whenever the compounds of the group V, VI and VII elements act as Lewis bases, π-bonding should be an important factor in the base strength of these compounds.

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12 December 1956

Distribution of Calcium in Adult Drosophila melanogaster

It has been shown by Levine (*1*) that the amount of crossing over observed in females of *Drosophila melanogaster* can be decreased by feeding adults on medium containing increased amounts of calcium. Similar effects are produced if the females are desiccated during pupal life. On the other hand, crossing over is increased if the females are fed during larval life on a medium containing the chelating agent, ethylenediaminetetraacetic acid. To explain these results, Levine postulated that calcium normally plays a role in the nuclei of *D. melanogaster* and that this role is to "stabilize" the chromosomes. Therefore, if the cellular calcium concentration is increased by feeding the fly extra calcium or by decreasing the water content of the cells by desiccation, the chromosomes are "stabilized" and show less crossing over. Conversely, if the calcium content is decreased by administering a chelating agent, then the chromosomes become "unstable" and show increased amounts of crossing over.

However, it is hard to reconcile this theory with certain information that is available concerning the calcium requirements of *D. melanogaster*. It is known, for example, that if calcium is required by *D. melanogaster* at all, it is necessary only in minute quantities (*2*). Furthermore, Yasuzumi and Sawada (*3*) have shown that, whereas calcium is present in the cytoplasm of the larval salivary gland cells of *D. virilis*, it is apparently absent in the chromosomes, and Poulson and Bowen (*4*), utilizing radiocalcium, found no evidence for nuclear localization of calcium in larvae of *D. repleta*. In addition, Poulson and Bowen state that "the rapid transfer of the element to storage areas of the malpighian tubules contraindicates the existence of any major calcium component in tissues generally."

The results of our autoradiographic studies (*5*) of Ca^{45}-localization in adult *D. melanogaster* are in complete agreement with the statement of Poulson and Bowen. Flies of the Oregon-R strain were fed during the larval and adult stages on *Saccharomyces cerevisiae* homogeneously labeled with Ca^{45}. Autoradiograms of adults showed Ca^{45} to be homogeneously distributed in the blood and tissue fluids and concentrated only in the terminal portions of the anterior malpighian tubules. Developed grains were equally abundant whether above the nuclei or the cytoplasm of the cells making up various tissues. There was no concentration of calcium in oocyte nuclei or sperm heads. The calcium content of the adults was 165 ppm. Males and females did not differ significantly with respect to the distribution of calcium. The distribution of calcium from yeast ingested during adult and larval stages was as follows: head, 0.131; thorax, 0.141; legs, 0.051; wings, 0.024; gut, 0.084; reproductive system, 0.037; malpighian tubules, 0.109; abdominal residue, 0.066; and liquid residue (mainly hemolymph), 0.357 (for adult flies 0 to 1 day old).

We conclude (i) that *Drosophila melanogaster* requires at most trace amounts of calcium, (ii) that the majority of the calcium taken in is rapidly transferred to and stored in the excretory organ of the insect, and (iii) that calcium is not required as a component of chromosomes in concentrations higher than those found in the cytoplasm and body fluids.

It follows that, if calcium plays a role in "stabilizing" the chromosomes of *D. melanogaster*, then extremely small amounts are required. It is difficult to see under these conditions why adding more calcium to the standard medium should have any effect. It also seems unlikely that ethylenediaminetetraacetic acid can reduce the available calcium in the standard medium below the traces in which it is required.

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Purification of Poliovirus with Fluorocarbon

When we were searching for a procedure for removing protein from crude viral suspensions, the report of Gessler *et al.* (*1*) came to our attention. Gessler *et al.* have described a procedure for the purification of vaccinia and Rous sarcoma virus in which infected tissues are homogenized in a high speed blender with a fluorocarbon mixture. On separation of the two phases, it was found that nonviral protein was removed from the aqueous layer, whereas the virus remained in it. This communication (*2*) reports results following the application of a similar procedure to the purification of poliomyelitis virus, type II, strain MEF-1, that was grown in a culture of HeLa cells.

The medium used for propagation of

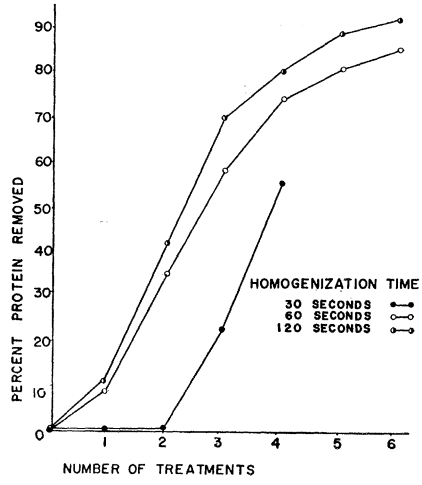

Fig. 1. Protein removal by fluorocarbon treatment.

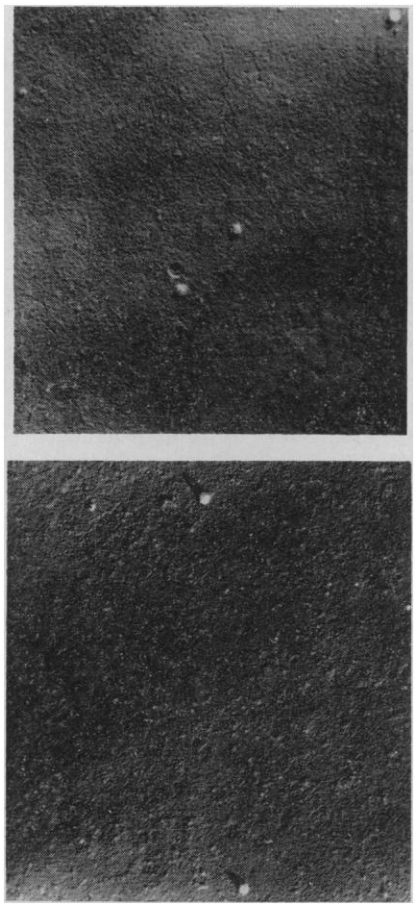

Fig. 2. (Top) Crude suspension of poliovirus, type II, strain MEF-1 (× 18,400). (Bottom) Poliovirus, type II, strain MEF-1, purified by one fluorocarbon treatment (× 18,400).

the host tissue was Eagle's basal medium (*3*) to which human serum was added to a final concentration of 10 percent. The following method was employed in the purification procedure. All operations with infectious materials were carried out in a hood equipped with an exhaust system. Freon 112, 1,2 difluorotetrachloroethane (*4*), was dissolved in *n*-heptane to yield a solution with a specific gravity of 1.30. Equal volumes of aqueous virus suspension and fluorocarbon solutions, both cooled to 4°C, were then blended at top speed (14,500 rev/min) in a Serv-all Omnimixer. The blending chamber was immersed in an ice bath during the homogenization. The chamber was then allowed to stand undisturbed for 10 minutes to allow any aerosols that might have developed to settle. The homogenate was centrifuged for 10 minutes at 1000*g* and was found to have separated into three layers. Uppermost was a clear aqueous layer containing the virus; at the bottom was the clear fluorocarbon mixture; the protein accumulated at the interphase.

It is possible to remove 90 percent of the protein by repeatedly treating a sample of growth medium with the fluorocarbon mixture. In Fig. 1 are shown the effects of repeated homogenizations, as well as the effects of various times of homogenization, on the protein content of the aqueous phase. Protein was determined by the method of Lowry *et al.* (*5*). Since homogenization for as long as 10 minutes did not increase the efficiency of a single extraction, blending for 1 to 2 minutes was adopted as a routine procedure. However, an increase in the ratio of fluorocarbon mixture to aqueous suspension did increase the efficiency of extraction in one instance: in this experiment, a ratio of 10/1 was used, and almost 50 percent of the protein was removed in one step.

The residual concentrations of infectious virus were determined after successive fluorocarbon treatments had increased the purity of the viral material. In one typical experiment, 200 ml of crude poliovirus suspension containing $10^{6.50}$ tissue-culture infectious doses (*6*) were subjected to six successive fluorocarbon treatments. A sample of the supernatant fluid from each treatment was assayed for viral content. Tenfold serial dilutions were made in growth medium in which calf serum was substituted for human serum. One-milliliter aliquots of each dilution were inoculated into each of four tubes containing HeLa cells. After three days' incubation at 37°C, the presence or absence of virus was determined by observing whether or not the sheet of cells had disintegrated. The supernatant fluids obtained after each of the six successive treatments titered $10^{6.25}$, $10^{6.50}$, $10^{6.25}$, $10^{5.75}$ $10^{4.75}$, and $10^{4.75}$, respectively. Thus there was no significant decrease in titer until after three successive treatments.

Electron photomicrographs (*7*) of the supernatant fluids of cultures of strain HeLa cells that were infected with poliovirus before and after one fluorocarbon treatment (Fig. 2) show that one such treatment removes nonviral components to such a degree that viral particles can be readily distinguished.

This purification procedure was subsequently applied successfully to other viral suspensions; coliphage T5; poliovirus, types 1 and 3; feline pneumonitis virus; and certain members of the adenovirus group. By the same means, Hummeler and Hamparian (*8*), report that anticomplementary activity and host antigens are removed from poliomyelitis antigens derived from tissue culture. Werner (*9*) reports the purification of some adenovirus types by the fluorocarbon method. The foregoing method seems to have great applicability and shows promise of providing a relatively simple way of removing protein from viral suspensions without materially affecting the virus particle. Further work to assess the scope of usefulness of this procedure is being carried out in our laboratory (*10*).

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Removal of Anticomplementary Activity and Host Antigens from Viral Preparations by Fluorocarbon

Complement-fixing antigens derived from tissue cultures of HeLa or monkey renal cells infected with poliomyelitis or other viruses may reveal anticomplementary activity that renders them unsuitable. Since such preparations also regularly contain host materials, the specific reactions can be analyzed and standardized only with serums free of antibodies to host antigens. Gessler *et al.* (*1*) recently described a simple method of segregating virus from tissue homogenates by deproteinization with fluorocarbons. The virus particles remain in the aqueous phase, while proteins are retained in the organic phase. This technique has been applied successfully to removal of anticomplementary activity and of host antigens from infected tissue-culture preparations, without impairment of specific reactions (*2*).

Fluorocarbon Freon 112 (*3*) was adjusted to a specific gravity of 1.30 by admixture of *n*-heptane (*1*). Bottle cultures of HeLa or monkey renal cells infected with poliomyelitis viruses were harvested when the cell-sheets were completely destroyed. The culture fluids were treated without prior removal of the cell debris. Initially, 1 part of fluorocarbon-