The Effect of Neuraminidase on the Rabbit Urinary Interferon

The possible presence of carbohydrates in interferon has been repeatedly suggested[1–4]. Although the degree of purification has been very high in some cases, it does not warrant a definite conclusion classifying interferon as a glycoprotein[5]. Loss of activity after periodate treatment suggests[6,7] that the carbohydrate moiety may be important for the activity, but the aspecificity of action of this oxidizing agent[6,8] leaves the problem open to further investigation. It may also be mentioned that α-amylase[3] and lysozyme[7] had no effect on interferon.

In the Table we have summarized some data indicating that the removal of sialic acid from some glycoproteins can destroy either the biological activity, or reduce the electrophoretic mobility, or alter the biological function.

The rabbit urinary interferon has an isoionic point of 6 or lower and a very broad range of electrophoretic mobilities, most of the activity being located on the α–β globulin region in block and gel media[17]. If interferon contains sialic acid, and if this is cleaved by neuraminidase, the isoelectric point may be lowered and the electrophoretic mobility may be reduced.

First of all it was necessary to examine the effect of neuraminidase on the biological activity of interferon and, as we are not aware of such a study, we wish now to give a brief account of our results.

Urinary interferon was obtained from male rabbits after i.v. inoculation of Newcastle disease virus (NDV) as previously described[17,18]. 100 ml of dialyzed urine yielded 85–100 mg of proteins that were lyophilized and stored under refrigeration.

Interferon was titrated in baby rabbit kidney cell cultures by measuring viral inhibitory effect by plaque reduction method of vesicular stomatitis virus (VSV)[17]. The average specific activity of urinary interferon was 5000 U/mg urinary proteins.

Neuraminidase of *V. cholerae* (Batch No. 469D) was obtained from Behringwerke and the enzyme preparation was reported to be protease-free and contained 500 U/ml.

10 ml of urinary proteins containing interferon (3–4 mg/ml) dissolved in 0.15 *M* NaCl, 0.05 *M* sodium acetate-acetic acid buffer (pH 5.5) and 20 m*M* $CaCl_2$ were incubated at 37 °C in the presence of chloroform, for up to 9 days with a total of 1000 U of neuraminidase (usually the second lot of 500 U was added in the fourth day). Control samples were incubated without neuraminidase in the same conditions.

Aliquots were withdrawn from the sample at different times during incubation and were immediately frozen; those to be used for interferon activity measurement were also diluted tenfold with medium 199-serum (pH 7.4).

Sialic acid content was measured according to AMINOFF[19]. Total sialic acid content was measured from the same sample hydrolyzed in 0.05 *N* H_2SO_4 at 80 °C for 1 h. Residual sialic acid content and interferon activity are expressed as a percentage of the original at the beginning of incubation.

The Figure shows the change in sialic acid content and interferon activity for neuraminidase-treated and untreated rabbit urinary proteins as a function of time. The upper diagram (h) shows that the very rapid release of sialic acid from glycoproteins is not accompanied by a parallel loss of antiviral activity, the latter decaying similar to the control. The unlikely possibilities remain that either the bulk of contaminant glycoproteins binds most of the enzyme, or that interferon, like other glycoproteins, may contain sialic acid resistant to the neuraminidase used[20,21]. However, it can be noted that after complete desialization, or in the control at 8–9 days, about 30% of the original interferon activity is still present.

The conclusion has been drawn that if sialic acid is a constituent of interferon, it has no role in its activity.

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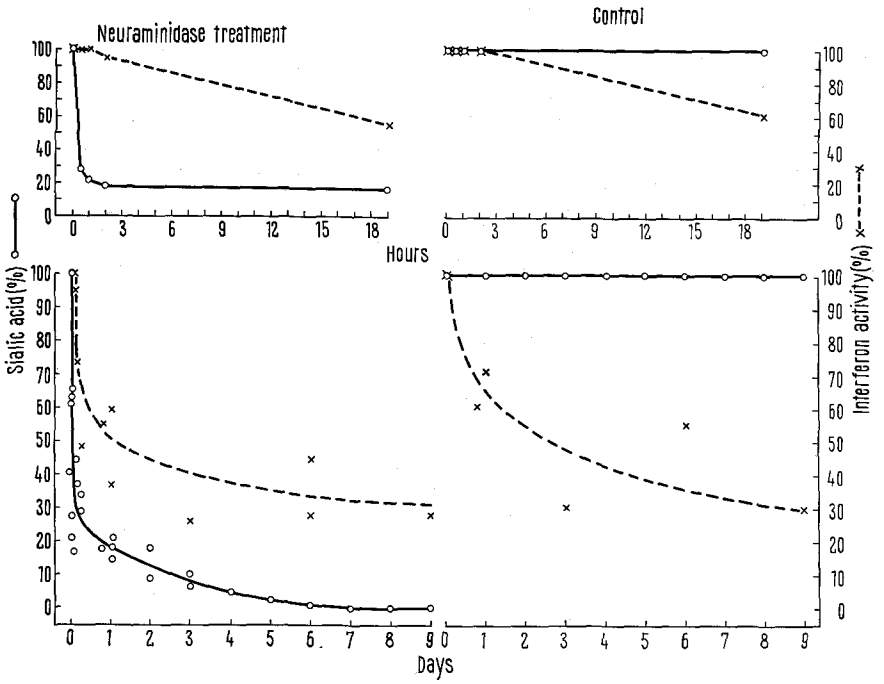
Effects of neuraminidase on some glycoproteins

Protein	Effect	Reference
Chorionic gonadotropin	Biological activity destroyed	GOT and BOURRILLON[9]
Erythropoietin	Biological activity destroyed	LOWY et al.[10]
Follicle-stimulating hormone	Biological activity destroyed	GOTTSCHALK et al.[11]
Tamm and Horsfall glycoproteins	Reduced electrophoretic mobility	PERLMAN[12]
Haptoglobin	Reduced electrophoretic mobility	SCHULTZE[13]
Transferrin	Reduced electrophoretic mobility	SCHULTZE[13]
Ceruloplasmin	Reduced electrophoretic mobility	POULIK[14]
Serum cholinesterase	Reduced electrophoretic mobility	SVENSMARK and KRISTENSEN[15]
Fibrinogen	Incomplete clotting	MESTER[16]



Residual sialic acid content and interferon activity (as a percentage of the original) of neuraminidase-treated and control samples of rabbit urinary proteins containing interferon.

Work now in progress based on isoelectric fractionation of neuraminidase-treated sample might give further information on this point. The progressive loss of activity is not due to bacterial contamination as samples were sterile and could be due to traces of proteolytic enzymes contained in the urine. The high susceptibility of interferon to proteases is well known[5].

In spite of extensive efforts[5], the complete purification of interferon still appears a very difficult task, and for the time being, considering the number of glycosidases available, the study of their specific effect on the interferon activity may be rewarding[22].

Riassunto. Proteine urinarie di coniglio contenenti interferone con una attività di 5000 U/mg sono state desializzate mediante neuraminidasi. La rapida e completa rimozione dell'acido sialico non si accompagna alla scomparsa della attività antivirale e pertanto, se l'interferone contiene acido sialico, quest'ultimo non ha un ruolo nell'attività biologica.

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The Effect of Acridines on the Synthesis of RNA in Isolated Nuclei and Intact HeLa Cells

Many acridines and related compounds have been used in chemotherapy, but their mechanisms of action remain obscure (see ALBERT[1] for a general review). Although the presence of different substituents in the heterocyclic nucleus profoundly affects activity, there is reason to suppose that there is a common mechanism of action. ALBERT[1] has shown a correlation between the bacteriostatic and basic properties of the acridines which indicates that activity depends on the presence of strongly basic amino groups. The aminoacridines interact strongly with nucleic acids[2] and other polyanions[3,4] and are powerful inhibitors of protein and nucleic acid metabolism in vivo[4,5]. Their effects in vivo resemble those produced by actinomycin D[5–7], and some of the aminoacridines which have been used in chemotherapy inhibit RNA polymerase in vitro[8]. Since the primary action of actinomycin D is probably the inhibition of DNA-dependent synthesis of RNA, it is possible that this may be a common mechanism of action of the acridines. A range of acridines has therefore been used to see if there is a correlation between inhibition of RNA synthesis in intact cells and in isolated nuclei.

Materials and methods. HeLa cells were grown and their nuclei isolated by methods described elsewhere[9]. Cells were labelled by incubating 10 ml culture containing 5×10^6 cells and 0.4 μC 8-^{14}C-adenine or G-^{14}C-guanosine (both of specific radioactivity 28 mC/m*M*) and acridines at a concentration of 61 μ*M*, for 30 min at 37 °C in the dark. Under these conditions over 90% of the radioactivity incorporated was in RNA; there was virtually no interconversion of the precursors. The cells were collected by centrifugation, washed with ice-cold 0.6 *M*-