of the influenza viruses. One *Staphylococcus aureus* and one *Aerobacter aerogenes* were also unaffected by any of the viruses. The reason for the refractoriness of some bacterial species and strains as well as the underlying mechanism responsible for the ability of viruses to effectuate bacterial inhibition remains to be elucidated.

This investigation was supported by U.S. National Science Foundation grant GB-2505.

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Further Simplification of the Purification Procedure of Influenza Virus on Barium Sulphate

A METHOD for the purification of influenza virus by adsorption to and elution from BaSO₄ with subsequent ultracentrifugation of the eluate has been published by Mizutani¹. We have found that this method can be further simplified by separation of the citrate (necessary for elution but interfering with adsorption) in the form of its calcium salt after elution from BaSO₄ has been achieved. By this step the ultracentrifugation of the eluate is eliminated and repeated purification cycles on the BaSO₄ can be performed.

Allantoic fluid of eggs was infected with PR8, Singapore, and Lee strains of influenza virus maintained in this Institute. CCA was established according to Salk², and infectivity by titrating serial dilutions of the preparations in embryonated eggs; N was determined by the Kjeldahl method as modified by Schulek³. In the adsorption stage the prescriptions of Mizutani were strictly adhered to but the time needed for adsorption was reduced to 1 h after it had been confirmed that during this period the virus content in the supernatant did not decrease, that is, the adsorption to BaSO₄ was complete. CaCl₂ solution, in quantities equivalent to the citrate content, was added to the eluate, the pH of which was adjusted to 7.2. After standing for 1 h in a refrigerator at 4° C Ca-citrate crystals separated. According to confirmatory tests these did not adsorb the virus. After sedimentation of the crystalline material by centrifugation a second cycle of adsorption to and elution from BaSO₄ could easily be carried out in the supernatant. The results are shown in Table 1.

It can be seen that in the case of the PR8 strain the results are similar to those of Mizutani. With the other two strains, however, the rate of recovery and the effi-

ency of purification were considerably lower. Mizutani's findings also indicate that the CCA/N value of strain 'Adachi' is five times greater than that of strain 'Setagaya'. Obviously differences concerning adsorption to and elution from BaSO₄ do exist between different strains. The data presented in Table 1, however, reveal that these differences are likely to originate from the different adsorptive capacity of the various strains to BaSO₄. Thus adsorption to and elution from BaSO₄ show a similar pattern to the adsorption to and elution from Al(PO₄) used by Miller and Schlesinger⁴. Some damaging effects on the adsorption of the yet unknown ballast substances in the allantoic fluid were also noted. There is no doubt that the peculiarities of the behaviour toward an adsorbent are determined by the surface of the viral particles, and might be changed by its variations.

The N/CCA values of the PR8 strain shown in Table 1, representing the efficiency of the purification process, compare favourably with the corresponding values found by Miller with his purest influenza virus preparations without substantial loss of infectivity. In view of the simplicity, effectiveness and technological advantages of autoclaving the adsorbent and eluent, further investigation of some other factors, such as pH, ionic strength, etc., influencing the adsorption to and elution from BaSO₄ of the various influenza virus strains seems to be justified.

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CYTOLOGY

Chloroplast Replication: Evidence for 5-Bromouracil Incorporation and Plastid Mutation in *Euglena gracilis*

THERE is now growing experimental support for the hypothesis that nucleoprotein genetic determinants located outside the nucleus confer photosynthetic activity on plastids. The first clear indication of nucleoprotein involvement in plastid mutation was derived from the action spectrum for ultra-violet inactivation of *Euglena* plastids which showed peaks at 260 and 280 mμ¹. This ultra-violet sensitive plastid determinant is photoreactivable², and evidence for its cytoplasmic nature has been reported¹⁻³. Comparison of wild-type and ultra-violet-induced mutants of *Euglena* strongly suggests that a DNA of different base composition from the nucleus is uniquely associated with the ability to form plastids⁴. Similar findings have been reported for plastids of other algae⁴⁻⁶ and higher plants^{6,7}. The recent observation¹⁰ that cells which incorporate azathymine in place of thymine exhibit increased resistance to ultra-violet inactivation of plastids is consistent with the reports that plastids have associated with them a specific DNA, and serves as a guide to the rational search for chemical agents that might be expected to induce plastid mutations. By analogy with other nucleoprotein genetic systems, we have postulated that incorporation of base analogues into the genetic material that determines plastids should lead to mutational changes, and that such changes would be reflected in altered plastid structure and function. This communication presents evidence for incorporation of 5-bromouracil into cells of *Euglena gracilis*, and describes conditions for obtaining stable colourless plastid mutants during replication of plastid

Table 1

Strain		Haemagglutinating units (HU)	Recovery virus (%)	N (mg)	HU/mg N	mg N/HU	Concentration
PR8	1	102.400	—	30.3	3,413	2.9 × 10 ⁻⁴	—
	2	80.0	0.8	29.5	27.1	3.7 × 10 ⁻⁵	—
	3	61.440	59.5	3.87	16,742	6.0 × 10 ⁻⁵	—
	4	0	—	1.28	0	0	—
	5	92.160	90.0	0.70	131,660	7.6 × 10 ⁻⁵	38.5
Singapore	1	51.200	—	31.3	1,610	6.2 × 10 ⁻⁴	—
	2	3,000	5.9	24.8	129	7.2 × 10 ⁻⁵	—
	3	15.360	29.9	3.79	4,053	2.5 × 10 ⁻⁴	—
	4	600	1.17	1.60	355	2.8 × 10 ⁻³	—
	5	11.520	22.6	1.49	7,732	1.3 × 10 ⁻⁴	4.8
Lee	1	12.800	—	36.9	346	2.9 × 10 ⁻³	—
	2	1.600	12.5	25.8	62	1.6 × 10 ⁻²	—
	3	3.840	30.0	4.5	853	1.2 × 10 ⁻³	—
	4	600	4.68	2.44	245.9	4.1 × 10 ⁻³	—
	5	5,760	45.1	1.86	3,096	3.2 × 10 ⁻⁴	9.0

1, Crude infected allantoic fluid.

2, Supernatant of No. 1 after adsorption to BaSO₄.

3, Eluate from BaSO₄.

4, Supernatant from II adsorption to BaSO₄.

5, Eluate from II adsorption to BaSO₄.