McCrady, W. B. The University of Texas, Arlington, Texas. Injection of concentrated extract from delayed recovery D. melanogaster.

Previous work (McCrady and Sulerud, 1964, Genetics 50:509-526) has shown that it is possible, although difficult, to transmit CO_2-sensitivity to resistant flies by injection of cell free extracts prepared from Texas Delayed Recovery (TDR) Drosophila melanogaster. TDR flies are known to be homozygous for gene Dly which is the primary factor responsible for the delay in recovery from CO_2 exposure of these strains. However, the above mentioned transmission of CO_2-sensitivity suggests an infectious agent which is suspected of being causally related to gene Dly. The poor success of transmission of CO_2-sensitivity attained in many injection series suggests a very low titer of infectious material within TDR flies. In attempting to determine if low titer is responsible for the lack of success of CO_2-sensitivity transmission, concentration of TDR extracts by high-speed centrifugation prior to injection was accomplished in several experiments. In each experiment several hundred TDR flies were crushed in 0.25M sucrose (200-250 flies/ml) buffered at pH 7.5 with Tris-HCl. Centrifugation of the crude extract at 1200XG for 15 minutes was followed by centrifugation of the supernatant at 6000XG for 10 minutes. The supernatant was then centrifuged at 40000XG for one to one and one-half hours with all centrifugation being completed in a Sorvall Superspeed RC2-B automatic refrigerated centrifuge at 0-3°C. The pellet was resuspended in a small volume of sucrose solution and then injected into resistant Oregon D. melanogaster.

All control flies injected with CO_2-sensitive fly extracts prepared in the above manner became CO_2 sensitive while none of those injected with plain sucrose solutions or heat-treated TDR extracts did. While control flies gave the expected results, the CO_2 response of flies injected with the concentrated TDR extracts was often not clear cut in a manner similar to the situation encountered when non-concentrated TDR extract is injected. Most TDR-injected flies did not develop classical CO_2-sensitivity, however in all three injection series some sensitivity was induced with death of the affected flies following CO_2 exposure. For example, in one test 13 of 54 injected flies did not recover within 15 minutes after CO_2 exposure completed 31 days after the injection. Some recovery occurred later, but 8 flies died within 24 hours. In most cases there were some flies which recovered slowly in a manner suggestive of true delayed-recovery. Other evidence supporting the conclusion that some transmissible agent is involved appears when comparisons of control and test flies which survived CO_2 exposures are made. Flies injected with TDR extracts were consistently weaker after CO_2 treatment than those injected with sucrose or TDR heat-treated extracts and were much less likely to survive for any given length of time after exposure. Following each CO_2 exposure many of the weakened flies of the TDR-injected test groups became stuck when returned to culture vials and died while few of the controls did. One striking example was the third injection series. After 45 days the 18 living control flies of a total of 26 injected with TDR heat-treated extract were quite healthy and active while only 13 of 108 TDR-injected flies of the test group were still alive and all were weak and inactive. The difference in survival percentages (69% survival for controls to 12% survival for the test group) as well as the observable differences in general health of the two groups indicates a difference in the two extracts injected. The fact that the two extracts were the same except for heat treatment prior to injection into the control flies must mean that the different responses are related to some heat-liable transmissible substance in the TDR extracts.

One conclusion which can be drawn from these observations is that the concentrated TDR extracts contain an infectious agent, not necessarily in small quantities, but essentially of a "weak" nature which results in chronic detrimental effects following CO_2 exposure that eventually kill most of the flies injected with such extracts. The differences between flies injected with TDR extracts and flies injected with extracts from CO_2-sensitive flies could be the result of a less virulent form of virus σ which normally kills infected flies immediately after CO_2 exposure. The possibility of a mutant form of σ in TDR flies may be related in some unknown way to the possible integration of the virus in the Drosophila chromosome as suggested by the presence of gene Dly.