Cyclosporin was given orally to one group of mice daily for 14 days at a dose of 100 mg/kg in olive oil, beginning 48 h before infection. A second control group of mice was dosed identically but was not infected. Serum and CSF drug levels were measured by radioimmunoassay. In our laboratory the assay can detect drug levels greater than 25 ng/ml. The serum levels shown in the figure are means for five mice sampled on each day. No significant drug levels were detected in the CSF of any of the mice in either group. The drug was effective peripherally since serum T-cell-dependent IgG antibody production was reduced or suppressed and the brain virus titers were consequent prolonged. The drug was not, however, effective within the CNS since an intense inflammatory response of at least the same severity as that normally seen after Semliki Forest virus infection was apparent in the drug treated mice by 7 days post-infection, with lesions of demyelination by the 9th day.

The inability of cyclosporin to cross the blood-brain barrier in significant quantities would seem to allow immunosuppressed lymphocytes recruited to the CNS from the circulation to escape the immunosuppressive effect of the drug. These results suggest that in the mouse cyclosporin does not cross the blood-brain barrier and does not prevent the Semliki Forest virus-induced immune response in the brain or the associated immune mediated demyelination. The failure to reduce the virus induced CNS inflammatory response in the mouse and the inability of cyclosporin to cross the blood-brain barrier in this species (and, probably, in man) should be taken into account if cyclosporin is to be used in the treatment of CNS inflammatory conditions such as multiple sclerosis.

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IMPACT OF NATIONWIDE ANTI-SMOKING CAMPAIGN

Sirs.-The Athens group (Sept 26, p. 712) may have been too optimistic about the impact of the Greek anti-smoking campaign. We have given too little information to judge whether the campaign was a decisive factor in reducing the annual rate of increase in tobacco consumption, and some information which suggests it may not have been.

Professor Dixitakis and colleagues report the relative prices of all commodities over time (CPB) and of tobacco (TPS), but no measure of purchasing power. If, under the government of the day, growth in incomes was restrained while prices rose, the public may have chosen to buy less tobacco. Income restraint may have been a reason why the government was defeated at the ensuing election. Faced with a discontented electorate a retiring government often boosts incomes before an election in the hope of regaining electoral favour. This might explain the upsurge in the rate of increase of tobacco consumption in pre-election, post-campaign Greece.

In interpreting the possible association of the campaign with tobacco consumption it would have helped to define tobacco consumption, particularly since whole-year statistics are used whereas policy changes were introduced mid-year. Was it in terms of retail sales or of products leaving warehouses? The lag-time in effects on these two indices may be appreciably different.

The graph of trends in growth of consumption has been inadequately explained. The reduction in growth between 1982 and 1985, when the campaign had been started, appears just as impressive as the effect between 1978 and 1979, the first year of the full-scale campaign. How is that to be explained? Also, the full-scale campaign continued to within 2-3 months of the end of 1981, but the increase in tobacco consumption in 1981 was as high as it had been in the pre-campaign period.

A measure of the "effectiveness" of the campaign was that 10-5% of those smoked regularly at the start of the campaign said they had given up by the end of spring, 1981. We are given no information about the drop-out rate from regular smoking in non-campaign years. Neither are we given any information about changes in the rate of recruiting new smokers or before or during the campaign year.

The paper does not claim a campaign effect on nofice levels of smoking. We need more data, however, before we can agree that there was a reduction even in the growth of tobacco use because of the campaign.

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CASE FOR CONCLUDING THAT HEAT-TREATED, LICENSED ANTI-HAEMOPHILIC FACTOR IS FREE FROM HTLV-III

Sirs.-LAV/HIII-III virus has been grown in vitro in 196 cells at concentrations of 10³ particles/mL of cell culture fluid. In limiting dilution experiments, such cell culture fluids in quantities as low as 1 ml of a 10⁻⁴ dilution will establish an infective dose of 3% of the cell cultures. (The Poisson distribution predicts that when an infectious dose (ID₅₀) is added to a series of cell cultures, 6.5% of them will be positive.)

Data available from over half a million samples tested in the United States up to April, 1985, suggest that about 0-2% of random blood and plasma donors are positive (repeatedly reactive) for antibody to LAV/HIII-III when tested with the ELISA assays currently being marketed. None also assumes that there are no false-positives among the 0-2% of donors with antibody to LAV/HIII-III and that everyone with antibody is virulent to the same degree as in optimised in-vitro systems, then 0-2% of the units in plasma pools used to produce antihemophilic factor (AHF) would contain LAV/HIII-III with 10⁵ particles/mL or 10¹⁰ ID₅₀. This is a worse-than-real situation because the proportion of false-positives is expected to be about two-thirds for currently licensed ELISA tests for antibodies to LAV/HIII-III when the prevalence of infection in the population being tested is 0-1%, and only 2 of 9 (22%) antibody-positive specimens from blood donors were positive for infectious LAV/HIII-III when tested in our laboratory. Large plasma pools will have a false average virus concentration of 2000 ID₅₀ per 500 dilution of the infectious units without non-infectious units.

Although a loss in titre of retrovirus has been reported in the preparation of AHF from plasma, it is possible that virus could be concentrated along with AHF during cryoprecipitation as well as in the later manufacturing steps. The yield of AHF may be calcu-
30% of the amount present in the starting material, so AHF activity in commercial products is concentrated about 100-fold relative to the original plasma protein concentration. If viral concentration without loss of infectivity were to occur along with concentration of AISE, then the AHF would contain 2000 ID50/100 (2 × 10^10 ID/ml).

Any treatment of AHF which is aimed at reducing the risk of transmission of LAV/HTLV-III should therefore be capable of inactivating 2 × 10^10 ID/ml of the virus. All of the above assumptions were used to calculate the amount of LAV/HTLV-III that might be present in AHF that were chosen in a worst-case situation, and a figure of 2 × 10^10 ID/ml therefore represents an overestimate since at this concentration and greater, one should easily be able to isolate the LAV/HTLV-III virus using current cell culture techniques. To date no one has reported success in isolating LAV/HTLV-III virus from commercial AHF.

Inactivation procedures which can be shown to reduce LAV/HTLV-III infectivity by 5 logs should therefore provide assurance that the preparation is no longer infectious. Heat inactivation kinetics data show that when LAV/HTLV-III is added to liquid AHF and the mixture is heated at 65°C, one log is inactivated in about half a minute.4 Lympholized AHF requires about 30 min of heating at 65°C for the inactivation of one log of added LAV/HTLV-III.4 Since the minimum temperature and time period required by commercial manufacturers of AHS are 60°C and 30 min, respectively, the extrapolated minimum reduction of virus would be 20 logs.

Even after taking into consideration the administration of doses of AHF as large as 100 ml, and the possibilities of non-linear inactivation kinetics at very low virus concentration and a human ID substantially smaller than that required for in-vitro assays, there still seems to be enough of a safety factor afforded by AHS to the best of our knowledge to permit the conclusion that infectious LAV/HTLV-III is unlikely to present in currently licensed heat-treated AHS and that these products should not result in infections, in addition to any possible AIDS in persons with hemophilia. LAV/HTLV-III is much more stable than other viruses, and efforts to improve virus inactivation procedures should continue, in cooperation with studies aimed at stabilizing and inactivating viruses in plasma derivatives.

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