Berkowitz, A.D. Finck, M.D. Hynes and S.H. Ngai, Anaesthesiology *51*, 309 (1979).
5 D. Lawrence and A. Livingston, J. Physiol. *301*, 42P (1980).
6 A.D. Finck, S.H. Ngai and B.A. Berkowitz, Anaesthesiology *46*, 241 (1977).
7 P.B. Bennett, Anaesthesiology *49*, 9 (1978).
8 B.A. MacLeod, F.C. Ping and L.C. Jenkins, Can. Anaesth. Soc. J. *27*, 29 (1980).
9 A. Beaumont and J. Hughes, A. Rev. Pharmac. Toxic. *19*, 251 (1979).

At least two toxins are involved in *Escherichia coli* mastitis

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Summary. Culture filtrate of *Escherichia coli* produces changes in the bovine udder identical to those seen in experimental infections with the same organism. *E. coli* endotoxin produces an acute inflammatory response but none of the cell damage induced by culture filtrate. It appears therefore that at least 2 toxins are involved in the disease.

Bovine mastitis caused by *E. coli* is characterized by a rapid local inflammatory response, pyrexia and changes in the composition of the udder secretions. Intramammary infusion of a small dose of purified *E. coli* endotoxin elicits a very similar response, and for this reason has been used for many years to produce mastitis experimentally without the associated problems of an infective agent[3]. Although the development of the clinical features of coliform mastitis has been studied extensively, little is known about the pathogenesis of the disease or how the response of the gland to endotoxin is related to that produced in a natural infection. However, we have recently demonstrated[4] that damage to the epithelium of the teat and lactiferous sinuses of the mammary gland occurred within 1 h of infusion of a large number of washed bacteria (approximately 10^9 colony forming units, cfu). After 2 h epithelial lesions were extensive and there was microscopical evidence of inflammation. By 4 h the inflammation was intense and large numbers of neutrophils were migrating through the lesions to the epithelial surface. The same sequence of events has now been observed over a longer period of time (14 h)[5], when small numbers of bacteria were infused (approximately 200 cfu). In none of these experiments was there any evidence of the attachment of *E. coli* to the epithelial lining of the gland, suggesting that the inflammatory response and epithelial damage were mediated by a soluble agent released from the bacteria. We now present evidence that fresh filtrate of the medium in which *E. coli* has been growing for 18 h (*E. coli* culture filtrate, CCF) elicits a response identical to that observed in experimental infections, but that purified endotoxin produces an inflammatory response without any indication of epithelial lesions. We