the new form will be found to be a semiconductor, as are the hexagonal forms of both $MoSe_2$ and MoS_2 (*4*).

The powder pattern of the 3R form is indexable with the assumption of a three-layer rhombohedral cell ($-h+k+l \neq 3n$ absent, hexagonal axes) in which lines with $-h+k \neq 3n$ are diagnostic of the new form. As conversion occurs the diagnostic lines for the 2H form disappear and are bracketed by a pair of lines from the 3R form. Thus in the zone $h0 \cdot l$ the line $(10.3)_{2H}$ disappears and is replaced by $(10.4, 10.\bar{5})_{3R}$, and $(10.5)_{2H}$ disappears and is replaced by $(10.7, 10.\bar{8})_{3R}$.

There is good agreement between observed and calculated structure factors for the diagnostic reflections (Table 1). The atomic positions are like those in 3R MoS_2, with space group R3m, and all atoms on 3 (a). Intensities were calculated by using Mo in ⅓, ⅔, 0; Se_1 in ⅔, ⅓, .083; and Se_2 in ⅓, ⅔, .250. Intensities were measured from peak heights on a diffractometer trace (0.4° and 0.2° per minute scanning speed) and corrected for Lorentz and polarization factors. Scattering factors were derived from Thomas and Umeda (*5*).

The lattice parameters for 3R $MoSe_2$, calculated from six of the large (76° to 80°) θ lines, are $a = 3.292$ Å and $c = 19.392$ Å (thickness of one layer = 6.46 Å). These values are similar to those reported for the 2H form (*6*), for which $a = 3.288$ and $c = 12.931$ Å (thickness of one layer = 6.46 Å). Calculated interatomic distances of nearest neighbors are the same as those of James and Lavik (*6*) since our unrefined z coordinates are the three-layer equivalents of their two-layer values.

Table 1. Observed and calculated structure factors for the 10. *l*, 20. *l*, and 21. *l* zones $MoSe_2$ (sec text).

hk.l	A	B	$F_{calc.}$	$F_{obs.}$
10. 1	−40	−11	41	55
10. 2	−31	+ 7	32	34
10. 4	− 2	+52	52	55
10. 5	+ 7	+67	67	63
10. 7	+ 6	+63	63	56
10. 8	− 2	45	45	41
10.10	−24	44	50	69
10.11	−31	− 7	32	38
10.13	−29	− 6	30	34
10.14	−22	+ 5	23	35
10.16	− 2	+36	36	63
10.17	+ 4	+45	45	61
10.19	+ 4	42	42	44
20. 1	+31	+ 8	32	46
20. 4	− 3	−41	41	40
20. 5	+ 6	−52	52	47
20. 7	5	−51	51	43
20. 8	−2	−39	39	34
21. 5	+ 4	+44	44	39
21. 7	+ 5	+44	44	31
21.13	−24	− 4	24	24

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Nucleohistone Dissociation by Ganglioside Micelles

Abstract. *The sialic acid–containing glycosphingolipids known as gangliosides can reverse the heat stabilization of DNA by histones. The ability of pure mono- and disialogangliosides to dissociate reconstituted nucleohistone is directly dependent on their sialic acid content; they are effective in concentrations above their critical micelle concentration.*

It is established that DNA is rendered less effective as a template for RNA synthesis in vitro when histones are added (*1*), and that removal of histones from chromatin preparations stimulates template activity (*2*). Such findings suggest that histones may function as repressors of DNA expression, and that disruption of the nucleohistone complex may constitute derepression. Mechanisms for the dissociation of nucleohistones are therefore of considerable current interest.

Frenster (*2*) has reported that addition of polyanions to chromatin increases its effectiveness as a template for RNA synthesis, and that "active chromatin" isolated from calf thymus has a higher content of polyanions than has "repressed chromatin." The polyanions probably function by changing the electrostatic attraction between DNA and histones. Gangliosides are anionic neuronal glycolipids, which have been shown to form complexes with basic proteins, including histones (*3*). McIlwain has demonstrated migration of histones from the nucleus to ganglioside-rich membranes in brain slices briefly exposed to cold (*3*). Thus it was considered of interest to determine whether gangliosides can interact directly with nucleohistones. We have observed that four pure gangliosides compete with DNA for its associated histones, as evidenced by reversal of the heat stabilization of DNA by histones.

Nucleohistone was prepared from calf-thymus DNA (*4*) and calf-thymus lysine-rich histone (*5*) by a method described (*6*). DNA (61.5 μg) and 114.0 μg of histone were dissolved in 4.0 ml of dilute saline-citrate buffer (2.8 m*M* NaCl, 3 m*M* sodium citrate, *p*H 7.3). Nucleohistone solutions were prepared by sequential addition of 0.4 ml of DNA stock (154 μg/ml), 3.05 ml of buffer, and 0.55 ml of histone stock (208 μg/ml). When the sample included ganglioside, a portion of $10^{-3}M$ ganglioside was subsequently added to the above mixture. The volume of buffer was reduced to maintain a final volume of 4.0 ml. By this procedure, precipitation of the complex was avoided. *N*-Acetylneuraminic acid (NANA) was determined by the modified resorcinol method (*7*). Gangliosides were isolated from human brain in a manner described (*8*).

The helix-coil transition of DNA was observed by measurement of the accompanying hyperchromic shift at 260 mμ. Samples were heated in the Beckman-DK2 recording spectrophotometer by means of an aluminum heating block; optical density (O.D.) was measured at ambient temperatures. T_m is defined as the temperature at which half the total hyperchromicity is reached. The percentage of total hyperchromicity reached at 74°C was determined in some experiments as

$$[\text{O.D. (74°C)} - \text{O.D. (25°C)}]/[\text{O.D. (98°C)} - \text{O.D. (25°C)}] \times 100$$

This determination requires O.D. measurements at three temperatures only. Samples containing gangliosides were always compared with blanks of identical ganglioside concentration.

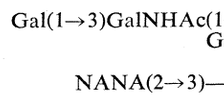
Under the ionic conditions of these

experiments, the T_m of free DNA is 65°C, and the percentage of hyperchromicity at 74°C is 88. Our standard nucleohistone is prepared at a DNA : histone ratio of 1 : 2 by weight, this mixture being completely resistant to denaturation at 74°C. The percentage of hyperchromicity at 74°C is zero; when less histone is used, some hyperchromicity is observed at 74°C, while higher concentrations of histone result in precipitation of the complex.

When standard nucleohistone solution is made $7.5 \times 10^{-5}M$ in ganglioside (in the manner described), the percentage of hyperchromicity at 74°C increases from zero to 88, the increase indicating that the nucleohistone is completely dissociated to free DNA (Fig. 1). As the concentration of ganglioside is raised above the critical micelle concentration of $1 \times 10^{-5}M$ (*9, 11*), a parallel rise in the percentage of hyperchromicity at 74°C is observed (Table 1). The increasing proportion of denaturation accomplished at temperatures below 74°C reflects increasing quantities of free DNA in the reaction mixture, as a result of withdrawal of histone by the ganglioside micelles.

Additional evidence of the formation of a ganglioside-histone complex came from an experiment in which the normal order of mixing was changed. When 185 μg of histone and 61 μg of DNA are combined in 4 ml of buffer, a precipitate results, but there was no precipitation when the histone was preincubated with $10 \times 10^{-5}M$ ganglioside (HG-4). The heat-denaturation curve of the ternary mixture was equivalent to that for free DNA. Apparently the histone was made inaccessible by its combination with the ganglioside.

The effects of mono- (HG-1), di- (HG-2 and HG-4), and tri- (HG-5) sialogangliosides on nucleohistone were compared. These gangliosides differ in the number of negative charges (NANA residues) per molecule, as indicated, and in the positions of the charged groups within the carbohydrate moiety (*10*). The structure of HG-1 is



where Gal is galactose; NHAc, *N*-acetyl; Glc, glucose; and Cer, ceramide. If the interaction between gangliosides and nucleohistone is due to electrostatic competition between the two polyanions (ganglioside and DNA) for the polycationic histone, then the NANA content of each ganglioside sample should directly determine its ability to dissociate the nucleohistone. This direct dependence on NANA rather than on molarity or micelle size was observed; therefore, on a molar basis, the disialogangliosides are twice as effective as the monosialoganglioside (see Table 1). The results imply that all the NANA residues in HG-1, HG-2, and HG-4 are equally accessible for interaction, while the residues of HG-5 appeared less effective.

It has been reported that only about 70 percent of the NANA residues of mixed ganglioside samples are titratable (*11*). In order to determine that NANA content of our ganglioside samples could be equated with net charge, each of the gangliosides was passed over a small column of Dowex-50W X-2[H^+] and titrated. All NANA residues (97 to 103 percent) of the mono- and disialogangliosides were titratable, while only 84 percent of the NANA residues of the trisialoganglioside (HG-5) were accountable for as acid equivalents. Thus it appears that a significant portion of the acid groups in the trisialoganglioside is bound in some form; this fact probably accounts for the less-effective interaction of HG-5 with nucleohistone.

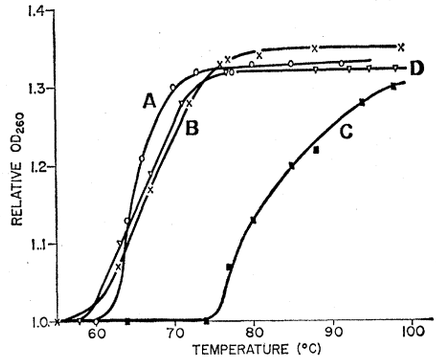


Fig. 1. Effect of ganglioside on heat denaturation of nucleohistone. (*A*) DNA; (*B*) nucleohistone plus $7.5 \times 10^{-5}M$ ganglioside (HG-4); (*C*) standard nucleohistone; solvent: 2.8 m*M* NaCl, 3.0 m*M* sodium citrate, *p*H 7.3; (*D*) DNA plus $7.5 \times 10^{-5}M$ ganglioside (HG-4).

The presence of gangliosides in cell membranes results in an array of anionic groups similar to those at the hydrophilic surface of a ganglioside micelle. Such membrane-bound charges may be capable of interaction with electrostatic complexes in the cell, by analogy with the ability of the micelles to dissociate dissolved nucleohistone. Such interactions are of interest in consideration of the functional role of these membrane constituents (*12*).

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Table 1. Dissociation of nucleohistone by four pure gangliosides; nucleohistone prepared at a DNA:histone ratio of 1:2 by weight.

Ganglioside	NANA residues per molecule	Ganglioside molarity ($\times 10^5$)		Total NANA in 4 ml (μg)	Hyperchromicity at 74°C, duplicates (%)	
		For 50 percent dissociation	Of sample			
HG-1	1	4.0	1.07	13.2	0	
			2.12	26.2	29	31
			3.71	45.9	41	48
			5.30	65.5	50	50
HG-2	2	2.0	1.93	47.8	39	48
			3.86	95.5	69	71
			5.81	143.8	81	81
HG-4	2	2.1	1.18	29.2	17	24
			2.36	58.5	52	54
			7.08	175.0	90	90
HG-5	3	2.0	1.07	39.7	29	
			2.13	79.0	46	
			3.21	119.0	67	68

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