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## Photodynamic Inactivation of the Vacuolating Virus, $SV_{40}$. (27162)

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The vacuolating virus, $SV_{40}$, isolated by Sweet and Hilleman(1), is a frequent contaminant of rhesus and cynomolgus monkey kidney cell cultures, in which it grows without any obvious cytopathic effect. The agent may be recovered by subculture on kidney cells from the green monkey, *Cercopithecus aethiops*, and in this system causes the cytoplasmic vacuolation from which its name is derived.

The prevalence of $SV_{40}$ constitutes a serious problem in manufacture of live attenuated poliovirus vaccine, since existing federal regulations(2) will permit no detectable foreign virus in the product. Attention has been focused upon methods of differential inactivation which might be employed to kill $SV_{40}$ (and perhaps other simian viruses as well) without destroying the infectivity of attenuated poliovirus.

In studying the inactivation of viruses by visible light in presence of photodynamic dyes(3,4) we were impressed by the remarkable selectivity of this process, and suggested (5) that it might be useful for differential inactivation of B-virus in suspensions of live poliovirus. The experiments described here were undertaken to determine whether or not $SV_{40}$ is also amenable to differential inactivation by this method.

*Methods.* $SV_{40}$, strain 776, was obtained from Dr. Maurice Hilleman, who also kindly provided a supply of rabbit antiserum. Some of the experiments were performed with strain 777, isolated in this laboratory(6) from a sample of Salk poliomyelitis vaccine. The identity and purity of this strain were established by neutralization tests using the antiserum obtained from Hilleman.

The virus was propagated in monolayer cultures of trypsinized cercopithecus kidney cells in medium 199(7) with no serum added. The culture fluids were harvested after incubation for 5-6 days at 36°C and were centrifuged at 600 g to remove cell debris.

In certain of the experiments endogenous wild virus was collected by pooling the fluids from cultures of rhesus kidney cells containing tissue from 8 to 12 monkeys. The infectious agent in these fluids was identified as $SV_{40}$ on the basis of the specific cytopathic effect on cercopithecus cells. For one experiment, $SV_{40}$ was added so that a final concentration of $10^3$ $TCID_{50}$/0.2 ml was present in a suspension containing $10^{7.3}$ PFU/0.2 ml of live poliovirus, type 1(LSC). The poliovirus

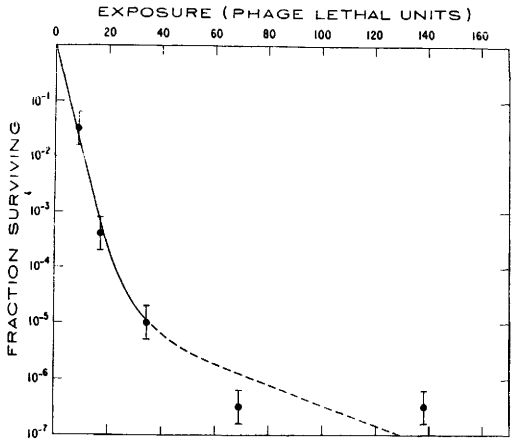


FIG. 1. Survival curve for $SV_{40}$ irradiated with visible light in presence of toluidine blue, 6 $\mu$g/ml.

component of this mixture was assayed by the plaque method in rhesus kidney cell monolayers; the $SV_{40}$ component was assayed in cercopithecus kidney cell tube cultures, as described below, with rabbit antiserum (prepared against poliovirus, type 1, which had been grown in HeLa cells) present to neutralize the poliovirus component.

Virus titrations were performed in tube cultures of cercopithecus (vervet) kidney cells maintained with 1.3 ml of Eagle's minimum essential medium(8) and 1% calf serum. Each of 4 tubes per dilution was inoculated with 0.2 ml of virus suspension. The tubes were incubated at 37°C in a roller drum for 14 days and were observed for appearance of characteristic cytopathic changes.

After addition of 6 $\mu$g/ml toluidine blue the fluids to be treated were held at 4°C in the dark for 45 minutes or more prior to irradiation. For determination of the survival curve, replicate 4 ml samples of virus with dye added were irradiated in Pyrex test tubes between two 350W Photoflood lamps as previously described(4). Larger volumes (250 to 1500 ml) of infective material were irradiated in a continuous flow apparatus(4) consisting of a glass helix surrounding a 1500W incandescent filament lamp. Both systems were calibrated using T3 coliphage as an actinometer(3). The effective exposure (quantum density) for the batch irradiator was 1.72 phage lethal units (PLU) per second and for the continuous-flow device was approximately 80 PLU at a flow rate of 150 ml/min. (One phage lethal unit is defined as the intensity of light which will in 1 second reduce the survival ratio of a standard phage suspension to $e^{-1}$.)

*Results and discussion.* The results shown in Table I were obtained by irradiating 4-ml batches of $SV_{40}$ at constant intensity for graded time intervals. As shown in Fig. 1, the survival curve constructed from these data departed significantly from the exponential form when examined over a concentration range of 7 logarithmic units. Reduction of the survival ratio to less than $10^{-6}$ appeared to be feasible at exposures of 80 PLU or greater.

Experiments with the continuous-flow apparatus led to the results summarized in Table II. All of these experiments were done at the constant flow rate of 150 ml/min., corresponding to a quantum density of 80 PLU

TABLE I. Inactivation of $SV_{40}$ by Graded Exposures to Light in Presence of Toluidine Blue (6 $\mu$g/ml).

| Exposure | | | Fraction surviving |
|---|---|---|---|
| Time, sec. | PLU* | $TCID_{50}$/.2 ml | |
| 0 | 0 | $10^{7.0}$ | $10^{0.0}$ |
| 5 | 8.6 | $10^{5.5}$ | $10^{-1.5}$ |
| 10 | 17.2 | $10^{3.6}$ | $10^{-3.4}$ |
| 20 | 34.4 | $10^{2.0}$ | $10^{-5.0}$ |
| 40 | 68.8 | $10^{0.5}$ | $10^{-6.5}$ |
| 80 | 138 | $10^{0.5}$ | $10^{-6.5}$ |
| 160 | 276 | $<10^{0.0}$ | $<10^{-7.0}$ |

* Phage lethal units.

TABLE II. Inactivation of Virus Suspensions by Continuous-Flow Irradiation.

| Material treated | $TCID_{50}$/.2 ml Before treatment | $TCID_{50}$/.2 ml After treatment | Titer reduction, log units |
|---|---|---|---|
| $SV_{40}$, strain 776 | $10^{4.0}$ | $<10^{0}$ | >4.0 |
| " " 777 | $10^{6.7}$ | $10^{1.7}$ | 5.0 |
| Rhesus kidney tissue-culture fluids, pooled from 8 or more pairs of kidneys | $10^{3.5}$ | $<10^{0}$ | >3.5 |
| | $10^{3.0}$ | $<10^{0}$ | >3.0 |
| Mixture of poliovirus, Sabin type I, and $SV_{40}$, strain 776 | | | |
| Polio component | $10^{7.3}$ | $10^{7.1}$ | .2 |
| $SV_{40}$ | $10^{3.0}$ | $<10^{0}$ | >3.0 |

and a titer reduction of 6 logarithmic units predicted from Fig. 1. The mean titer reduction actually obtained was probably closer to 5 logarithmic units, which would indicate that the effective quantum density at a flow-rate of 150 ml/min was somewhat less than the calculated value. These experiments demonstrate, however, that differential inactivation of $SV_{40}$ in poliovirus preparations is feasible at flow rates applicable to commercial production. Experiments on a full production scale using naturally infected tissue culture fluids would be necessary to determine whether or not this process, or some variation of it, can contribute appreciably to the safety of live attenuated poliovirus vaccine. Additional information would also be needed to verify that stability and immunogenic potency of the poliovirus are not appreciably reduced by the treatment.

*Summary.* The vacuolating virus, $SV_{40}$, is inactivated by visible light in the presence of toluidine blue (6 μg/ml) at quantum densities which have previously been shown to have almost no effect upon infectivity of poliovirus. The possible application of this process as a means of differential inactivation of $SV_{40}$ in suspensions of live poliovirus was demonstrated by continuous-flow experiments.

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## Calciphylactic Muscular Dystrophy Induced by DHT plus 5HT.* (27163)

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Calciphylaxis is a condition of induced systemic hypersensitivity in which, during a "critical period" after sensitization by a systemic calcifying factor (*e.g.*, vitamin-D compounds, parathyroid hormone), treatment with certain challengers (*e.g.*, metallic compounds, albumen, mastocyte depletors) causes acute local calcification with inflammation and sclerosis(1,2). Recently we found that in certain calciphylactic reactions, the precipitation of calcium within the target area may be minimal and evanescent, although even then it apparently plays an important pathogenetic role. Following sensitization with a single oral dose of dihydrotachysterol (DHT) a generalized and often fatal muscular dystrophy with minimal calcinosis can be produced in the rat by a single subcutaneous injection of 5-hydroxytryptamine (5HT).

*Methods.* 160 female Holtzman rats with a mean body weight of 196 g (180-212 g) were subdivided into 10 groups and treated as indicated in Table I.

Dihydrotachysterol, "DHT" (Calcamin®, Wander S.A.) was invariably administered at the dose of 2 mg in 0.5 ml corn oil by stomach tube on the fifth day. 5-Hydroxytryptamine, "5HT" (serotonin creatinine sulfate, Abbott), was given as a single dose of 5 mg in 0.5 ml water, subcutaneously but on different days, to determine the "critical period" for its calciphylactic challenging effect. Of necessity, the intensity of the muscle lesions had to be gauged semiquantitatively in terms of an arbitrary scale 0 to ++++. The means

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