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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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-

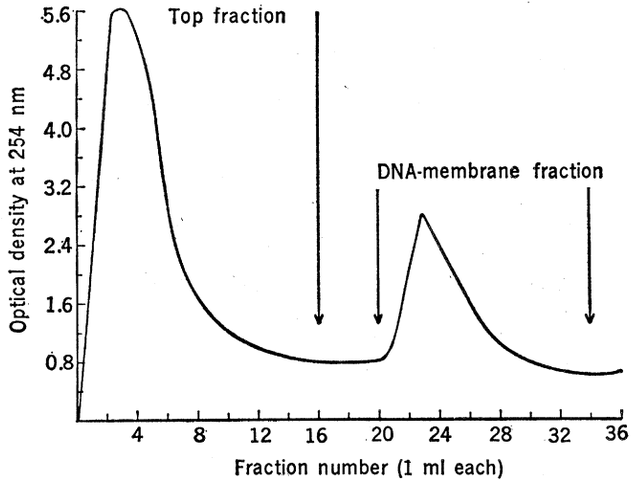

Fig. 1. Ultraviolet tracing of a two-layer sucrose centrifugate of a cell lysate from virulent pneumococci. After incubation of cell suspensions for various periods, 4.2 ml of washed cells (in a 0.01*M* tris, 0.01*M* magnesium acetate, 0.1*M* KCl buffer, final *p*H 7.0) were layered on top of equal volumes 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.

tion in a sucrose gradient (Fig. 1). Further evidence for the existence of an association between DNA and membrane components was obtained by centrifugation of the extracted complex in a cesium chloride density gradient (*3*) before and after treatment with enzymes (*5*) that break such a complex. Before treatment, DNA in the complex remained at or near the meniscus after centrifugation. However, after incubation of the complex with either pronase (Cal Biochem-B grade) or ribonuclease (ribonuclease A, Sigma) (1 mg of each per milliliter of complex) for 12 hours at 37°C, approximately 40 to 60 percent of the DNA sedimented at the buoyant density of free DNA (1.701).

Table 1 shows that there is relatively little difference between supplemented and control cell suspensions with respect to the percentage distribution of RNA, protein, and phospholipid in the top or DNA-membrane fraction after the various periods of incubation. However, the DNA contents of supplements and controls are significantly different after 25 and 50 minutes of incubation, that of the DNA-membrane fraction being the greater. Only after a 10-minute incubation is the percentage distribution of DNA approximately the same in supplements and controls.

Although both top and DNA-membrane fractions from supplements and controls exhibit deoxyribonucleotide kinase and DNA polymerase activity, the specific activity of these enzymes is significantly greater in the DNA-membrane fraction than in the top fraction of both supplements and controls (Table 2). More important is the observation that the DNA-membrane fraction from supplemented cell suspensions exhibits greater specific activity than controls for almost all the enzymes examined, including deoxyguanylate kinase, the enzyme induced by oligomers of adenylic acid (*2*). Finally, when cells are lysed by grinding with microglass beads (*1*) and otherwise treated identically with respect to centrifugation and assay of kinase activity and DNA polymerase activity, no DNA-membrane complex is detected, and no enrichment of enzyme activity is seen in that part of the gradient which would contain this complex.

Although these observations suggest that DNA replication occurs in the DNA-membrane fraction, several

Table 1. Analysis of the top and DNA-membrane fractions from supplemented and unsupplemented cell suspensions of pneumococci. Supplement consists of poly A (100 μg per milliliter of cell suspension) plus all eight naturally occurring deoxyribonucleosides and deoxyribonucleotides (200 μg of each per milliliter of cell suspension). Total DNA was measured by the method of Burton (*8*), or by steady-state labeling of acid-insoluble material with [2-^{14}C]deoxycytidine (0.1 μc per milliliter of cell suspension). Total RNA was measured by the orcinol method of Drury (*9*) or by steady-state labeling of acid-insoluble material with [2-^{14}C]uridine (0.1 μc per milliliter of cell suspension). Total protein was measured by the method of Lowry *et al.* (*10*). Phospholipids (indicative of cell-membrane material) were labeled by inorganic [^{32}P] phosphate (0.01 μc per milliliter of cell suspension). Fractions were extracted four times with butanol and then washed twice with water. Radioactivity for all fractions was assayed in a Nuclear Chicago liquid-scintillation counter with appropriate phosphors (i) after trichloroacetic acid precipitates were filtered onto Millipore filters (HA) and washed four times with additional trichloroacetic acid in the case of nucleic acids, and (ii) after samples were added to filters in the case of phospholipids.

Fraction	Incubation time at 37°C (min)	Percentage of total cell content			
		Total DNA	Total RNA	Total protein	Total phospholipids
		Supplemented suspension			
DNA-membrane	10	14	20	9	20
DNA-membrane	25	40	27	11	21
DNA-membrane	50	55	26	11	29
Top	10	86	80	91	80
Top	25	60	73	89	79
Top	50	45	74	89	71
		Control			
DNA-membrane	10	15	23	10	18
DNA-membrane	25	30	25	13	16
DNA-membrane	50	25	25	10	24
Top	10	85	77	90	82
Top	25	70	75	87	84
Top	50	75	75	90	76

Table 2. Enzymatic activity in supplemented and unsupplemented top and DNA-membrane fractions of pneumococci. Supplement is the same as in Table 1. Pooled top and DNA-membrane fractions were examined for DNA polymerase activity and deoxyribonucleotide kinase activity in supplemented and control cell suspensions after a 25-minute incubation. For the former, each fraction was dialyzed for 24 hours against a buffer consisting of tris (0.02 mole/liter, *p*H 8.5), albumin (1 mg/ml), dithiothreitol (0.29 mg/ml), and glycerol (20 percent). Activity was measured as described by Steuart *et al.* (*11*). For the latter, each fraction was dialyzed for 24 hours against a potassium phosphate buffer (0.01*M*, *p*H 7.5); activity was measured as described in references (*2*) and (*3*). dCMP, deoxycytidylate; dGMP, deoxyguanylate; dAMP, deoxyadenylate; TMP, thymidylate.

Enzyme assayed	Cell suspension	Product (nm/mg protein)
	DNA-membrane fraction	
dCMP kinase	Supplemented	10.1
dCMP kinase	Control	4.6
dGMP kinase	Supplemented	51.6
dGMP kinase	Control	16.2
dAMP kinase	Supplemented	28.3
dAMP kinase	Control	21.0
TMP kinase	Supplemented	22.0
TMP kinase	Control	12.6
DNA polymerase	Supplemented	10.2
DNA polymerase	Control	4.9
	Top fraction	
dCMP kinase	Supplemented	0.8
dCMP kinase	Control	2.4
dGMP kinase	Supplemented	11.4
dGMP kinase	Control	5.7
dAMP kinase	Supplemented	30.1
dAMP kinase	Control	22.9
TMP kinase	Supplemented	3.0
TMP kinase	Control	0
DNA polymerase	Supplemented	1.7
DNA polymerase	Control	1.1

points must be considered before this conclusion is established definitively. Among the most important is the fact that although the amount of DNA in the membrane fraction increases with incubation time in supplemented cell suspensions, the amount of DNA in the top fraction, which is initially greater, decreases with incubation time. This inverse correlation could indicate that DNA from the top fraction is released selectively into the membrane fraction with increasing incubation time. For example, deoxyribonuclease activity could increase during incubation of cell suspensions and degrade DNA in the top fraction in quantitative amounts after cell lysis, or DNA could become more sensitive to shear with incubation time. However, no manipulation of cell lysates occurred after addition of the sarcosyl detergent that might cause shearing of the DNA. In addition, if substantial deoxyribonuclease activity were present, little or no DNA would be present in any of the fractions, since acid-washing of the extracts before assay of DNA would presumably remove the products of deoxyribonuclease digestion. Even if some large oligodeoxyribonucleotides were not removed by acid-washing, they would have sedimented in the sucrose gradient in a heterogeneous manner instead of in two main peaks in the top and membrane fractions. Another possibility is that since phospholipids, kinases, and the DNA polymerase are present in the top fraction, DNA is first synthesized in the top fraction and then subsequently bound to the membrane fraction. Although this possibility cannot be eliminated, it must be viewed in relation to the fact that greater amounts of DNA polymerase and deoxyribonucleotide kinase activities are present in the DNA-membrane fraction than in the top fraction. A third possibility, which we favor, is that DNA is replicated in a discontinuous manner, as hypothesized by Okazaki *et al.* (*7*), in the membrane fraction. This would imply (i) that DNA in the top fraction is derived originally from DNA in the membrane fraction but has not yet been covalently bound to DNA in the latter fraction, (ii) that the molecular weight of DNA in the top fraction during early incubation times (less than 10 minutes) is smaller than that of DNA in the membrane fraction, and (iii) that the decrease in percentage of DNA in the top fraction with incubation time reflects the binding of the segments of DNA.

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Selective Destruction of Target Cells by Diphtheria Toxin Conjugated to Antibody Directed against Antigens on the Cells

Abstract. *Monkey-kidney cells bearing new surface antigens induced by infection with mumps virus were lysed selectively by diphtheria toxin conjugated to antibody against mumps antigens.*

Despite evidence that animal and human tumors carry unique antigens (*1*), the conceptual promise of immunotherapy for the control of cancer has resisted practical exploitation. Once autochthonous tumors develop, host immune responses to them are rather weak; furthermore, as they are mediated primarily by delayed hypersensitivity, they are difficult to regulate and to transfer passively to tumor-bearing hosts. Although easier to manipulate, humoral antibodies against tumor-specific antigens have so far proved ineffectual in vivo, despite evidence from sensitive assay methods that they can injure tumor cells in tissue culture; more often than not, circulating antibodies serve to enhance tumor growth (*2*). We now report a method for killing antigen-bearing cells with antibody, using the antibody as a distinctive means for directing a potent cytotoxin to the target cells.

As an optimal model system toward which the antibody-toxin complex could be aimed, we first explored the use of antibodies against viral antigens that appear in abundance on the surface of cells infected with mumps virus, as this was operationally less difficult than working with antibodies against tumor-specific antigens.

Primary cultures of rhesus monkey kidney (*3*) were infected with about 10^4 tissue culture infectious doses of mumps virus, and maintained at 36°C in 1.5 ml of Eagle's minimum essential medium (MEM) which contained 2 percent fetal calf serum that had been previously inactivated at 56°C for 30 minutes. Under these circumstances, mumps virus replicates slowly, so that the cells appeared morphologically intact and excluded a vital dye (erythrosin) for at least 7 to 8 days after infection, although viral antigen in the cell membrane was demonstrable by hemadsorption as early as 4 days after infection.

Diphtheria toxin was purified by the method of Goor and Pappenheimer (*4*), except that we used 0.15*M* phosphate buffer, *p*H 6.5, to elute the toxin from the diethylaminoethyl (DEAE) cellulose column, and phosphate-buffered saline, *p*H 7.4, as our final solvent. The final preparation contained 2000 flocculating (Lf) units per milliliter and 2.5 μg of protein per Lf unit. The immunoglobulin G (γG) fraction was isolated from guinea pig antiserum to mumps virus (5) by chromatography on DEAE cellulose (*6*, pp. 322–25).

Toluene diisocyanate (TDIC) was used to conjugate antibody to toxin (*6*, pp. 150–55). Liquid TDIC (0.1 ml) was added to 20 to 25 mg of γG in 5 ml of phosphate buffer, *p*H 7.5, ionic strength 0.1, and the mixture was stirred at 0°C for 30 minutes. The precipitated TDIC was then removed by centrifugation, and the supernatant was filtered through a Millipore HA filter. After standing at 0°C for another 50 minutes, the clarified solution was then mixed with diphtheria toxin (0.75 mg per milligram of antibody; 1.8 moles