with the expected result (*7*). The average percentage of T-3 found in the circulating fluid for four perfusions was 5.5 ± 1 of the [^{14}C]T-4. This amount was obtained almost immediately (5 minutes) and it only increased slightly during the remaining 90 minutes of the perfusion. Analysis of the labeled T-4's used in these experiments (New England Nuclear and Isolab, Inc.) indicated less than 0.1 percent of T-3 present as an impurity. Perfusion of the glassware with the doubly labeled T-4 and carrier T-3 in the absence of heart tissues yielded (after 90 minutes) only 0.1 to 0.2 percent of doubly labeled T-3. After the perfusions, the heart tissues contained over 45 to 55 percent of the ^{14}C and 40 to 50 percent of the ^{125}I in original solutions.

We conclude that the isolated surviving heart is capable of rapidly removing iodine from T-4. The exact amount of T-3 formed is difficult to determine because of the great amount of newly formed T-3 retained by heart fibers. The conversion of 5 to 6 percent of T-4 into T-3, as observed by our technique, would be an estimated lower limit of this activity.

Chromatography of the perfusing solutions indicated the liberation of free iodide (3 to 6 percent of the initial amount of ^{125}I). As calculated from the ^{14}C content, yields of 10 to 15 percent of tetraiodothyroacetic acid and 12 to 16 percent of mono- and diiodothyronine were obtained. Other iodinated materials were observed but not identified.

These results confirm the postulate of Sterling and others (*3, 7, 8*) that conversion to T-3 is an early step in the metabolism of T-4. The high activity of deiodinase in the heart may contribute to the tachycardia observed in some individuals.

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Nonencapsidated Infectious DNA of Adeno-Satellite Virus in Cells Coinfected with Herpesvirus

Abstract. *Adeno-associated satellite viruses produce antigen detectable by immunofluorescence but not infectious virus in tissue culture cells coinfected with herpes simplex virus. Analysis of DNA extracts from these infected cells shows that large amounts of infectious satellite virus DNA are produced but not encapsidated in the system. This result indicates that satellite virus may be defective at the maturation step.*

Adeno-associated satellite viruses are small, defective DNA viruses which produce infectious progeny only in cells coinfected with adenoviruses (*1*). Herpesvirus can provide a more limited helper function than competent adenoviruses (*2, 3*). Satellite viruses types 1 and 3 produce antigen detectable by immunofluorescence but not infectious virus or complement-fixing antigen in tissue culture cells coinfected with herpes simplex virus. No evidence was offered in these studies for synthesis of adeno-satellite DNA in these herpesvirus-satellite infected cells. The studies of Atchison (*2*) indicate that although no infectious satellite virus or even empty viral capsids are produced, abundant satellite virus structural antigens, presumably capsid proteins, are sequestered in the satellite-herpesvirus system. If some form of satellite virus DNA is replicated it is obviously not encapsidated and would therefore appear to be ideally suited for release from infected cells by mild extraction procedures.

We have recently studied the intracellular DNA species produced during satellite virus infection of green monkey cells in the presence of simian adenovirus SV15 and have developed an assay for satellite virus DNA which is capable of detecting 0.025 μg of infectious satellite DNA (*4*). Satellite virus DNA is infectious when the inoculum contains forms that are either double-stranded or single-stranded (mixtures of "plus" and "minus" strands) (*4*). Below we describe how these methods have been applied to demonstrating that infectious satellite virus DNA is indeed produced in cells coinfected with type 1 satellite virus and herpes simplex virus.

The simian adenovirus SV15 and adeno-satellite virus type 1 preparations used have been described previously (*5*). The satellite preparation was treated at 56°C for 15 minutes prior to use, to inactivate the adenovirus helper. The KOS strain of herpes simplex virus type 1 was used. Assay for competent satellite particles was performed on human embryonic kidney (HEK) cells by using adenovirus type 7, free of satellite, as previously described (*5*). Satellite antigen was assayed by the micro–complement fixation test (*6*). The isolation and assay of infectious satellite virus DNA was done on green monkey BSC-1 cells. To isolate satellite DNA, drained BSC-1 monolayers in 16-oz (473-ml) bottles were infected with herpesvirus and satellite at a multiplicity of infection of 10. After adsorption for 1 hour at 37°C, the cells were washed three times before maintenance medium was added.

Low molecular weight DNA was selectively extracted from control and infected cells 12 to 18 hours after infection, by using the sodium–dodecyl sulfate–1*M* sodium chloride precipitation technique of Hirt (*7*). The 1*M* NaCl-SDS fractionation procedure separates DNA molecules on the basis of their molecular weight. Thus cell DNA is precipitated while SV15 adenovirus

Table 1. Detection of infectious adeno-satellite DNA by immunofluorescence.

	Inoculum mixture*	Prior treatment of test cells with helper adenovirus†	Percent of positive cells by immuno-fluorescence	Infectious adeno-satellite type 1 recovered
No. 1	Hirt supernatant from herpes-infected control cells, 0.4 ml	+	0	Negative
No. 2	Hirt supernatant from cells infected with satellite and herpesvirus, 0.4 ml	+	30	Positive
No. 3	Supernatant No. 2, 0.4 ml + deoxyribonuclease, 0.4 ml	+	0	Negative
No. 4	Double-stranded type 4 satellite DNA, 0.4 ml	+	2	Not done
No. 5	Satellite virus, 0.4 ml	+	80	Positive

* Deoxyribonuclease I was twice crystallized, obtained from Nutritional Biochemicals Co. It was prepared as a 300 μg/ml solution in 0.025*M* veronal buffer, *p*H 7.5, with 0.004*M* $MgSO_4$. Supernatants mixed with deoxyribonuclease were incubated at 37°C for 1 hour prior to addition of DEAE-dextran and PBS⁻ and inoculation. PBS⁻ was added to each mixture to give a final volume of 0.9 ml. To this, 0.1 ml of the DEAE-dextran (10 mg/ml) was added. Controls comprising culture medium from cells with and without prior treatment with adenovirus were negative by both direct and indirect fluorescence procedures. † In the absence of helper adenovirus, no cells became immunofluorescent.

DNA (molecular weight 23 × 10^6) has been found in the supernatant (*4*). Single-stranded DNA is not readily soluble in 1*M* NaCl and has not been found in supernatants prepared under these conditions (*4*).

Crude supernatant fractions were dialyzed against SSC (0.15*M* sodium chloride, 0.015*M* sodium citrate, 0.001*M* ethylenediaminetetraacetate, *p*H 7) for 48 hours at 4°C. They were then assayed for infectivity of satellite virus DNA according to the schedule and with the controls described in Table 1.

DNA assays were carried out in young BSC-1 monolayers prepared on cover slips in petri dishes and maintained in Eagle's medium supplemented with 2 percent fetal calf serum. Essentially the diethylaminoethyl-dextran (DEAE-dextran) method of Pagano *et al.* (*8*) as modified by Kit *et al.* (*9*) was used. Four cover slip cultures were grown in each petri dish. After infection with helper adenovirus SV15 (also at a multiplicity of infection of 10) 12 hours previously, they were treated with various inoculation mixtures as described in Table 1. After 1 hour of adsorption at room temperature the cultures were washed with PBS buffer lacking calcium and magnesium (PBS⁻), then covered with fresh maintenance medium and incubated at 37°C.

Sixteen hours after inoculation of the above mixtures, which is the time required for a single cycle of satellite virus replication, the cover slips were harvested. A typical adenovirus cytopathic effect (CPE) involving approximately 25 percent of the cell sheet was clearly evident by this stage in cover slips pretreated with SV15. All the cover slips were dried at room temperature, fixed in acetone for 10 minutes, and redried. As a known infectious DNA control, double-stranded type 4 "renatured" satellite virus DNA stored for 9 months at 4°C (*10*) was assayed by coinfection of cells with adenovirus as above. Cells were stained for type 1 satellite capsid antigen by the indirect fluorescent antibody procedure with a 1 : 10 dilution of a monovalent type 1 satellite antiserum prepared in rabbits and a 1 : 5 dilution of baboon fluorescein-conjugated globulin prepared from a baboon inoculated with rabbit globulin. Cover slips infected with type 4 satellite were stained by the direct fluorescence procedure as described previously (*11*).

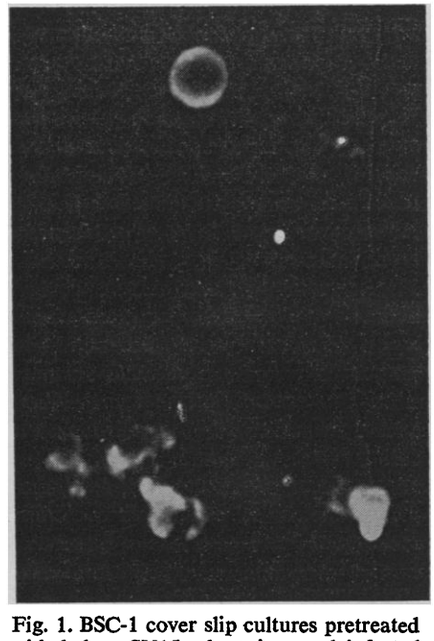

Fig. 1. BSC-1 cover slip cultures pretreated with helper SV15 adenovirus and infected with Hirt supernatant No. 2; indirect fluorescent antibody procedure. Nuclei are brilliantly stained for specific satellite virus antigen.

As can be seen in Table 1, when helper adenovirus was preinoculated, satellite virus antigen was readily detected in cells inoculated with the Hirt supernatant prepared from the harvest of cells coinfected with satellite and herpesvirus. The fluorescence was nuclear and brilliant, and was identical in site and appearance to that described previously for cells infected with whole satellite virions and adenovirus helper (*11*). Specific fluorescence appeared in 30 percent of cells in the monolayer (Fig. 1).

Development of fluorescence was completely obliterated by the presence of deoxyribonuclease, indicating that it was indeed initiated by satellite DNA. By contrast, only 2 percent of the cells inoculated with approximately 0.25 μg of highly purified type 4 native satellite DNA (*4*) developed specific fluorescence. Extraction of free satellite virus DNA, presumably in the double-stranded configuration in the fraction containing low molecular weight DNA, appears to be a relatively efficient method for collecting highly infective satellite DNA.

Examination of the crude Hirt supernatant in the electron microscope with the microtechnique (*12*) revealed no evidence of herpes or satellite virus particles.

Additional evidence that maturation of satellite virus had not taken place in the satellite-herpesvirus system was obtained by electron microscope examination of negatively stained preparations and complement-fixation tests of cultures companion to those destined for Hirt extraction. Satellite virus was not isolated after two passages in HEK cells from either DNA supernatant or from 16-oz bottles incubated for 12 to 18 hours with herpesvirus and satellite. In contrast, type 1 satellite virus could be recovered from SV15-infected cover slip cultures which had been inoculated with the satellite virus DNA (conditions same as in Table 1, No. 2) but held until 4+ SV15 CPE was observed. Deoxyribonuclease-treated preparations (conditions same as in Table 1, No. 3) did not yield virus.

The production of double-stranded infectious satellite DNA plus satellite subviral antigen but not complete nucleocapsids in the satellite-herpesvirus system indicates that transcription of satellite structural proteins can take place, but that assembly does not occur. Replication of "plus" and "minus" strands no doubt also occurs, but rapid renaturation results in "renatured" DNA

either in the cell or during precipitation with 5*M* NaCl in the isolation procedure.

A number of possibilities exist to explain the failure of the final assembly of protein and DNA leading to complete satellite nucleocapsids.

1) It is possible that the satellite proteins produced by herpes complementation are faulty and differ either in amino acid composition or molecular weight from the truly functional capsid proteins produced by adenovirus complementation. This possibility is open to experimentation using polyacrylamide gels.

2) The proteins made in the herpesvirus complementation system are the same as those made in the competent adenovirus complementation system. However, maturation is not just a self-assembly process but rather an adenovirus-controlled satellite virus specific morphopoetic factor. A maturation activating factor, such as that found in the R17 phage system (*13*), or a genetically controlled step, such as the attachment of the fibers to the base plate in the phage T4 (*14*) may be missing in the herpesvirus but present in the adenovirus system. It is also possible that instead of lacking a particular function, herpesvirus may be providing a specific repressor action.

Herpesviruses shut down cell DNA synthesis (*15*), and may therefore turn off some cellular function necessary for satellite maturation. This hypothesis is open to test in a three-component system—herpesvirus inoculation followed by challenge with satellite virus together with helper adenovirus. An active repression step by herpesvirus rather than a paucity of genetic information would be expressed by a failure to replicate satellite virus in this three-component system.

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Linkage of Genes Controlling the Rate of Synthesis and Structure of Aminolevulinate Dehydratase

Abstract. *The rate of synthesis of δ-aminolevulinate dehydratase in mice is controlled by alleles at the levulinate* (Lv) *locus. Two structural mutations modifying the sensitivity of δ-aminolevulinate dehydratase to heat have been identified. Genetic analyses established that the structural locus for δ-aminolevulinate dehydratase was either physically close to or identical with the locus that controls its rate of synthesis.*

The enzyme δ-aminolevulinate dehydratase (ALD) is involved in the biosynthesis of heme, specifically converting δ-aminolevulinic acid to porphobilinogen. In mice, the activity of ALD is under the control of alleles at the levulinate (*Lv*) locus in Linkage Group VIII (*1–3*). Inbred mouse strains homozygous for the Lv^a allele have approximately three times as much ALD activity per gram of tissue (liver, kidney, and spleen) as do those mice homozygous for the Lv^b allele. These differences in activity are attributable to differing amounts of enzyme protein rather than to any structural changes that modify enzyme activity (*4*). Enzyme purified from the livers of representative Lv^a (AKR/J, DBA/2J) and Lv^b strains (C57BL/6J) is indistinguishable on the basis of all properties studied (*3, 4*). These include electrophoretic mobility, sedimentation coefficient, stability to heat and trypsin, specific activity, K_m, and immunochemical criteria. Studies on ALD turnover (*4*) have demonstrated that the *Lv* locus effects its control over enzyme amount by increasing the rate of ALD synthesis two- to threefold in strains of mice homozygous for the Lv^a allele. No effect on the rate of enzyme degradation is apparent.

Genetic control of the rate of enzyme synthesis by a single gene is rare in mammals and the nature of this control can only be explained fully when other genetic loci affecting ALD activity (particularly the structural locus) are identified. This report describes the properties of structurally modified ALD from livers of two inbred strains of mice and establishes that the genetic factor responsible for the structural modification is linked and possibly allelic to the *Lv* locus.

Hutton and Coleman (*2*) classified nearly 30 inbred strains of mice with respect to ALD activity levels. For the most part, all strains fell into three distinct classes: high (Lv^a/Lv^a), low (Lv^b/Lv^b), and an intermediate class designated as Lv^c/Lv^c. This latter class could represent either a third allele at the *Lv* locus or a structural modification of ALD that either increases or decreases the specific activity of the enzyme produced. Thus all strains of mice with atypical enzyme activity levels were considered as potential sources of structural mutations and were each studied carefully with respect to many physical chemical properties, including the rate of heat denaturation.

Enzyme was prepared from the livers of the strains of interest, purified to the ammonium sulfate stage, and compared with that isolated from C57BL/6J mice which served as an arbitrary standard. All enzyme purifications and assays were carried out as previously described (*3*). At 78°C, ALD from the C57BL/6J strain had a half-life of 13.6 ± 0.5 minutes, while enzyme from the C57BR/cdJ strain was somewhat more heat labile ($t_{1/2}$ = 7.7 ± 0.5 minutes). Enzyme from the SM/J strain was considerably more heat resistant ($t_{1/2}$ = 26.7 ± 0.8 minutes) than enzyme from either the C57BL/6J or C57BR/cdJ strains.

The SM/J and C57BR/cdJ strains were chosen for genetic analysis because the differences in the rate of heat