*Summary and Conclusions.* Antigastric mucosal antibody reduces iron absorption in normal and anemic rats. This appears to result from the formation of large antibody–antigen complexes with the iron which are not easily absorbed. Such a mechanism might account for the idiopathic hypochromic microcytic anemia associated with complete achlorhydria and circulating parietal antibody. In this situation lack of acid might prevent degradation of the large molecules to a more easily absorbed form. A simple technique for studying iron binding *in vitro* was devised.

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## Effect of Neuraminidase on the Accumulation of Alpha-Aminoisobutyric Acid in HeLa Cells* (33927)

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(Introduced by R. L. Vernier)

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Sialic acid residues are major constituents of the surface of plasma membranes and contribute to the negative change of the surface membranes (1, 2). Several functional alterations of cells may be induced by removal of sialic acid from the surface membrane. These include enhanced phagocytosis (2, 3), inhibition of viral (4) or mycoplasma (5) induced hemagglutination, unmasking of cell surface antigens (6), inhibition of cell aggregation (7), and increased cell deformability (8).

The present studies illustrate how membrane composition can selectively be changed and related to discrete functional interrelationships of cells with each other and their environment. This method can similarly be utilized to study the effect of altered plasma membrane composition as applied to the function of the membrane as a barrier to the passage of simple chemicals. The effect of removal of surface membrane sialic acid from HeLa cells on the transport of alpha-aminoisobutyric acid was studied.

*Materials and Methods.* HeLa cells in monolayer culture (plaque bottle) were incubated in Eagle's basic media with Hanks' BSS (10% fetal calf serum) and used during the logarithmic phase of growth. The growth media were removed and the monolayer was washed twice with Hanks' BSS (pH 7.4). Four ml of Hanks' BSS containing 0.1–0.2 $\mu$Ci $^{14}$C-1-alpha-aminoisobutyric ($^{14}$C-AIB) acid (New England Nuclear Corp., sp act 5.5 mCi/mmole) were added and the cells were incubated at 37° for 30 or 60 min. Incubation media were quickly removed and the cells washed three times with cold BSS. The cells were suspended in 2.0 ml of 0.02% $Na_2EDTA$. An aliquot was taken for determination of protein (9) and the remainder

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TABLE I. Uptake of $^{14}$C-Alpha-Aminoisobutyric Acid by HeLa Cells.

| Treatment | (cpm/g of prot N)[a] | Percentage of control |
|---|---|---|
| Ouabain, $10^{-4}$ *M* | 246 ( 202– 330)[b] | 18 |
| Control | 1374 (1216–1632)[b] | |
| 2,4-Dinitrophenol, $10^{-4}$ *M* | 1694 (1352–1950)[c] | 55 |
| Control | 3065 (2736–3247)[c] | |
| Incubation, 4° | 142 ( 110– 172)[b] | 12 |
| Control | 1136 (1006–1260)[b] | |
| Neuraminidase, | | |
| 25 units/ml of BSS | 530 ( 498– 583)[b] | 70 |
| 50 units/ml of BSS | 375 ( 330– 405)[b] | 48 |
| Control | 756 ( 655– 862)[b] | |
| Ribonuclease, 0.05 mg/ml of BSS | 1084 ( 969–1221)[b] | 98 |
| Control | 1108 (1075–1156)[b] | |

[a] Mean (range) of at least 4 incubations.
[b] Incubation, 30 min.
[c] Incubation, 60 min.

was boiled for 10 min at 100° and centrifuged at 1500 rpm. The radioactivity in the supernatant was determined by addition to Bray's solution and counted in a Packard liquid scintillation spectrometer. The net accumulation of amino acid was expressed as cpm/$\mu$g of protein nitrogen. Efflux was determined by incubation of the HeLa cells in BSS with $^{14}$C-AIB for 60 min at 37°, aspiration of the incubation media, washing with BSS (room temp) three times; and replacement of the media with BSS without radioactivity. The incubation media and the wash were combined and radioactivity was determined. Efflux was expressed as the percentage of the counts remaining the cells at each interval as compared with a control group incubated with $^{14}$C-AIB for 60 min. Viability of the cells was determined by exposure to trypan blue after brief incubation with 0.02% trypsin. All determinations were in quadruplicate.

*Results.* The $^{14}$C-AIB accumulation was inhibited by ouabain, dinitrophenol, and reduced temperature (Table I), characteristics which are similar to amino acid transport with other cells and organ systems. Addition of neuraminidase (*Vibrio cholerae*, strain Z4, 500 units/ml or 10 units/$\mu$g of protein nitrogen, General Biochemicals) to the incubation media at concentrations of 25 or 50 units/ml of BSS decreased $^{14}$C-AIB uptake at 30 min to 70 or 48% of control values, respectively; 95% of the enzyme treated cells were viable by trypan blue exclusion studies. There was no significant neuraminidase induced change in the efflux of $^{14}$C-AIB from preloaded cells (Fig. 1). Although ribonuclease had been shown to alter the net change and calcium binding of some cells (10); incubation of HeLa cells with ribonuclease (Sigma, 75 Kunitz units/g at a concentration of 0.05 mg/ml fo BSS was not associated with any change in $^{14}$C-AIB uptake.)

*Discussion.* The mechanism of the inhibitory effect of neuraminidase on the $^{14}$C-AIB uptake is not clear. "Surface sialic acid" is the primary target of neuraminidase treatment of intact cells (11). The resultant change in the negative charge of the cell surface (1, 2) or the close relationship of

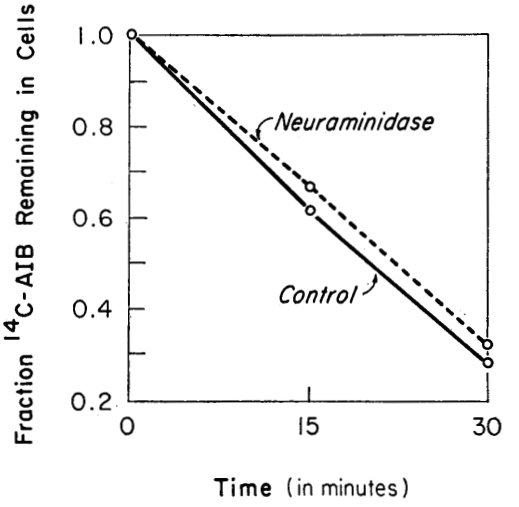


FIG. 1. The effect of neuraminidase (50 units/ml of BSS) on the efflux of $^{14}$C-alpha aminoisobutyric acid ($^{14}$C-AIB) from HeLa cells: BSS washed cells were incubated for 60 min with $^{14}$C-AIB, washed with BSS, and incubated with BSS (without $^{14}$C-AIB) in the presence or absence of neuraminidase. Efflux is expressed as the fraction of the $^{14}$C remaining in the cells.

transport-related enzymes (e.g., $Na^+$, $K^+$ $Mg^{2+}$–ATPase) to the plasma membrane (12) may have relevance to the maintenance of normal transport mechanisms.

*Summary.* An amino acid transport system in HeLa cells utilizing $^{14}C$-1-alpha-aminoisobutyric acid is described. Neuraminidase treatment of HeLa cells decreases the net accumulation of amino acid without alteration of the rate of efflux of preloaded cells. These studies suggest that the sialic acid residue of the external cell surface may have a significant role in amino-acid transport in human cells.

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## Suppression of Antibody Synthesis by Chloramphenicol Analogs* (33928)

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We have previously demonstrated that chloramphenicol can effectively inhibit protein synthesis in mammalian systems (1, 2). Significant inhibition of mammalian protein synthesis *in vivo* occurs only in those situations where cells are being committed to newly induced protein synthesis and where new messenger RNA (mRNA) is being formed and deposited on ribosomes. The ability of chloramphenicol to inhibit newly induced mammalian protein synthesis is reflected in its ability to suppress immune responses in animals and in humans when used in pharmacologic amounts during the inductive phase of antibody synthesis (2–5).

Both the antimocrobial properties of chloramphenicol and its inhibitory effect on protein synthesis are dependent upon maintaining the steric configuration of the molecule. Jardetzky (6) demonstrated, by nuclear magnetic resonance studies, that chloramphenicol has the configuration of a pyrimidine analog. Modification of the propranediol moiety of chloramphenicol alters the steric configuration with a resultant loss of antimicrobial activity. In contrast, substituion of the *p*-nitrophenol moiety of chloramphenicol by various alkyl groups does not alter the steric configuration of the molecule and such compounds retain antimicrobial properties. A number of such chloramphenicol analogs with antimicrobial properties have been prepared but their effectiveness as inhibitors of mammalian protein synthesis has not been determined.

The present study was undertaken to compare the effectiveness of chloramphenicol and a number of chloramphenicol analogs with respect to their effectiveness in inhibiting primary antibody synthesis *in vivo*. The comparative effectiveness of these compounds in prolonging the survival of skin homografts was also determined. Certain analogs of chlo-

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