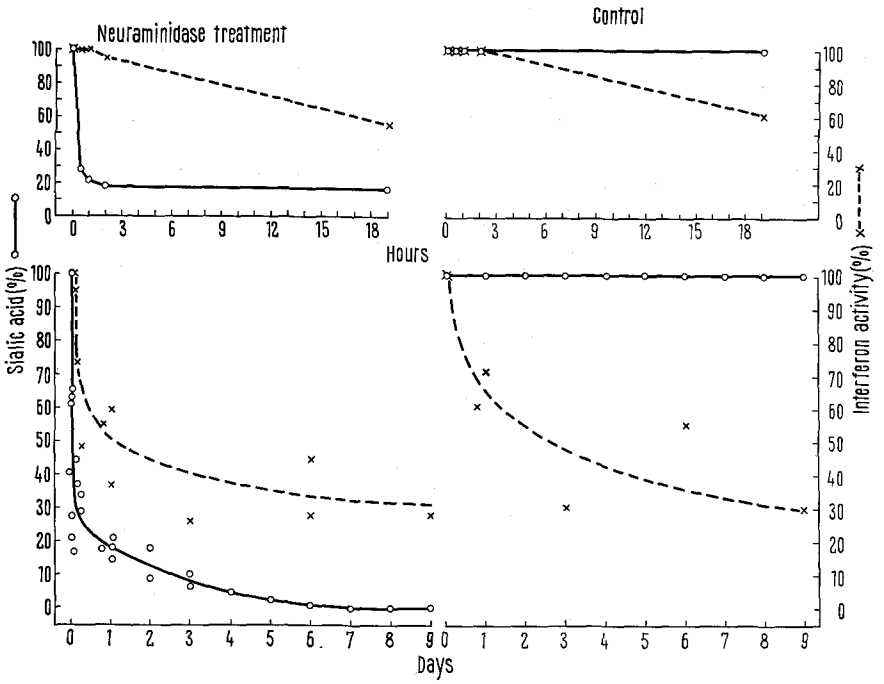

Residual sialic acid content and interferon activity (as a percentage of the original) of neuraminidase-treated and control samples of rabbit urinary proteins containing interferon.

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*-

The effect of acridines on RNA synthesis in isolated nuclei and whole cells

Compound	RNA synthesis (% of controls) Whole cells labelled with ^{14}C-adenine	^{14}C-guanosine	Isolated nuclei	Bacteriostatic index	pK_a
4-methoxyacridine	104	101	115	3	5.0
9-methoxyacridine	96	102	116		
9-mercaptoacridine	92	86	103		
Ethidium bromide	75	50	31	–	
3,6-diacetamidoacridine	72	40	112		
Acridine orange	52	32	62	17	10.1
Acriflavine	32	22	49	22	12
Rivanol	25	15	73	20	11.1
Acridine orange^R	22	13	46	13	–
Acridine yellow	20	8	86	21	9.8
4-hydroxyacridine	12	8	110	12	5.2
Mepacrine	6	6	113	10	10.0
9-aminoacridine	9	5	81	25	9.6
Proflavine	8	4	67	22	9.3

trichloroacetic acid and water. RNA was then extracted with 3 ml 10% aqueous sodium chloride solution for 1 h at 100 °C, and precipitated with 3 vols. of ethanol.

Isolated nuclei were labelled by incubating nuclear suspension equivalent to 150 µg DNA for 45 min at 37 °C in the dark in 0.5 ml of reaction medium that contained 0.1 *M* *Tris*-HCl buffer (pH 7.5), 0.1 *M* sucrose, 0.2 *M* $(NH_4)_2SO_4$, 5 m*M* $MgCl_2$, 3 m*M* $MnCl_2$, 60 m*M* KCl, 20 m*M* NaF, 1 m*M* CTP, 1 m*M* GTP, 1 m*M* UTP, 0.01 µmoles 8-^{14}C-ATP (specific radioactivity 30 mC/m*M*), and acridines at a concentration of 180 µ*M*. Under these conditions incorporation of radioactivity into RNA continued for over 1 h. The reaction was stopped by addition of 2.5 ml ice-cold 0.6 *M* trichloroacetic acid, and RNA was extracted by the method used with whole cells.

The radioactivity in RNA was determined with a scintillation counter[9]; all counts were corrected for internal quenching.

Results and discussion. The Table shows the effects of different acridines on the incorporation of labelled precursors into the RNA of isolated nuclei and intact cells. In order to assist comparisons, the concentrations of acridines were chosen to give only partial inhibition of incorporation. The values for pK_a and bacteriostatic index are taken from ALBERT[1]; the index is a measure of the activity of the acridine against several bacterial species and roughly parallels pK_a.

Considering first the inhibition of RNA synthesis in isolated nuclei, it appears that the acridines fall into 2 main groups: the aminoacridines with high pK_a and bacteriostatic index strongly inhibit RNA synthesis in vitro, whilst the reverse is true of acridines with low pK_a and bacteriostatic index. The single exception to this is the heavily substituted aminoacridine mepacrine which at this concentration does not inhibit RNA synthesis. Higher concentrations (400 µ*M*) of mepacrine are, however, very inhibitory. There is thus a broad correlation between pK_a, bacteriostatic index, and inhibition of RNA synthesis in vitro, although substituents in the molecule also influence activity.

The incorporation into intact cells of both ^{14}C-adenine and ^{14}C-guanosine was examined to ensure that effects produced by the acridines did not simply reflect inhibition of turnover of adenine in the end-groups of transfer RNA. Both precursors gave parallel sets of results, although in general the incorporation of ^{14}C-guanosine was inhibited more than was that of ^{14}C-adenine. The figures for incorporation of precursors into whole cells show that pK_a, bacteriostatic index and incorporation in vitro are again correlated although there are obvious exceptions. Thus mepacrine is considerably more active in vivo than expected from the other parameters. In contrast, the phenanthridine ethidium bromide is relatively less active in vivo than in vitro. 4-Hydroxyacridine is quite exceptional, since the pK_a and activity in vitro would indicate low activity in vivo; as the Table shows, however, it is very inhibitory to incorporation of precursors into the intact cell and has a higher bacteriostatic index than mepacrine.

It seems reasonable to conclude, therefore, that inhibition of RNA polymerase is often a major feature in the action of the aminoacridines, but other factors considerably modify this property. The presence of substituents in the acridine molecule affects not only its diffusion into the cell and nucleus, but also its ability to inhibit both RNA polymerase and other enzyme systems.

Zusammenfassung. Eine Reihe von Acridinen wurde auf ihre Hemmung der RNS-Synthese intakter Zellen und isolierter Kerne untersucht. In Kernen wurden ähnliche Hemmungsverhältnisse gefunden wie in Bakterien, während in intakten Zellen die Reihenfolge der Stärke der Hemmwirkungen sehr verschieden ist.

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