The isolate obtained from the pool collected in Brooks was sent to Dr. Telford Work, Communicable Disease Center, Atlanta, for identification and was found by the gel-diffusion method to be a member of BFS-283, La Crosse, Snowshoe Hare subgroup. In our laboratory, neutralization tests were performed with both isolates, and both the WE and the CE immune serums were used. No neutralization of infectivity was observed with WE immune serum. The isolates were neutralized by both CE serums—the serum prepared in our laboratory and the one obtained from Atlanta, as shown in Table 1. Two CE immune serums were used because we wanted to compare results obtained with the serums prepared in our laboratory and the one obtained from the Communicable Disease Center. California encephalitis virus was isolated from two pools of *C. inornata* collected in mammalian burrows of Alberta. This is the first isolation of CE virus from *C. inornata* in Canada.

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Sialic Acid in HeLa Cells: Effect of Hydrocortisone

Abstract. *HeLa S_3 cells grown in the presence of hydrocortisone contain more sialic acid than their corresponding untreated controls. The percentage of the total sialic acid present in the sedimentable fraction obtained from homogenates of cells treated with hydrocortisone is smaller than that of the corresponding fraction of control cells. In addition to* N-*acetylneuraminic acid, the chromatographic analysis suggests that HeLa S_3 cells probably contain trace amounts of* N-*glycolylneuraminic acid.*

Several lines of human cells undergo biochemical changes when cultured in the presence of hydrocortisone (*1*). Cell growth in this steroid-rich environment results in alterations of membrane structure (*2*). It has been postulated that modifications of the membrane structure affect membrane function and thereby the metabolism of dividing cells (*3*).

Sialic acid residues play an important role in the maintenance of membrane structure of dividing cells and are responsible for the electronegative charge of the cells as revealed by boundary electrophoresis (*4*). We now report the sialic acid content of HeLa S_3 cells growing exponentially in monolayer cultures in the presence and absence of added hydrocortisone.

HeLa S_3 cells were grown in monolayer culture with Waymouth medium supplemented by 10 percent of calf serum, 50 units per milliliter of penicillin, and 50 μg per milliliter each of streptomycin and kanamycin (*5*). These cells, found to be free of pleuropneumonia-like organisms (*6*), were cultured in Roux bottles with (treated) and without (control) hydrocortisone hemisuccinate (2 μg/ml) at 37°C for 72 hours and were harvested by gentle scraping. The cells were centrifuged, suspended in saline with tris(hydroxymethyl)-aminomethane (0.001*M*, *p*H 7.4), and a small sample was taken for cell counting. These samples were treated with an equal volume of 0.0025 percent trypsin in 0.3 m*M* ethylenediaminetetraacetate (*p*H 9 to 10 for 5 minutes at 37°C) to insure complete cell disaggregation, prior to counting in a Levi hemocytometer. The cells from each bottle were packed by centrifugation and suspended in 2 ml of deionized distilled water at 0°C. They were then disrupted by two 15-second bursts of ultrasound delivered by the microprobe of a Bronson Bio-Sonicator at 70 percent efficiency with a 14-kc output. The protein content was determined (*7*) with crystalline bovine serum albumin as the standard. The homogenates were subjected to acid hydrolysis with 0.1*N* sulfuric acid at 80°C for 1 hour to release bound sialic acid, and the sulfuric acid was then precipitated with an equivalent amount of saturated barium hydroxide. The supernatant fraction plus one water washing of the sediment were passed successively through columns (4 by 30 mm) of Dowex 50W (H^+) and Dowex I (formate). The columns were washed with distilled water, and sialic acid was eluted from the Dowex I (formate) column with

Table 1. Sialic acid and protein content (per culture) of HeLa S_3 cells grown in the presence and absence of added hydrocortisone.

Sialic acid (nmole)	Protein (mg)	Cell count $\times 10^{-7}$ (mean ± S.D.)	Sialic acid			
			Nmole/ mg protein	Nmole/ 10^8 cells	Sedimented (%)*	Non-sedimented (%)*
		Control cells (Expt. A)				
102.93	22.0		4.68	1.72		
93.66	21.0	6.00 ± 0.70	4.46	1.56	63.8	36.2
115.71	26.0		4.45	1.93	61.9	38.1
		Control cells (Expt. B)				
131.38	20.0	6.82 ± 0.40	6.57	1.93		
129.38	20.0		6.47	1.90		
		Hydrocortisone-treated cells (Expt. A)				
151.01	20.0		7.55	3.78		
146.09	18.0	4.00 ± 0.40	8.12	3.65	54.9	45.1
138.86	25.0		5.55	3.47	51.7	48.3
		Hydrocortisone-treated cells (Expt. B)				
137.17	19.2	3.64 ± 0.22	7.14	3.77		
128.95	16.8		7.68	3.54		

* Percentages of sialic acid in pellet and supernatant fraction, respectively, obtained by subjecting the sonicated cells to ultracentrifugation at 100,000*g* for 1 hour. Values are expressed with reference to the total sialic acid recovered. The overall recovery with respect to the unfractionated material was 90.6 ± 5.12 (mean ± S.D.).

0.3*N* formic acid. The eluate was lyophilized to remove formic acid, and the residue was dissolved in distilled water. The sialic acid content was determined by the thiobarbituric acid method (*8*) with crystalline *N*-acetylneuraminic acid as the standard. The chromogen produced by the sialic acid isolated from the cells and that produced by standard *N*-acetylneuraminic acid showed identical spectral characteristics. The sialic acid obtained from both control and induced cells was identified as *N*-acetylneuraminic acid by means of a chromatographic system for the identification and quantitative resolution of mixtures of sialic acids (*9*). Another spot also detected in these chromatograms by the thiobarbituric acid spray reagent (*10*) revealed traces of a material which had an R_F identical to that of standard *N*-glycolylneuraminic acid.

The sialic acid content, when referred to the protein content of the cells, shows a moderate but consistent increase in the preparations treated with hydrocortisone (Table 1). This difference is more striking when the sialic acid content is expressed per cell, in view of the pronounced difference in the cell count between control and hydrocortisone-treated cells. The cell count and protein content reflect the increase in size of the treated cells (*1*). The increase in both the size and the sialic acid content of hydrocortisone-treated cells is consistent with the observations of Kraemer (*4*) who found a direct correlation between cell volume and sialic acid content of synchronized hamster cell lines. The distribution of sialic acid betwen the sedimentable and unsedimentable fractions points out an additional difference between these two types of cells.

The consistent detection of trace amounts of a sialic acid with the chromatographic characteristics of *N*-glycolylneuraminic acid is of considerable interest because this type of sialic acid has never been found in human material (*11*).

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Choline in the Cell Wall of a Bacterium: Novel Type of Polymer-Linked Choline in Pneumococcus

Abstract. *Radioactive choline is incorporated by pneumococcus (strain R36A) into a polymeric substance from which it can be quantitatively recovered as free choline, after hydrolysis by strong acid. The polymeric substance is insoluble in lipid solvents and can be degraded by periodate. Fractionation studies and chemical analyses suggest that choline is linked to a polysaccharide component of pneumococcal cell wall.*

Diplococcus pneumoniae strain R36A has a nutritional requirement for choline (*1*). When pneumococci growing in a chemically defined medium containing choline (*2*) are transferred to choline-free medium, growth continues until the bacterial mass is approximately doubled; after this time protein and ribonucleic acid syntheses come to a halt and growth stops (Fig. 1).

At least a major part, if not all, of choline incorporated by these bacteria appears to be linked to a polymer which is associated with the cell wall fraction and which resembles a polysaccharide. Evidence for this conclusion came from the following experiments.

1) A culture of *Diplococcus pneumoniae* R36A clone R6 was grown at 37°C in a synthetic medium (*2*) supplemented with 0.05 μc (in 2 μg) of radioactive choline per milliliter (*3*). At the end of the logarithmic phase of growth, the cells were harvested by centrifugation, washed three times and suspended in saline at *p*H 8. This cell suspension was used in a number of analyses.

A portion of the washed cells was extracted with a mixture of chloroform and methanol (2 : 1) first at room temperature and then at the boiling temperature of the mixture under reflux. Only about 1 to 5 percent of the incorporated radioactivity became soluble by these treatments. The chloroform-methanol extract was concentrated under nitrogen, and was chromatographed on paper coated with silicic acid (Eastman chromogram sheet, type K301-R-2) with the solvent system consisting of diisobutylketone, acetic acid and water (8 : 5 : 1) (*4*). No more than traces of lecithin could be detected when the chromatograms were developed either by spraying with 2′, 7′-dichlorofluorescein in ethanol or by autoradiography. The major phospholipid component detected by the fluorescein stain had an R_F of 0.77 (lecithin $R_F = 0.41$) and had no radioactivity. Over 99 percent of the radioactivity of the chloroform-methanol extract remained at the origin of the chromatogram. A portion of the cell suspension with radioactivity of 1.02×10^6 count/min was extracted with 5 percent trichloroacetic acid (TCA) at 90°C for 15 minutes. After centrifugation, all of the radioactivity was found in the supernatant. The rest of the cell suspension was lysed by the addition of a few drops of 5 percent deoxycholate (DOC). A portion of this crude lysate was hydrolyzed in 6*N* HCl at 100°C for 10 hours. Chromatography of the hydrolyzate in four solvents which can resolve the methylated derivatives of ethanolamine and serine yielded single radioactive spots with mobilities identical to those of authentic choline (Fig. 2). Another portion of the DOC lysate was passed through a Sephadex G-75 column. The material containing the radioactivity was completely excluded by this gel filter. TCA (in a final concentration of 5 percent) was added to a third portion of the DOC lysate. After 30 minutes extraction at 0°C, the precipitate was removed by centrifugation. A variable but substantial fraction of the total label (between 50 to 90 percent in four separate ex-