virulence or decreased stability, or both, might be easily selected in an in vitro system. The use of such mutant populations could reduce the danger of airborne contamination of laboratory workers, experimental animals, and other viral or cell culture materials. Venezuelan equine encephalomyelitis virus, another arbovirus, has been shown to lose its virulence in vivo as a result of its serial passage in vitro (3, 4). The question of whether this applies to other arboviruses can be determined only after an adequate number of suitable tests have been performed.

LITERATURE CITED

Discussion

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Your supposition that a Dutch bacteriologist would have more experience of yellow fields of tulips than of yellow fever is absolutely correct. It is thus with great respect that I have read the careful and laborious experiments of Dr. Hearn on which he certainly is to be complimented.

Dr. Hearn described variations in yellow fever virus after passage in HeLa cells: (i) variation in growth rate, growth capacity, and the appearance of cytopathic effects; (ii) attenuation of virulence for monkeys; and (iii) variation in aerosol stability. These correlations are obvious and important. The HeLa cultures were passed at high multiplicity so that mixed populations were studied. Consequently, the bulk of the particles in the atypical first passage (aTy 1) might well have lost the lethality for monkeys, but the population might still contain a few per cent of virulent particles. Again, the difference between the Ty 3 and aTy 3 might be due to interference or a von Magnus phenomenon. Thus, though the populations seem unstable, the variants might be quite stable genetically. Admittedly, yellow fever virology is very difficult, but, unless these variants are isolated from single plaques or passed at limiting dilutions, it is difficult to discuss these variations in terms of genetic markers. I sincerely hope that Dr. Hearn will find opportunity in the future to work in this direction.

The aerosol work is again of the highest level.
The monkeys were exposed to aerosols of different ages. The dose was expressed in terms of MICLD_{50}. (Let us hope this unit is constant before and after HeLa passage or aerosolization.) This means that, especially at lower doses, the monkeys receive a few viable particles and very many inactivated particles. Whether these inactivated particles still contain active ribonucleic acid is not known. It might well be then that the large number of inactivated particles in these experiments produced some kind of interference. The additional attenuation by aerosolization itself (Table 5) might also point in this direction. It would be interesting to know what happened when monkeys were exposed to various doses of aerosols of the same age.

All this, of course, detracts nothing from the fact that an important step has been taken in the direction of immunization with an avirulent yellow fever virus. In this connection, it would be important to know whether this virus could multiply in mosquitoes.