found in a living bird receiving the high treatment), it seems likely that even the low "background" quantities found in wild birds (*7*) could have an effect on thyroid activity.

An increase in thyroid size with hyperplasia associated with a reduction in colloid content of the follicles may reflect either a hyper- or hypofunctioning gland. A hyperfunctioning gland may be the result of (i) stimulation of the pituitary or thyroid to produce excess thyroid-stimulating hormone (TSH) or thyroxine or (ii) a reduction in the concentration of circulating thyroxine causing the production of TSH by the pituitary. This in turn would then stimulate the thyroid to accelerate the formation and secretion of thyroxine. A reduction in the amount of circulating thyroxine would be brought about by increased hepatic activity, as indicated by the increase in liver size, causing increased hepatic metabolism of thyroxine. A second possibility for the reduction of thyroxine is that isomers of DDT may have an estrogenic activity (*8*), as estrogen increases the destruction of the circulating hormone (*9*). A hypofunctioning gland may result if (iii) DDT acts as a goitrogen. Goitrogens, such as thiouracil, produce the same histological picture as above, and the gland is hypofunctioning as the formation of thyroxine is suppressed within the gland (*10*).

With cause (i) the animal concerned would be in a hyperthyroidal state, and with cause (iii) in a hypothyroidal state. With cause (ii), as long as a sufficient supply of thyroxine remained available, signs of hypothyroidism would not develop. If the thyroid could not achieve a balance with the breakdown, symptoms of hypothyroidism, and, possibly with overstimulation, even hyperthyroidism would develop. Fregly *et al.* (*11*) concluded that the increased thyroid weights in rats fed *o,p'*-DDD were producing symptoms of hypothyroidism. In Bengalese finches the symptoms suggested hyperthyroidism (*1*), though some reactions such as decreased egg weight can be produced by both hyper- and hypothyroidism (*12, 13*). The reduction in eggshell weight in birds of prey (*14*) and its correlation with DDT and its metabolites (*15*) suggests that many wild birds are in a hypothyroidal state (*13*). The decision on whether experimental birds are in a hyper- or hypothyroidal state awaits metabolic rate examinations because chemical analysis of protein-bound iodine in the blood as a measure of changes in iodine-containing hormones appears to be insensitive in birds (*16*). Marked differences in species responses to changes in thyroxine concentrations are likely, as opposite results are obtained with different strains of hens (*17*).

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1. Abbreviations are: *p,p'*-DDT: 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane; *p,p'*-DDE: 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene; *o,p'*-DDD: 1,1-dichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)ethane.
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18. We thank Dr. L. E. Mawdesley-Thomas for carrying out the Quantimet examinations at Huntingdon Research Centre, Huntingdonshire, England, and Miss J. Relton for taking the photographs.

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Plus and Minus Single-Stranded DNA Separately Encapsidated in Adeno-Associated Satellite Virions

Abstract. *Based on physical and chemical determinations, the molecular weight of the type 4 adeno-satellite virus is 5.4×10^6 daltons, and the virion contains 1.4×10^6 daltons of DNA. Denaturation and renaturation studies indicate that the viral genome is a single-stranded DNA molecule and that each virion contains either a minus or a plus strand. Upon extraction, the minus and plus strands unite to form double-stranded DNA molecules with no obvious excess of unpaired strands.*

Adeno-associated satellite viruses are biologically defective and replicate only in the presence of competent adenovirions. When isolated from the virion, the DNA of satellite virus had been shown previously to be double-stranded by hydrodynamic and biochemical analyses (*1, 2*).

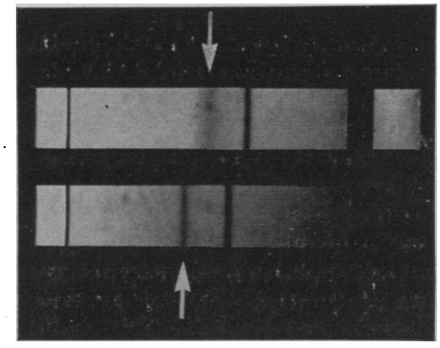

Fig. 1. Ultraviolet photograph of satellite virus DNA (white arrows) at equilibrium in CsCl (44,770 rev/min, 25°C, 23 hours). (Upper) Photo after osmotic release in presence of subtilis-phage 2C DNA. (Lower) Photo after 15 minutes at 67°C in 2 × SSC. Internal reference (2C DNA) is on the right of the satellite band.

However, satellite virions gave staining patterns with acridine orange (*3*) and ultraviolet reaction patterns with dilute formaldehyde (*4*) consistent with patterns of a single-stranded DNA structure, whereas patterns on purified extracted satellite DNA confirm a double-stranded helix. Recent studies using agar diffusion techniques (*5*) yielded a strong reaction between satellite virus DNA *in situ* and rabbit antiserums prepared specifically against single-stranded DNA. Adenovirions (double-stranded DNA) gave no reaction to the test, whereas X14 rat virus and bacteriophage ΦX174 (single-stranded DNA's) gave strong positive reactions. Purified DNA extracted from satellite virions did not react (*5*). Apparently, "quasi" single-stranded regions exist in the virion which are "renatured" to form

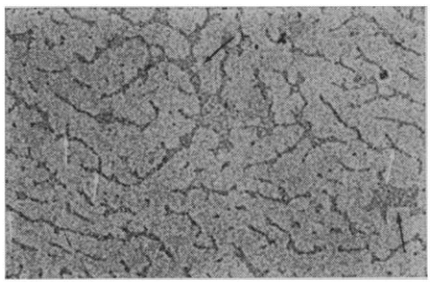

Fig. 2. Electron micrograph of satellite DNA during "renaturation." The collapsed puddles (black arrows) suggest single-stranded regions. Double-stranded regions show evidence of branched molecules (white arrows). Specimens examined at time 0 showed collapsed puddles only. Specimens stained with 1 percent uranyl acetate (× 45,000).

a firmly hydrogen-bonded macromolecule after extraction (*4*).

Experiments testing the possibility that satellite virions contain single-stranded DNA with complementary single strands in different particles (*4, 6*) are reported here.

Satellite type 4 virus was grown in tissue cultures of established green monkey kidney cells (BSC 1) by using simian adenovirus SV15 as the helper. Purification procedures were those already described (*7*). The experimental material comprised CsCl-banded virions at a buoyant density of 1.43 g/cm³. A constant 260/280 nm ratio of 1.42 was characteristic of these purified preparations. The absorption spectrum is typical of that found for small viruses containing single-stranded DNA (*4, 8*).

The sedimentation coefficient ($S_{20,w}$) of satellite virions was determined by band sedimentation in a Spinco analytical model E ultracentrifuge (*9*). A value of 137*S* was found. A diffusion coefficient ($D_{20,w}$) of 2.0×10^{-7} cm/sec was obtained essentially by the method of Valentine and Allison (*10*). The partial specific volume of the satellite virion was determined from chemical data (*2*) to be 0.69 cm³/g. The molecular weight of the satellite type 4 virion computed from these chemical and hydrodynamic data was 5.4×10^6 daltons.

These data indicate that the calculated molecular weight (3.0×10^6 daltons) for type 4 satellite DNA based on a double-stranded structure is probably not correct (*2*). The chemical composition of type 4 satellite virus indicates an average value of 26.5 percent DNA (*2*). On the basis of our molecular weight figure for the complete virions (5.4×10^6 daltons), this would be equivalent to a genome of 1.4×10^6 daltons, a value of one-half the published figure. However, the published calculation assumes that the genome is in the form of a duplex molecule. The data (*2*) obtained from sedimentation of extracted native DNA, alkaline-denatured DNA, and DNA obtained from whole virus by alkali treatment, as well as that obtained from contour length measurements of selected extracted DNA molecules in the electron microscope, are consistent with a molecular weight value of 1.5×10^6 daltons if a single-stranded genome is postulated for the intact virion. In the same way, the data for type 1 satellite DNA (*1, 6*) should also be reconciled. In addition, the low yields of double-stranded DNA could easily be the result of selective losses of highly charged single-stranded materials during the purification procedures.

Satellite type 4 DNA prepared by the method of Parks *et al.* (*2*) was homogeneous and had a buoyant density of 1.719 g/cm³ and physical properties, both in the ultracentrifuge and by electron microscopy, consistent with a native (double-stranded) configuration. After heat treatment, denatured satellite DNA banded at 1.728 g/cm³. Control bacteriophage DNA [2C from *Bacillus subtilis* (*11*)] had a buoyant density of 1.742 g/cm³ and gave two bands after heat denaturation (1.756 g/cm³ and 1.770 g/cm³) (Table 1).

Kinetic studies after the release and renaturation of DNA from satellite virions indicated the following:

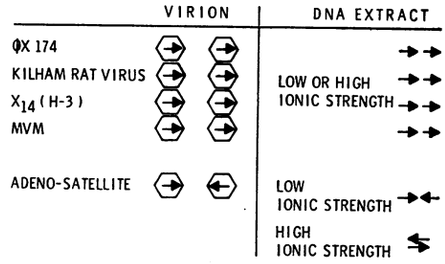

Fig. 3. Diagrammatic representation of states of DNA in selected pico-DNA virions. Direction of arrow indicates the polarity of the strand (for convenience, → signifies a plus strand and ← a minus strand). Formation of the double-stranded structure (⇆) at high ionic strength results from an association of a plus and a complementary minus strand and is predicted to be time- and concentration-dependent.

(i) When purified type 4 satellite virus is lysed at low ionic strength (1/10 standard saline citrate) by 0.1 percent sodium lauroyl sarcosinate at temperatures approximately 12° to 18°C below the T_m of satellite DNA in sodium lauroyl sarcosinate (Table 1), only single-stranded satellite DNA (density 1.729 g/cm³) is obtained, whereas control native bacteriophage 2C DNA contained in the mixture remains double-stranded despite the abnormally low melting temperature of this control sample.

(ii) When purified satellite virus is ruptured by shock at low concentration with 12*M* ammonium acetate (*12*) and pronase treatment (100 μg/ml) at mildly elevated temperatures, again single-

Table 1. Densities in CsCl of satellite and control DNA. Abbreviations: D, double stranded; S, single stranded; SSC, standard saline citrate (0.15*M* NaCl plus 0.015*M* Na_3 citrate, *p*H 7.0); SLS, 0.1 percent sodium lauroyl sarcosinate.

Material	Buoyant density (g/cm³)	DNA strandedness	
		Satellite	Phage control
Satellite DNA, extracted "native"	1.719	D	
The above, heated 100°C, 10 minutes, chilled in ice	1.728	S	
Subtilis-phage 2C DNA, native	1.742		D
The above, heated 100°C, 10 minutes, chilled in ice	1.756, 1.770		S
Satellite virus + 2C DNA in 1/10 SSC + SLS, 57°C*	1.729, 1.742	S	D
Satellite virus + 2C DNA in 1/10 SSC + SLS, 63°C*	1.728, 1.742	S	D
Satellite virus + 2C DNA in 1/10 SSC + SLS, 100°C*	1.729, 1.756, 1.770	S	S
Satellite virus + 2C DNA in 1/10 SSC + SLS, 100°C*, adjusted to 2 × SSC, 70°C, 6 hours	1.716, 1.742	D	D
Satellite virus + 2C DNA, 12*M* NH_4 acetate (diluted 30-fold, pronase 50°C, 30 minutes)			
Experiment 1	1.728, 1.742	S	D
Experiment 2	1.724, 1.742	S	D

* Heating time was 10 minutes. The T_m values were as follows: T_m 2C DNA in SSC = 79°C; T_m 2C DNA in 1/10 SSC = 64°C; T_m 2C DNA in 1/10 SSC + 0.5 percent Na lauroyl sarcosinate = 65.6°C; T_m satellite type 4 DNA in SSC = 92°C; T_m satellite type 4 DNA in 1/10 SSC = 75°C.

Table 2. Formation of double-stranded DNA of satellite virus from plus and minus single strands. S, single stranded; D, double stranded. Conditions: Satellite virus in standard saline citrate (SSC) is diluted to 1/10 containing 0.1 percent sodium lauroyl sarcosinate (SLS), heated to 100°C for 10 minutes, then cooled in ice water, and brought to 2 × SSC in concentration; then 2C DNA is added (final concentration 0.5 μg/ml). Final concentration of satellite DNA is about 1 μg/ml. Satellite DNA is heated in water bath at 67°C.

Time (min)	DNA density (g/cm³)	Appearance of band	Strandedness
0	1.729	Fuzzy	S
15	1.724	Broad	S → D
30	1.718	Sharp	D
60	1.719	Hypersharp	Aggregated D
120	1.718	Hypersharp	Aggregated D
180	1.718	Hypersharp	Aggregated D

stranded satellite DNA is obtained, whereas control native DNA (bacteriophage 2C) contained in the mixture remains double-stranded (Table 1).

(iii) It is known that the rate of renaturation of fully denatured DNA is a second-order reaction and that the reaction rate is at a maximum at T_m − 25°C and high ionic strengths (*13*). To study renaturation kinetics of satellite DNA, material prepared as described in (ii) at a concentration of 1 μg/ml was heated to 100°C for 10 minutes and immediately cooled in ice. Solvent concentration was then brought to twice standard saline citrate, marker 2C DNA was added, and the preparation held at 67°C. Samples were withdrawn, added to solid CsCl, and adjusted to a mean density of 1.71 g/cm³. The buoyant densities achieved at various time intervals are shown in Table 2 and Fig. 1.

Electron microscopic examination (*12*) of DNA during renaturation showed conversion of the collapsed coil characteristic of single-stranded DNA to a linear rigid form characteristic of a double-stranded helix within 15 minutes after initiating renaturation (Fig. 2). Published data on molecular weight from alkaline band sedimentation and contour length measurements [molecular species is linear and 1.5 μ long (*2*)] together with the fact that renaturation is not instantaneous (Table 2) militate against the possibility that the molecule is self-complementary with a hairpin-like reflexion. Renaturation data and the physical data presented here and in a previous publication (*2*), therefore, are consistent with the possibility of a single-stranded linear satellite genome with complementary plus and minus strands in different particles (Fig. 3).

"Renaturation" kinetics for satellite DNA proceed at an extremely high rate, indicative of two completely complementary linear molecules, and show no evidence of residual single-stranded material on completion of the double-stranded form.

Wetmur and Davidson (*13*) have shown that the second-order renaturation coefficient for DNA is $k_2 = 3.5 \times 10^5\ L^{0.5}/N$ at $T_m - 25$°C, where N is the complexity and L the average number of nucleotides per single strand. The k_2 also increases slightly with the guanine-cytosine content of the DNA and is proportional to the reciprocal of the molecular weight. Assembly of double-stranded satellite DNA proceeds at a high rate, indicating presence of linear complementary molecules of low molecular weight with the possibility of many redundancies (low N). The band-sharpening observed during "renaturation" and the rate of decrease in buoyant density during "renaturation" are consistent with these findings (*14*). The hypersharp profile achieved (Table 2) is consistent with the possibility of an aggregated molecular species and may indicate the presence of permuted ends. The branched molecules observed in the electron microscope during renaturation are consistent with this finding. Circular molecules which might be expected to be present in a permuted population (*15*) were not observed.

There are now numerous reports in the literature on the single-stranded nature of the DNA of autonomous pico-DNA, or parvo viruses (*3, 6, 16*). Their DNA, however, remains single-stranded after extraction, indicating that the normal rules for replication of single-stranded molecules are being followed. Adeno-satellites are unique among the small DNA viruses in that they are defective. Their DNA's also are unique. Study of the replicative mechanisms leading to encapsidation of complementary plus and minus single-strands in separate particles could be important in unraveling the mystery of viral defectiveness.

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Altered Ribosomal Protein in Streptomycin-Dependent Escherichia coli

Abstract. *We have compared the 30S ribosomal proteins of strains of* Escherichia coli *sensitive to and dependent on streptomycin and identified a single protein that is functionally altered in the ribosomes dependent on streptomycin. This protein (30S-15) is the same protein that is functionally altered in ribosomes resistant to streptomycin.*

The functional analysis of genetically altered ribosomal proteins has been made possible by the development of procedures for the purification of individual ribosomal proteins (*1, 2*) and for reconstituting functional 30*S* ribosomal particles from 16*S* RNA and the 30*S* proteins (*3*). Consequently, genetic