| Compound | Concentration | Sex treated | Pairs crossed | Egg-pod per pair | Percent hatch of eggs | Percent corrected sterility | Mortality after 7 days |
|---|---|---|---|---|---|---|---|
| GDW | Control | Both | 5 | 1.00 | 90.00 | - | 10 |
| 51,240 | 5% | Both | 5 | 0.80 | 53.57 | 40.48 | 30 |
| | 1% | Both | 5 | 0.80 | 69.77 | 22.48 | 10 |
| GDW | Control | Both | 5 | 1.00 | 90.00 | - | 10 |
| 61,914 | 0.5% | Both | 5 | 1.80 | 44.44 | 47.77 | 30 |
| DMSO + $H_2O$ (1:1) | Control | Both | 5 | 2.60 | 61.54 | 27.67 | 20 |
| GDW | Control | Both | 5 | 3.00 | 85.08 | - | 0 |

GDW, glass distilled water; DMSO, dimethyl sulfoxide.

*Results and discussion.* (table): The compounds 1, 2, 4-dithiazolium-3-(dimethylamino)-5-[(2-hydroxyethyl) methylamino] iodide (62206), 2-(isopropylamino) ethanol (24220) and bis (dimethyl-amino) dithiazolium chloride (51160) has been found most effective at different dose levels, (compound 62206 at dose levels of 3% and 1%, 24220 at dose levels of 2.5% and 1% and 51160 at dose levels of 1% and 0.5%). The compound N, N, N', N'-tetramethyl-P-piperidinophosphonic diamide (51007) and hexamethyl phosphoric triamide i.e. hempa (50882) exhibit intermediate effectiveness at dose levels of 2.5% and 4% respectively. Compound 3-amidino-dithiocarbazic acid (61914) along with the solvent dimethyl sufloxide (DMSO) is only slightly effective at a dose level of 0.5%. Other compounds viz., 2, 4-diamino-6-(dimethylamino)-s-triazine hydrochloride (50994), boric acid (2406) and pentamethylmelamine hydrochloride (51240) are almost non-effective, although little effect is recorded at dose levels of 2%, 4% and 5% respectively.

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## Kinetics of interferon action[1]

H. Koblet[2], R. Wyler[3] and U. Kohler[2]

*Institute of Medical Microbiology, University of Berne, Friedbühlstrasse 51, CH-3008 Berne; and Institute of Virology, University of Zurich, Winterthurerstrasse 266A, CH-8057 Zurich (Switzerland), 1 September 1977*

*Summary.* A kinetic analysis of the action of interferon with different preparations in chick embryo fibroblast cell culture gives additional evidence for interaction of interferon with the cell surface, compatible with the idea that interferon is not taken up by the cells. With certain assumptions the binding constant is in the range of $10^{13}$ [l/Mol].

The host protein interferon is supposed to need an intact synthesis of host macromolecules to establish within hours a full state of inhibition of viral growth. The increase of resistance can be interrupted at intermediary levels, if actinomycin D is given at any time in the course of the development of the 'antiviral state'[4-6]. If interferon has a single mechanism of action, it might be summarized[7] as follows. Interferons bind to a specific receptor on the cell surface. This causes changes in the cell membrane, which, possibly through a cyclic adenosine-3':5'-monophosphate effect, result in the production of an inactive precursor of an antiviral substance. After viral infection and formation of a double-stranded viral RNA, this precursor is activated in a step involving phosphorylation to produce an antiviral substance that selectively inhibits a step in the initiation of translation of viral mRNA (review, Friedman[7]). However, it is still not clear whether interferon delivers the signal for this induction at the surface of the cellular membrane or within the cell. Several types of experiments give circumstantial evidence for the former mechanism[8-11].

The purpose of this paper is to add evidence for one of these possibilities on the basis of kinetic data. The rationale for the experiment is as follows.

After adding interferon to cells, a full state of protection is established after about 5-6 h. The degree of protection is given by the reduction of viral replication as compared to uninhibited replication. During the development of the protection, the time-dependent intermediary levels can be estimated by blocking any further increase of protection with actinomycin D; therefore the rate of increase of protection can be estimated. This rate is linear; the rate and final levels are dependent upon concentration of interferon. A presence of a few min is sufficient to trigger a protection[15,16]. Now, if there is an intracellular accumulation, the concentration of interferon should increase within the cell as a function of the time of contact. Therefore, unequal

times of contact should result in unequal rates of development and unequal final levels of protection, even if the outside concentrations of interferon are equal.

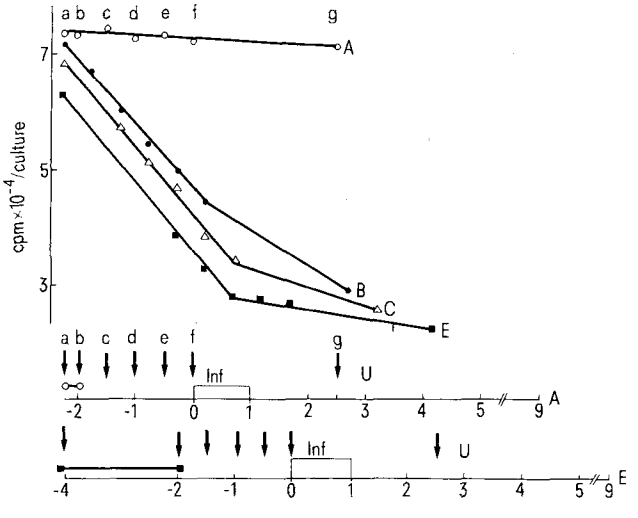
On the other hand, a mere surface interaction without transport should provoke equal rates and final resistance levels despite unequal times of presence, given equal outside concentrations. This outside concentration has to be high to saturate surface sites within short time intervals.

*Materials and methods.* Cell cultures, media and infection with Semliki Forest Virus (SFV), Zurich strain, were as described[12]. Interferon preparations were from allantoic fluids and chicken embryos after infection with Newcastle Disease Virus according to established methods. Interferon 14, the standard preparation, contained 50 units/mg protein; interferon 10/1[13]:20,000 units/mg. Antiviral states were assayed by measuring the incorporation of [$^3$H]-uridine[14] into acid-precipitable material of SFV-infected chick embryo fibroblast (CEF) monolayers treated with actinomycin D (RNA-test[12]). All experiments were done at 37 °C. The time-table (addition of interferon, actinomycin, uridine and infection) is given in the figure. The following terms are used: 'presence' means that interferon is in the culture fluid; 'induction period' is the time between addition of interferon and actinomycin and may be shorter or longer than, or equal to, the time of presence. Induction does not imply that Jacob-Monod types of regulation are involved. Different preparations yield the same results; therefore results with interferon 14 are shown.

*Results and discussion.* The figure shows that in every experiment the slopes of the resistance development are the same, and the end points at full protection approach an equal level. The main set A makes an exception (presence of interferon 15 min): no resistance developed. A presence of 10–15 min seems to reflect the critical time at our concentration[15,16], needed to establish the changes at the cell membrane.

Our conclusion is that a membrane-action is much more probable than an intracellular action as a signal for the induction of resistance. Therefore the following calculations are possible. Knight[17] purified human interferon to about $2\times10^8$ units/mg protein. Based on this degree of purification, the contamination factor of our interferon 14 is $4\times10^6$. 1 µg/ml of interferon 14 (=0.05 units) can be assayed with the RNA-test[12] and contains $2.5\times10^{-7}$ µg of active principle. Assuming a mol.wt of 25,000[12] and $10^7$ cells per culture, this corresponds to an input of not more than 1 molecule per cell as upper estimate for a minimal action. Based on these assumptions and the mass equation, an equilibrium constant for the binding reaction (37 °C) can be derived of about $10^{13}$ [l/Mol].

We point here to the peculiar fact that a modest level of resistance can be found even if actinomycin is added together with interferon. This resistance increases with the time of presence, as can be seen by comparing the levels of the subsets a. This phenomenon has been described as the direct effect of interferon preparations which are not highly purified[18].




Development of resistance levels; concentrations constant, times of presence and of induction variable (RNA-test).

CEF cultures were divided into 7 main sets A–G, 4 of which are shown. Set A (○—○) had 60 µg/ml (3 units) of interferon 14 for 15 min, set B (●—●) for 30 min, set C (△—△) for 1 h, set D (not shown) for 1.5 h, set E (■—■) for 2 h, set F (not shown) for 2.5 h, set G (not shown) for 3 h. Then interferon was removed, cells were washed to stop a possible further uptake and Medium Eagle was overlayered. Infection (Inf) for 1 h with SFV to test replication (m.o.i.=1) started at 0 time, always 2 h after the end of the presence of interferon. 3 h later [$^3$H]-uridine (3 µCi/culture) was added (U) for 6 h; harvest was therefore always 9 h after the start (0 time) of infection. Radioactivity in acid-precipitable material (viral RNA) of cultures was determined. – In order to vary the periods of induction and to 'freeze' the intermediary resistance levels, the main sets were divided into 7 subsets *a–g* with 3 cultures each; they received actinomycin D (1 µg/ml) at the time indicated by an arrow. To illustrate the principle, the full time scales for the main sets A and E are given. Times of presence of interferon are indicated with the same symbols (○—○, ■—■) as used for the corresponding curves *A* and *E*. As the arrows show, subsets *a* were not allowed induction because interferon and actinomycin were added at the same time; subsets *b* had an induction period as long as the presence time; and subsets *c–g* had induction periods longer than presence times. Evidently, points are gained at the end of the experiment, but they are drawn in the time scales at the time of the actinomycin additions. Tacit assumption is that antiviral states are 'frozen' at the time of addition of actinomycin without further change.

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