freshwater mollusk species have populations which differ significantly from each other in the extent to which they *do* concentrate calcium from the environment. Secondly, this example of variation in calcium metabolism between different populations of a single species of freshwater snail can be added to the other types of physiological variation already known for freshwater mollusks, which include aspects of respiration (*2, 3, 5* and references therein), patterns of shell growth (*17*), and cycles of growth and reproduction (*1, 18* and references therein). The fundamental significance of such extensive physiological variability has been related to the temporal and spatial nature of the freshwater environment (*1, 3*). It can be claimed that the short-term, small-scale genetic isolation which occurs is not only responsible for the extensive interpopulation infraspecific diversity (with little full speciation) but also for the existence of much adaptive plasticity in several aspects of the physiology of such freshwater animals (*1–3*).

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Mutations, Chromosomal Aberrations, and Tumors in Insects Treated with Oncogenic Virus

Abstract. *An increased incidence of lethal mutations, visible mutations, chromosomal losses, chromosomal nondisjunctions, and tumors resulted when drosophila were placed in medium containing Rous-sarcoma virus. In the group treated, a few mosaics of the eye and translocations appeared as well. There is a suggestion, from preliminary data, that the size of chromosomal puffs may be reduced by this RNA virus, but the difference is not significant. Tests for the persistence of virus in progeny have been negative so far.*

The Bryan (*1*) high-titer strain of Rous-sarcoma virus containing Rous-associated virus 2 was mixed with yeast and added to the surface of medium on which 2-day-old *Drosophila melanogaster* larvae ($sc^8.Y.B^8/y^3w^1ct^6f^1$) were feeding. Two different concentrations of virus were used—0.2 ml of undiluted material and the same volume of a 50-fold dilution of virus in drosophila Ringer's solution without calcium. We checked the activity of virus by determining the production within 10 days of characteristic sarcomas in chicks after they had been subcutaneously injected. Germ cells of male flies remaining on the virus-yeasted food throughout larval, pupal, and imaginal stages of development were tested for mutations and chromosomal abnormalities by methods (*2*) adapted from Muller and Muller and Oster.

Table 1. Production of tumors in drosophila by Rous-sarcoma virus (RSV) in dilutions of 1:1 and 1:50.

Treatment	Animals with tumors (%)	Dead animals (%)	Total studied (No.)
RSV 1: 1	4.9	30.4	491
RSV 1:50	8.7	29.4	1245
Control	0.4	2.8	508

The mortality was moderately high among those larvae treated, and some of the larvae and pupae exhibited black, pigmented bodies (*3*) adjacent to the gut (Table 1). The largest were in the pupae and reached a size equal to three-quarters of the diameter of the body. None of the individuals with these tumors survived. Melanization of pericardial cells were found in progeny of flies exposed to Rous virus (*4*).

An increased rate of lethal mutations on the X chromosome was found when males were exposed to Rous virus in the medium and tested by the Muller-5 method (Table 2). Increased nondisjunction and losses of the Y chromosome, along with more numerous visible mutations, were found when tested

Table 2. Mutations, mosaics, and chromosomal abnormalities in drosophila treated with Rous-sarcoma virus (RSV) in dilutions of 1 : 1 and 1 : 50.

Aberrations	Treated with RSV 1:1		Treated with RSV 1:50		Untreated	
	Total studied (No.)	With aberration (%)	Total studied (No.)	With aberration (%)	Total studied (No.)	With aberration (%)
Loss of Y	1181	0.01	4442	0.27	711	0.0
Nondisjunction	3191	0.38	10960	0.61	2473	0.11
Mosaics (eye)	3191	0.00	10960	0.03	2743	0.0
Visible mutations	3191	0.13	10960	0.12	2743	0.0
Lethal mutations	426	3.29	1083	2.87	648	0.0
Translocations	245	0.00	794	0.63	202	0.0

Table 3. Effect of Rous-sarcoma virus (RSV) on mean size of chromosomal puffs.

Puff	Control			Treated with RSV		
	Number measured	Size (μ)	Ratio*	Number measured	Size (μ)	Ratio*
X:2-B	20	8.3	1.48	25	7.6	1.36
III L:71	16	6.4	1.43	14	5.9	1.28
III L:72	16	5.9	1.28	15	5.3	1.19
III L:74	19	7.4	1.44	19	5.9	1.23
III L:75	19	7.3	1.41	17	5.6	1.24

* Ratio of puff to adjacent band.

by matings of the treated males ($sc^8.Y.B^8/y^2w^ict^6f^1$) to $Y\ sc^{81}\ In49\ sc^8;\ dp\ bw;\ st\ p^P$ females (Table 2). The absence of one wing or leg among the visible mutants was especially striking. Also a few eye mosaics were observed, suggesting that somatic abnormalities occurred concomitantly with the germinal havoc detected. Only a few translocations were recovered.

Comparison of the mean transverse diameter of puffs (*5*) X:2-B; III L:71; III L:72; III L:74; and III L:75 of treated and untreated chromosomes showed possible diminution in size resulting from treatment with this RNA virus (Table 3). However, these differences in preliminary results are not significant. Inoculation, into chicks, of material extracted from progeny of flies exposed to Rous virus so far has failed to elicit avian tumors.

Several reports of induction of tumors in mammalian tissue by this virus (originally discovered to affect fowls) have appeared (*6*). Our studies offer proof of more distant transgeneric effects of Rous virus. The well-known role of insects as vectors of disease and the possibilities offered by use of drosophila in studies on the mode of action of oncogenic and other viruses in tumorigenesis and mutagenesis are thought to be of general interest. The analytical methodology available with drosophila suggests that study of the behavior of viruses, helpers, and hybrids (*7*) in metazoan cells may be approached quantitatively and more precisely now that an oncogenic virus is known to interact with the dipteran genome.

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Seizure Discharges Evoked in Vitro in Thin Section from Guinea Pig Hippocampus

Abstract. *A thin section prepared from guinea pig hippocampus produced, in chloride-free medium, a train of seizure discharges in response to a single shock applied to the section. Generation of these discharges was ascribed to the lack of inhibitory processes in an absence of chloride ion.*

Eccles and his associates demonstrated that the inhibitory postsynaptic potential (IPSP) in the spinal motoneuron was brought about by an increase in permeability of the membrane to chloride ion, and the same ionic mechanism has been found to play a main role in generating IPSP's in the higher centers as well (*1*). It is deduced, therefore, that if chloride ion is removed from the extracellular space of the brain, the IPSP's are either changed to the depolarizing potentials or abolished (*2*). Thus, because of a lack of inhibitory processes, the brain neuronal network may be brought into such a highly excited state that it tends to generate the seizure discharges. Although this is an interesting surmise, it has not been tested because it is not possible to remove chloride ion completely from the extracellular space in the in vivo experiments. Recently, it has been found that mammalian brain tissue can exhibit electrical activity even if it is excised from the brain and maintained in a chemically defined medium (*3*). Since in this experimental situation chloride ion in the extracellular space can be readily removed, it is possible to test whether the seizure discharges are generated in an absence of chloride ion. Actually, in our experiments, which were carried out on a section from the guinea pig hippocampus, a single shock applied to the slice evoked a train of seizure discharges in the chloride-free medium.

After stunning the guinea pig by a blow on the back of the neck, the brain was removed and divided sagitally along the midline. The surface of the hippocampus facing the thalamus was exposed by removing the brainstem. A slice 0.35 mm thick was prepared from the exposed portion of the hippocampus with a razor blade and a slicing guide, in the same way in which a slice from the cerebral cortex is usually made (*4*). In Fig. 1B, the approximate portion of the hippocampus from which the slice was obtained is schematically shown by the dotted line. The slice was unfolded on nylon mesh with its cut surface upward; it was incubated at 37°C in glucose-saline medium saturated with

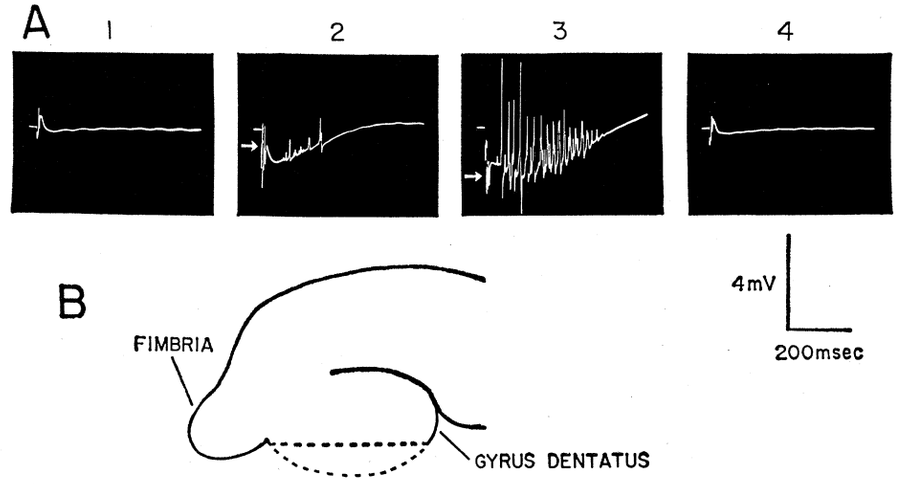

Fig. 1. Generation of seizure discharge in chloride-free (propionate) medium. (A-1) Recorded in normal medium; (A-2) 8 minutes after changing the solution to medium containing chloride at a concentration of 13 m*M*; (A-3) 8 minutes after immersing the slice in chloride-free medium; and (A-4) after returning the slice to normal medium. Note that the seizure discharge in record 2 is much smaller than it is in record 3. Arrows in records 2 and 3 indicate sharp deflections evoked with a short latency. (B) Approximate portion of the hippocampus from which the slice was taken is shown schematically by dotted line.