Table 1. The effects of tylosin tartrate and tetracycline on hybrids between the Santa Marta (SM) and Mesitas (M) strain of *Drosophila paulistorum*. Samples of 100 males were dissected in each generation in each experiment.

Cross	Percentage of males with motile sperm		
	Tylosin	Tetracycline	Controls
F_1 of SM ♀♀ × M ♂♂	0	40	0
BC_1 (SM × M) F_1 ♀♀ × M ♂♂	35	20	8–10

effective against the mycoplasma-like structures associated with plants infected with mulberry dwarf disease (*11*) and against aster yellows disease (*8*). Table 1 reports the percentages of the males in the F_1 and in the first backcross generation showing motile sperm upon dissection. In the controls, the mothers of these males had developed as larvae on food free of antibiotics, and in the experimental series the mothers were given antibiotics in their food. Treatment with tetracycline, but not with tylosin, results in the appearance of motile sperm in a part of the F_1 generation hybrid males. Both tetracycline and tylosin increase the frequency of motile sperm in backcross males. To test the fertility of the males, 30 cultures were made with eight females and eight males from each experiment reported in Table 1. No fertile cultures were obtained in the control experiments or in the F_1 hybrids treated with tylosin. In the other experiments, where many males had motile sperm, some of them were also fertile. However, the backcross males in the control experiments were all sterile, although some of them had motile sperm, as reported (*4, 12*).

Ultrastructurally, no mycoplasma-like inclusions were observed either in the F_1 or in the backcross males whose mothers were raised on the food that contained the antibiotic. However, the F_1 males treated with tylosin do show some disturbances in the symmetry of the axial filaments in the spermatids (Fig. 3). Males from the other treated series are not distinguishable (by electron microscopy) from the control males. The evidence is, therefore, consistent with the supposition that there is a causal relation between the presence of numerous mycoplasma-like bodies and the sterility of the hybrid males (*13*). The possibility cannot however be ruled out that the mycoplasma-like bodies are symbionts which thrive particularly in the cytoplasm derived from the degenerating spermatids in the hybrid males. On the other hand, the presence and the rate of multiplication of the mycoplasma may be genetically controlled; discord between the genotype of the host and the symbiont may allow the latter to multiply excessively and result in the sterility of the males. The enhancement of fertility in hybrid males whose mothers had been treated with antibiotics known to inhibit the multiplication of mycoplasma provides additional support for the hypothesis of causal relationship.

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13. In *D. paulistorum* females, mycoplasma-like bodies are also found intracellularly in the cytoplasm of developing follicles in the ovaries.
14. Supported by the Bio-medical Fund, Graduate School, and SUNY Research Foundation grants 8202, 8223, and 0232; and PHS award 2K03 HD09033-06. We thank Mrs. Isabel Leader, Chief of Microbiology, Beekman Downtown Hospital, for advice about the administration of the antibiotics, and Professor Th. Dobzhansky, The Rockefeller University, for suggestions concerning the manuscript.

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Tubules of Globoid Leukodystrophy: A Right-Handed Helix

Abstract. *Morphologic similarity between the cytoplasmic tubules of globoid leukodystrophy and Gaucher's disease (as demonstrated by thin sectioning, negative staining, and shadowing techniques) and their resemblance to negatively stained beef cerebroside are presented as evidence favoring the accumulation of cerebrosides in globoid cells. The tubules of globoid leukodystrophy have a 60-angstrom periodic banding similar to the tubules of Gaucher's disease. A right-handed helical twisting of the tubules is observed in both diseases.*

Information concerning the nature of the metabolic abnormality in any of the leukodystrophies may help to elucidate myelin metabolic pathways. The leukodystrophies are a heterogeneous group of genetically determined disorders of myelin metabolism. Each specific type has characteristic chemical alterations which are reflected in its morphology.

Globoid cell leukodystrophy (Krabbe's disease) is inherited as an autosomal recessive condition affecting infants usually in the first year of life. Progressive motor regression and mental deterioration become fatal within 1 or 2 years. Extensive demyelination is characteristically associated with the accumulation of a large number of globoid cells in the white matter of the brain.

The significance and origin of the globoid cells is not clear and has been the subject of extensive histochemical and biochemical studies (*1*). One hypothesis regarding the underlying enzymatic abnormality is that a deficiency of cerebroside sulfotransferase causes a block in the conjugation of cerebroside with sulfate to form sulfatide (*2*). This results in an increase in the ratio of cerebroside to sulfatides. More recently a deficiency of the enzyme galactose cerebrosidase has been reported in the tissues and leukocytes of patients with Krabbe's disease (*3*). This supports the idea that cerebroside is stored but not that sulfatide synthesis is blocked.

Three recent papers (*4, 5*) disclosed the presence of tubular structures in the cytoplasm of globoid cells. Two of them (*4*) described only straight to slightly arched polygonal structures

with striking cross-sectional appearance characterized by variable, multiangular profiles punctuated by electron-opaque dots (Fig. 1). In addition to that type, we (*5*) described smaller, twisted tubules that freely interlaced at short intervals with adjacent ones and had 300-Å round to rectangular cross sections (Fig. 1). When viewed longitudinally, both types of tubules had periodic banding every 60 Å which corresponded to the dots seen in cross sections. In twisted areas the periodicity was frequently less than 60 Å. This could have resulted from compression of fibrils or oblique views.

The ultrastructural resemblance of the tubules to beef cerebroside suggests that galactose cerebroside accumulates in these cells, possibly the result of defective myelin metabolism. Reexamination of Gaucher's tubules showed the same type of round to rectangular cross-sectional appearance with a similar periodicity, but multiangular profiles were not observed. Morphologic resemblance to the tubules of Gaucher's disease is significant because in this disease glucose cerebroside accumulates in the cytoplasm of reticuloendothelial cells due to a deficiency of glucose cerebrosidase.

Further characterization of the tubules of Krabbe's disease was undertaken by means of negative staining and shadowing techniques and comparison of these tubules with those of beef cerebroside and Gaucher's disease. Material for this study was obtained at autopsy of the patient previously reported (*5*). Four hours after death, brain tissue was frozen at −25°C. After thawing, the tissue was ground in a 1 percent solution of ammonium acetate with a Teflon pestle. Negative stains were performed with 1 percent phosphotungstic acid and carbonized, coated copper grids. After calibration of the magnification of the electron microscope by means of a grating replica, the size of the tubules, periodicity of the fibrils, and the pitch of the helix were measured and compared to those of tubules from Gaucher's disease and of beef cerebroside.

The existence of two types of tubules in globoid cells of Krabbe's disease was corroborated with this technique (Figs. 2–5). The slender tubules had a periodic twisting approximately every 2500 Å along their lengths. The large tubules appeared more rigid. They were straight or slightly bent and occasionally twisted at long intervals. Both types had a fibrillar substructure more evident at the points of twisting and at the ends of the large tubules.

The overall appearance of the tubules contrasted sharply with that of the adjacent concentric laminated structures, probably phospholipids, and is quite different from sulfatides, which accumulate in metachromatic leukodystrophy. On the other hand, both types of tubules are similar in size and shape to those of beef cerebroside and the twisted tubules are similar in appearance, size, and periodicity to those of Gaucher's disease.

Shadow casting provided an opportunity to determine the direction of the twist. With this technique the slender tubules are seen to be twisted in a right-handed helix (Fig. 6), the same as those of Gaucher's disease.

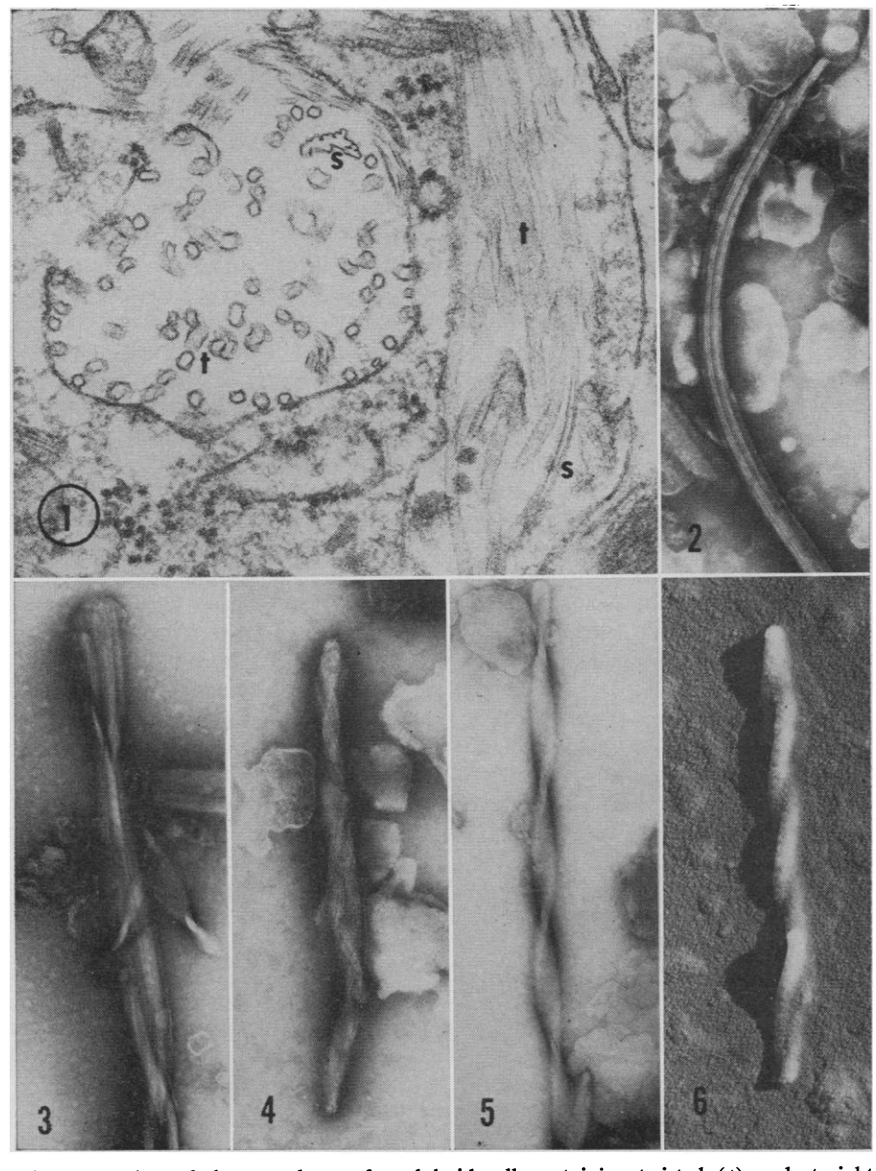

Fig. 1. Portion of the cytoplasm of a globoid cell containing twisted (*t*) and straight (*s*) tubules. Cross sections of both types of tubules are seen within the large sac on the left. Longitudinal views are present on the right (× 82,500). Figs. 2–6. Phosphotungstic-acid-stained (Figs. 2–5) and carbon-shadowed (Fig. 6) preparations from unfixed brain of a patient with globoid leukodystrophy. Fig. 2. The fibrillar character of this arched tubule is evident (× 80,500). Fig. 3. Large tubule with occasional twisted areas (× 58,500). Fig. 4. Slender twisted tubule with 60-Å periodic banding (× 96,600). Fig. 5. Twisted ribbonlike structure with a 2500-Å helical pitch. This was the more common type of twisted tubule seen (× 80,000). Fig. 6. The right-handed twist of a slender tubule is clearly demonstrated in this shadowed preparation (× 69,000).

The striking resemblance of the material deposited in globoid cells to Gaucher's tubules and beef cerebroside lends credence to the idea that galactose cerebroside deposits in globoid cells, possibly due to an abnormality in the metabolism of cerebrosides.

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10 March 1970

DNA-Membrane Complex: Macromolecular Content and Stimulation of Enzymatic Activity by Polyadenylic Acid

Abstract. *A DNA–cell membrane complex has been isolated from cell suspensions of virulent pneumococci by sarcosyl lysis followed by centrifugation on a sucrose gradient. When polyadenylic acid plus the eight naturally occurring deoxyribonucleosides and deoxyribonucleotides are added to cell suspensions, the percentage of total DNA in the complex increases with incubation time. This increase is not observed in unsupplemented cell suspensions. However, the percentages of RNA, protein, and phospholipid do not increase with incubation time in either supplemented or control complexes. A variety of deoxyribonucleotide kinases and the DNA polymerase are also detected in the DNA-membrane complex, and their specific activities are greater in complexes extracted from supplemented cell suspensions than in those extracted from controls.*

Polyadenylic acid (poly A) or polycytidylic acid (poly C), or oligodeoxyribonucleotides derived from a variety of DNA's treated with deoxyribonuclease, plus all eight of the naturally occurring deoxyribonucleosides and deoxyribonucleotides can induce virulent (but not avirulent) pneumococci to synthesize genetically competent DNA in excess of the normal complement (*1–3*). A number of studies (*2, 3*) suggested (i) that oligodeoxyribonucleotides and poly A or poly C (which were degraded to oligomers in vivo after uptake) induced higher amounts of specific enzymes (deoxycytidylate and deoxyguanylate kinases) involved in DNA synthesis and (ii) that deoxyribonucleosides and deoxyribonucleotides act as precursors for these reactions. However, the possibility that this induction may only be part of the mechanism of action of the supplement in stimulating DNA synthesis is suggested by the observation that poly A enhances DNA synthesis (in conjunction with deoxyribonucleosides and deoxyribonucleotides) almost as well as poly C, despite the fact that poly A induces higher amounts of only one kinase (deoxyguanylate) whereas poly C induces both deoxyguanylate and deoxycytidylate kinases (*2*).

To further determine the functions of the oligomers and the possible mechanisms involved in control of DNA synthesis in pneumococci, we have studied a DNA–cell membrane complex isolated from bacteria (*4, 5*); this complex may have physiological significance with respect to the site of initiation of DNA replication. Our approach was based on a suggestion of Maaløe (*6*), who hypothesized that the replication point consisted of a complex of DNA-synthesizing enzymes which were actually in association with DNA during replication. If the oligomers somehow functioned in initiation of DNA synthesis by acting on the formation or stabilization of this complex, the stimulatory effects of the oligomers on specific kinases could be part of such a phenomenon. Accordingly, cell suspensions of virulent pneumococci (type III, strain A66), prepared as described previously (*2*), were shaken in a gyrotory water bath for 10, 25, or 50 minutes at 37°C in the presence or absence of the supplement (poly A plus the eight deoxyribonucleosides and deoxyribonucleotides). After each period, a DNA–cell membrane complex was extracted by the procedure of Tremblay *et al.* (*4*) by which cell lysates are separated into two fractions, a "top" fraction and a DNA-membrane fraction, after centrifuga-

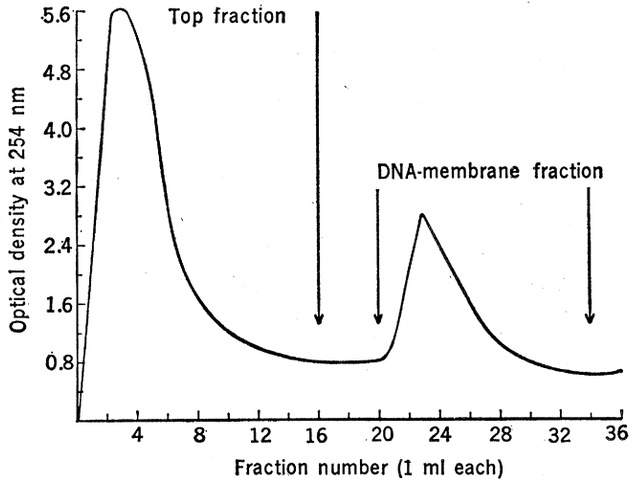

Fig. 1. Ultraviolet tracing of a two-layer sucrose centrifugate of a cell lysate from virulent pneumococci. After incubation of cell suspensions for various periods, 4.2 ml of washed cells (in a 0.01*M* tris, 0.01*M* magnesium acetate, 0.1*M* KCl buffer, final *p*H 7.0) were layered on top of equal volumes of a two-layer sucrose solution (15 percent over 40 percent in tris buffer) contained in 40-ml cellulose acetate tubes. The cells were lysed completely by gentle stirring in 0.5 ml of a 0.1 percent solution of sodium lauroyl sarcosinate (Geigy Chemical Corp.). This concentration of detergent produced the greatest and most reproducible extraction of the DNA-membrane fraction. After a few minutes at room temperature, the lysates were centrifuged for 30 minutes at 15,000 rev/min at 4°C in the SW 27 rotor of the Spinco L-2 ultracentrifuge. After passing the contents of each tube (by pushing the gradient from the bottom) through a Gilford recording spectrophotometer at 254 nm to detect the various components, we collected 1- to 3-ml fractions and analyzed them for macromolecular content.