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TITLE: DEFECTIVE VIRUS RNA IN HALOTHANE EXPOSED CELLS

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Introduction. Previously we have reported the inhibition of measles virus replication in tissue culture cells exposed to halothane. Replication of the virus is inhibited for the duration of the halothane exposure, however, when the anesthetic is removed, replication apparently proceeds from the point of the blockage.[1] Similar results occur using enflurane treated cells. Subsequently we have analyzed the virus RNA produced in cells which received previous halothane or enflurane treatment and compared this RNA to identical control cultures never exposed to an anesthetic. Significant changes in the virus RNA were observed in halothane treated cultures only. These results are described below.

Methods. Measles virus was propagated in vero cells, a continuous primary cell line as described previously.[2] Briefly, the cells were grown in sealed glass bottles, and cultures were exposed to 95% air-5% CO_2 with or without 2% halothane or 4.4% enflurane. Anesthetic vapor concentrations were assessed by gas chromatography; anesthetic loss from the bottles was less than 5% over a 24 hr. period. Cultures were innoculated with virus and allowed to incubate for one hour prior to the exposure of the cells to the anesthetic. The cell cultures were then exposed to actinomycin D and (5-^{3}H)uridine label 24 hrs. after infection. 40 hrs. following infection the cultures were harvested and a cytoplasmic extract was prepared first by swelling the infected cells in a hypotonic buffer followed by disruption with a Dounce homogenizer. Cell nuclei and debris were removed by centrifugation and labeled structures in the decanted cytoplasmic extracts were separated by rate zonal centrifugation through sucrose gradients. Gradients were fractionated and radioactivity incorporation into RNA was determined by liquid scintillation counting.

Results. The molecular weight of measles virus RNA is 6.2×10^6 daltons and it possesses a sedimentation coefficient through sucrose of 50 S. As figure 1 demonstrates, full length RNA was recovered from the labeled RNA of measles virus grown in vero cells not exposed to halothane. Similar results appeared in enflurane treated cells. However, when virus replication occurred following the removal of 2% halothane the RNAs species observed were substantially reduced in size. Pretreatment of the virus with 2% halothane before infection had no effect on the RNA products synthesized. Thus, halothane appears to block cellular ability to process mature virus particles, rather than acting directly on the virus particle.

Discussion. We observed that although virus infectivity rebounds following the removal of inhibiting doses of either anesthetic, enflurane or halothane, the newly synthesized virus particles from the halothane exposed cell cultures showed a preponderance of defective types. These defective particles possess a virus genome which is substantially smaller than those of the normal virus. Clinically defective virus particles of this kind have been implicated in a number of progressive viral diseases.[3] Since treatment of the virus with halothane prior to infection has no effect on defective particle production, it appears that the anesthetic may alter the cells ability to assemble intact virus particles properly. This may be due to the fact that some cellular component necessary for proper virus processing requires additional time for recovery or repair following the anesthetic removal. The present study suggests that some step in measles virus replication is effected by treatment of the cells with halothane during the course of acute infection.

References.

1. Knight PR, Nahrwold ML, Bedows E: Anesthetic action and virus replication. Replication of measles virus in cells exposed to halothane. Antimicrob Agents Chemother. May 1980, In press.
2. Bedows E, Knight PR, Nahrwold ML, Cohen PJ: Halothane alterations in viral RNA-protein synthesis. (Abstr) Am Soc Anesth Annual Meeting 1979, pg.S57.
3. Huang A, Baltimore D: Defective viral particles and viral disease processes. Nature (London) 226:325-327, 1970.

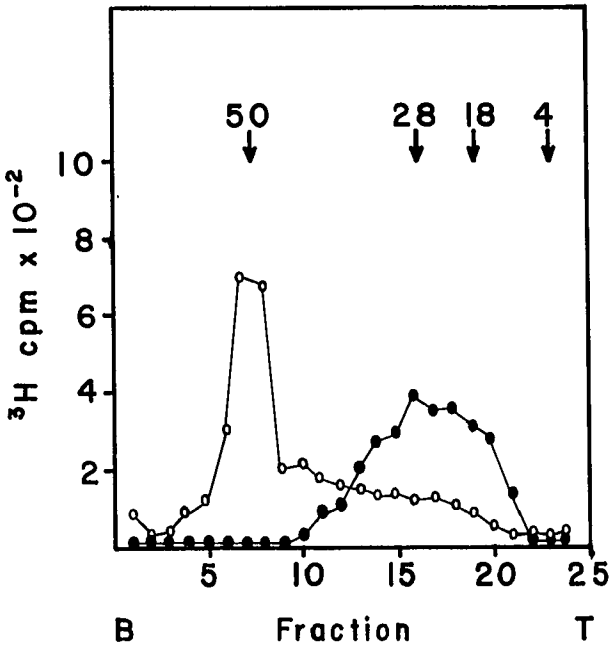

Figure 1. RNA profile of measles virus RNA isolated from cells which were treated with (o) or without (o) 2.0% halothane. Profiles were determined as described in Methods. The bottom of the gradient (B) is to the left and 50 S, 28 S, 18 S and 4 S size markers are indicated by the arrows.