Discussion

Conventional treatment with inhaled beta$_2$-agonists, aminophylline, and steroids has reduced the frequency of acute severe asthma attacks requiring mechanical ventilation. Slowly deteriorating patients who require intubation may show a significant improvement merely when the work of breathing is taken over by a ventilator, thus allowing the cumulative effect of various medications to improve the bronchospasm. Nevertheless there are a small number of patients who require immediate intubation, ventilation, and additional therapy as in the cases above.[1]

Experiments in animals and man have demonstrated that halothane has bronchodilator activity.[2,3] It relaxes smooth muscle and antagonises the bronchoconstrictor effects of both histamine and acetylcholine in tracheal muscle isolates. The specific mode of action has not been fully elucidated. An effect similar to beta-sympathomimetic agents has been postulated, although there is no evidence of increase in catecholamine levels.[4] In common with many other inhalational anaesthetics, halothane has a negative inotropic effect and predisposes to catecholamine-induced cardiac arrhythmias, which may be aggravated by concomitant acidosis and hypoxia.[5] In case 1 we were reluctant to increase the inspired concentration of halothane above 2% because of the risks of dangerous cardiac arrhythmias in the presence of severe respiratory acidosis. In case 2 electrocardiographic evidence of first-degree heart block accompanied the administration of halothane and was associated with a fall in systolic blood pressure. In both cases, the administration of ether resulted in prompt bronchodilatation with clinical and arterial blood gas improvement, and no adverse cardiac effects were observed.

In 1912 Trendelenberg observed a relaxation effect of diethyl ether on excised bronchial tissues obtained from animals.[6] In the 1930s two reports appeared of the use of diethyl ether in status asthmaticus.[7,8] In those cases the ether was mixed with olive oil in equal parts and administered per rectum. In 1952 Tausig et al showed an improved response when ether was administered by inhalation rather than rectally,[9] and in 1973 Mountford advocated its use intravenously.[10] In the past 20 years the use of ether for anaesthesia has declined dramatically, and halothane has been recommended for severe status asthmaticus.[5,11] Our two cases indicate that despite the potential risks of explosion and flammability, ether may also have a role in such life-threatening situations, particularly if conventional halothane administration has failed.

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INACTIVATION OF LYMPHADENOPATHY-ASSOCIATED VIRUS BY HEAT, GAMMA RAYS, AND ULTRAVIOLET LIGHT

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Summary Lymphadenopathy associated virus is inactivated by heating at 56°C for 30 min, and is not inactivated by 2×10^5 rad gamma irradiation or 5×10^3 J/m^2 ultraviolet irradiation.

Introduction

AN earlier paper[1] described the effects of various chemical disinfectants on lymphadenopathy-associated virus (LAV), a possible cause of the acquired immunodeficiency syndrome (AIDS).[2] We report here the effects on the virus of heat, gamma rays, and ultraviolet light.

Methods

LAV was obtained by infection of T lymphocytes from a healthy adult.[1,2] The cells were stimulated with phytohaemagglutinin (PHA) for 3 days, infected with LAV (5000 cpm equivalent reverse transcriptase per 10^6 cells), and grown in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS), 10% T cell growth factor (TCGF, Biotest), and anti-human-α-interferon and antibiotics (PSN Flobio).

Reverse Transcriptase Assay

LAV reverse transcriptase (RT) was measured in 0·1% Triton X 100 disrupted high speed pellets. The polymerase reaction mixture (50 µl) contained 50 mmol/l "tris" HCl pH 7·8, 20 mmol/l KCl, 1 mmol/l dithiotreitol, 5 mmol/l $MgCl_2$, 10 µCi tritiated thymidine triphosphate (30 Ci/mmol), and 0·1 OD/ml of poly-A-oligo-dT$_{12-18}$ as template-primer.[3]

Viral Infectivity Assays

4×10^6 T cells from a healthy donor were stimulated with PHA (1/500) for 3 days, infected with treated or untreated LAV (5000 cpm

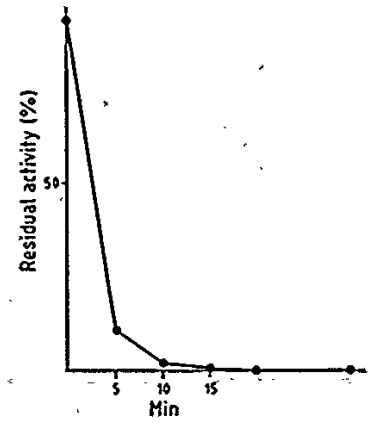


Fig 1—Inactivation of reverse transcriptase activity at 56°C.

10 µl of concentrated virus inactivated for different times at 56°C. Thermal inactivation was stopped by putting samples in ice.

TABLE I—DETERMINATION OF LAV INFECTIVITY AFTER HEATING AT 56°C FOR 30 MIN

Day after infection	Control virus RT (cpm)	Control virus in human serum RT (cpm)	Heated virus without serum RT (cpm)	Heated virus in presence of serum RT (cpm)
6	11 638	10 519	361	566
10	33 699	41 460	564	635
13	13 265	14 519	469	217
17	22 739	9944	270	314
20	3596	6316	652	584
23	2174	1994	362	524
26	4293	2290	313	381
30	—	—	326	595

TABLE II—EFFECTS OF ULTRAVIOLET IRRADIATION

UV dose ($J/m^2 \times 10^3$)	RT activity (cpm)	Infectivity*
Shadow irradiation	51 086	+++
Control (kept at +4°C)	45 000	+++
5	36 252	+++
10	33 207	—
100	8193	—
250	1742	—

*After infection, virus production was followed in the cell-free supernatant twice a week by measurement of RT activity;
+++ = virus replication;
— = no virus production.

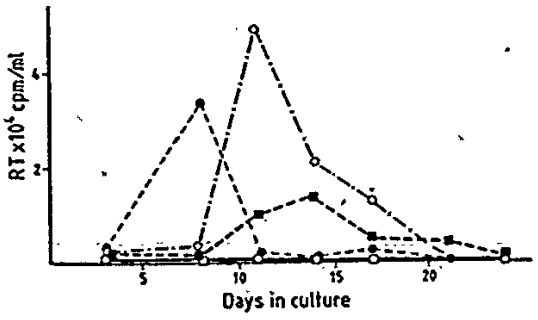
RT equivalent/10^6 cells), and grown in the above culture medium. Virus was sought in the cell-free supernatant by testing for RT activity twice a week. All negative cultures were checked for at least 4 weeks. Unless otherwise stated, tests were done with viral samples of 20 000 cpm RT equivalent.

Results

Heat Inactivation

After 30 min incubation of viral samples in a waterbath, inactivation was 0% at 37°C, 40% at 42°C, 63% at 48°C, and 100% at 56°C. At 56°C, RT activity reached zero after 20 min (fig 1).

Addition of 50% human serum caused an inhibition of RT activity which was probably due to the high concentration of proteins present (unheated virus without serum, 59 000 cpm; virus kept at −4°C before RT determination in 50% human serum, 7632 cpm; virus kept at 56°C for 30 min in 50% human serum, 690 cpm). Table I shows, however, that 100% of LAV infectivity was inactivated by incubation in the presence of normal human serum at 56°C for 30 min.



●--●=control and virus irradiated with less than 10^3 rad; ○-·-·-○=virus irradiated with 10^3 rad; ■—■=virus irradiated with 2×10^3 rad; □——□=virus irradiated with $2\cdot5\times10^3$ rad.

Inactivation by Gamma and Ultraviolet Irradiation

Nucleic acids are radiosensitive, and inactivation of viruses by radiations is essentially based on the sensitivity of their nucleic acid to such physical agents whereas the viral proteins, such as RT, are relatively resistant.

The viral enzyme itself was unaffected by $7\cdot5\times10^5$ rad gamma irradiation (cobalt-60 source, 645 rad/min). Doses delivered to viral samples were increased from $2\cdot5\times10^3$ rad, and at less than 10^5 rad infectivity was not modified. When viral samples were exposed to more than 10^5 rad before infection of normal T cells, virus production was delayed, suggesting inactivation of an increasing amount of viral particles, and samples exposed to $2\cdot5\times10^5$ rad were non-infectious in our assay (fig 2).

LAV RT was slightly sensitive to UV radiations, the enzyme activity decreasing as a function of the delivered dose (table II). No infectious virus was detected in our infectivity assay in samples irradiated with more than 5×10^3 J/m^2.

Discussion

These results indicate that LAV resembles other retroviruses[4,5] in its sensitivity to heat. The data cannot, however, be extrapolated to lyophilised products since our experiments were conducted in liquid medium.

The virus was heat inactivated even in the presence of 50% human serum. As a safety measure in hospital routine laboratories, sera from patients with AIDS or AIDS-related complex could be heat inactivated before analysis because this treatment does not interfere with ELISA for LAV antibodies (F. Brun-Vézinet, C. Rouzioux, personal communication) or hepatitis B surface antigen. LAV also resembles other retroviruses in its radio resistance.[4-6] After exposure to less than $2\cdot5\times10^5$ rad gamma rays, the virus is still infectious for human lymphocytes. The amount of radiation used for foodstuffs and sera is generally at least 10 times lower than this.

It is noteworthy that LAV was not inactivated by ultraviolet radiation in doses much higher than those usually employed under laminar hoods, in operating theatres, or in laboratories.

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