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.

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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

The effect of infusing either endotoxin, sterile CCF or culture medium into 13 lactating quarters of 7 cows

Cow and quarter number		Infusion*	Time of exposure (h)	Neutrophil response	Epithelial damage
K461	1	900 µg E	1.5	±	-
	2	100 µg E	10	+ + + +	-
J23	3	100 µg E	11	+ + + +	-
K960	4	5 mg E	3.25	+ + + +	-
F685	5	1 mg E	3.5	+ + + +	-
J21	6	5 mg E	4	+ + + +	±
	7	5 mg E	2	+ +	-
	8	CCF 3.5 ml	4	+ + +	+ + + +
	9	CCF 3.5 ml	2	-	+ + +
L75	10	CCF 2 ml	3.5	+ +	+ + + +
F703	11	CCF 2 ml	4	+ +	+ + + +
	12	Medium 2 ml	4	-	-
	13	Medium 2 ml	19	-	-

* E, *E. coli* endotoxin dissolved in pyrogen-free water. CCF, culture filtrate of *E. coli* strain B117. Medium, fresh bacterial culture medium.

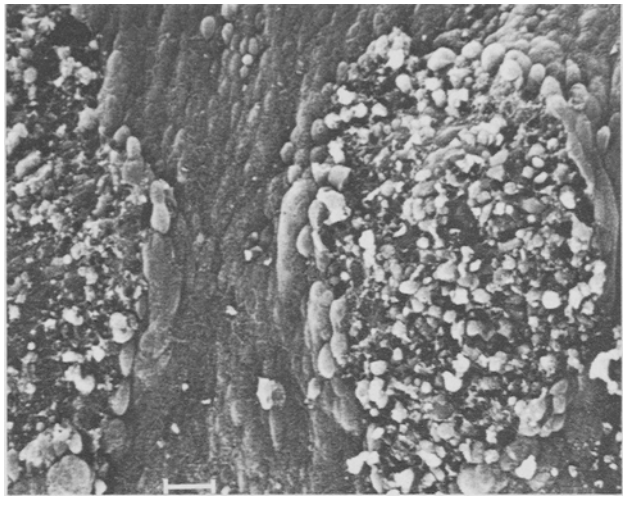

Fig. 1. Two large, round lesions in the lactiferous sinus epithelium 4 h after infusion of CCF. Bar = 20 µm.

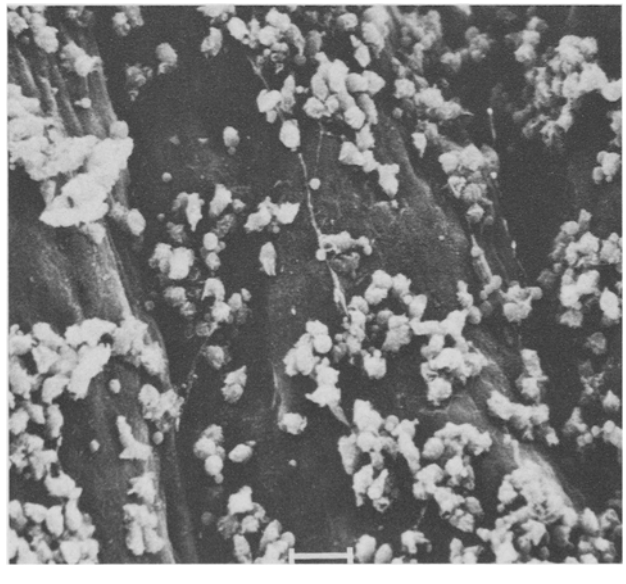

Fig. 2. Numerous polymorphs on the surface of the teat sinus epithelium 2 h after infusion of endotoxin. There is no evidence of epithelial lesions. Bar = 20 µm.

suggest that in the mammary gland of the cow, unlike any other organ so far studied, a labile toxin produced by *E. coli* is capable of causing epithelial damage.

Materials and methods. Healthy Friesian cows at varying stages of lactation were used in all experiments; the glands were free of bacterial infection and the somatic cell count in the milk had been constantly below 200,000/ml before inoculation. The CCF was prepared by growing *E. coli* strain B117 (08:K85:K99) for 18 h at 37 °C in a semi-defined medium[6], followed by sterilization of the resulting medium by centrifugation and filtration through a 0.45 µm filter (Millipore); the endotoxin was a commercial preparation from *E. coli* (Difco). The cows were slaughtered at appropriate times after the intraductal inoculation of each mammary gland with CCF or endotoxin and the udder was carefully removed intact. The relevant glands were gently infused through the teat duct with 3% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) until both teat and lactiferous sinuses were slightly distended. After fixation for 1 h, the udders were dissected and tissue removed for light and electron microscopy as described elsewhere[4].

Results and discussion. The treatments of the infused quarters and the results obtained are summarized in the table. Following infusion of CCF a mild inflammatory response was detected using light microscopy, but in all cases scanning electron microscopy (SEM) showed that there was extensive damage to the epithelium (figure 1). Both layers of the epithelium were lost, exposing the basement membrane and by 4 h polymorphs were beginning to appear at the lesions. This sequence of events was identical to that observed in experimental infections of the mammary gland with *E. coli*[4]. Storage of CCF for 36 h at 4 °C or 18 h at −20 °C resulted in the loss of all lesion-forming activity. In contrast, infusion of low doses of purified endotoxin produced intense inflammation and oedema but there was no damage to the epithelium. Using SEM, polymorphs could be seen all over the surface, but they were not associated with lesions (figure 2); using light microscopy neutrophils were seen packed against the basement membrane and between the 2 layers of apparently healthy epithelial cells. In one quarter (Number 6) infusion of a large dose of endotoxin did result in damage to small numbers of epithelial cells, but this was mild and confined to the rounding of single or small groups of cells in the superficial layer of the epithelium.

These experiments provide evidence of the presence in the culture filtrate of *E. coli* strain B117 of a cytotoxic agent whose effect on the bovine mammary gland is quite different from that of endotoxin. The total effect of CCF may

be attributed to the combined action of this cytotoxic factor and of endotoxin that is released during growth of the bacteria in culture[7]. The lability of this lesion-producing factor is in marked contrast to the stability of endotoxin. Strains of *E. coli* are known which produce heat stable and heat labile enterotoxins and a cytotoxin that destroys Vero cells in vitro[8-10], but there are few toxins known to produce tissue damage in vivo. Although the cytotoxic agent in CCF has yet to be characterized, the present results suggest that the assumption made by previous investigators that intramammary infusions of endotoxin produce a reaction indistinguishable from *E. coli*[3] is not justified.

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Effects of dihydroergocriptine on mouse and rat resistance to acute anoxia: influence of repetition of treatment

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Summary. The antihypoxic activity of DHEC mesylate, as evaluated in mice and rats submitted to hypobaric hypoxia and to asphyxic anoxia respectively, is noticeably stronger when the compound is administered according to a repeated treatment schedule than when administered only once. The results correlate well with human clinical observations.

For many years, the dihydrogenated alkaloids of rye ergot which belong to the ergotoxine group have been used to treat cerebral diseases caused by senescence or due to vascular insufficiency. In humans, their therapeutic efficacy has been somewhat difficult to assess under some circumstances, but in animals their activity in cerebral areas has been established using objective methods[1,2]. However, the influence of repetition of the administration on the pharmacological effect of the ergot alkaloids does not seem to have been given much attention. In humans, it has been established that several weeks are necessary before the therapeutic effect is apparent in geriatric patients[3,4] and the duration chosen for clinical studies is generally as long as 12 weeks[5]. As the dihydrogenated ergot alkaloids of the ergotoxine group are eliminated rather slowly (plasmatic $t_{1/2}\beta$ of 13 h for dihydroergotoxine, or 17 h for dihydroergocornine[6]) and as their digestive absorption is very poor[7], it seems clear that it would take several days to obtain the effective plasmatic (or tissular) concentration and to observe the therapeutic activity. We have studied the pharmacological effects of dihydroergocriptine (DHEC) after single and repeated administration for 5 days, on 2 simple experimental models of acute anoxia. Previous studies have suggested that among the 3 alkaloids constituting dihydroergotoxine (dihydroergocristine, dihydroergocornine, dihydroergocriptine) DHEC is the most active in counteracting the cerebral effects of acute anoxia in animals[1].

Material and methods. Hypobaric hypoxia in mice. These experiments were carried out on SPF male mice of the Swiss strain, weighing between 20 and 22 g (purchased by Iffa-Credo, Les Oncins, France). The methodology used was very close to the one described by Etesse-Carsenti[8]. Each experiment included 3 groups of mice (1 control group + 2 treated groups). The survival time (last visible respiratory or limb movement) of mice submitted to the hypobaric hypoxia (600 mm of mercury barometric depression reached in 100-110 sec) was studied on 3 mice simultaneously, each of them belonging to one of the 3 groups under experimentation. Treatments, dissolved in distilled water, were administered by the oral route (20 ml/kg b.wt). The dose of DHEC mesylate, administered once, that showed the optimum activity under these conditions was first determined (10 animals per group), using a large range of doses (3-3000 µg/kg) and retained for the next experiments. With the aim of studying the influence of the repetition of the treatment, DHEC mesylate has been administered (20 mice per group) either once daily for 5 consecutive days (0.3 mg/kg) or only on the 5th day (only the vehicle being administered on the 4 previous days); control animals were given 5 daily treatments of distilled water. 60 min after the last treatment, the animals were submitted to the hypobaric hypoxia test, using a set of 3 mice (see above). As variation of the body temperature exerts a strong influence on resistance to hypoxia, DHEC effects on this parameter were checked by measuring the rectal temperature before treatment and immediately before the test. Statistical comparisons on survival time and body temperature were established by the Wilcoxon's matched-pair test.

Asphyxic anoxia in rats: cerebral electric activity recording. These experiments have been carried out on SPF male rats of the Sprague-Dawley strain (purchased by Charles River, Saint-Aubin-lès-Elbeuf, France) weighing 300-400 g. 2 cortical electrodes (1 frontal, 1 parietal) made of silver wire were implanted through a hole in the skull of each animal, under diethylether anaesthesia, 3 days before testing. Drugs, dissolved in saline, were injected by the i.p. route (10 ml/kg b.wt, 16 rats per group). DHEC mesylate was injected either once daily for 5 consecutive days (0.3 mg/kg) or only on the 5th day, only the vehicle being administered on the 4 previous days; control rats were given 5 daily treatments of distilled water. The rats were submitted to the asphyxic anoxia 30 min after the last treatment. Under diethylether anaesthesia, they were tracheotomized, i.v. injected with gallamine triioethylate (20 mg/kg b.wt) and artificially ventilated (80 ml/min, 60 strokes/min). The