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Received June 6, 1968. P.S.E.B.M., 1968, Vol. 129.

Kinetics of the Development of Factors Responsible for Interferon-Induced Resistance (33363)

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In interferon (IF)-treated cells the development of antiviral resistance requires the integrity of cellular RNA and protein syntheses (1–4). This finding incidates that IF acts by inducing the cells to produce a peptide or a protein which is directly responsible for the antiviral state. Previous experiments from this laboratory support the view that the production of an antiviral substance (5) in IF-treated cells does not require synthesis of newly induced, intermediary proteins. Therefore, the most satisfactory working model of the induction by IF of the antiviral state would require at least the following sequential events: (a) derepression of the antiviral protein (AVP) cistron; (b) poduction of messenger RNA (mRNA) for AVP; and (c) translation of the mRNA into AVP.

Although this model identifies the main variables, it does not specify many of the kinetics of interaction during establishment of the antiviral state. The object of the present paper is to consider some interactions of the factors which are involved in development and maintenance of the antiviral state in IF-treated mouse embryo (ME) cells. For clarity of presentation the concept of mRNA and AVP will be used to help interpret the findings with the full realization that these concepts are indirectly derived and subject to future reinterpretation.

Materials and Methods. The IF was produced in the serum of NIH strain mice by intravenous injection of Newcastle disease virus (6). Primary ME cell cultures and continuous mouse L cell cultures were prepared as previously described (7). Antiviral resistance was measured by determining the inhibition of yield of vesicular stomatitis virus (VSV) in a single step growth cycle as employed previously (7). Actinomycin D (Merck Sharp and Dohme) was used at a final concentration of 1 μg/ml. Cycloheximide (Sigma Chemical Co.) was used at a final concentration of 10 μg/ml. Previous experiments showed that both drugs exerted 95% inhibitory activity on RNA synthesis and on protein synthesis, respectively, in these cell cultures (5, 7).

Results and Discussion. Estimation of time of production of mRNA for antiviral protein in interferon-treated mouse embryo cells. Figure 1 shows the curve of development of the antiviral state in ME cells treated with different doses of IF. The cells were treated with IF at time 0. At each indicated interval of time, one set of tubes was washed carefully to remove IF and challenged with VSV in

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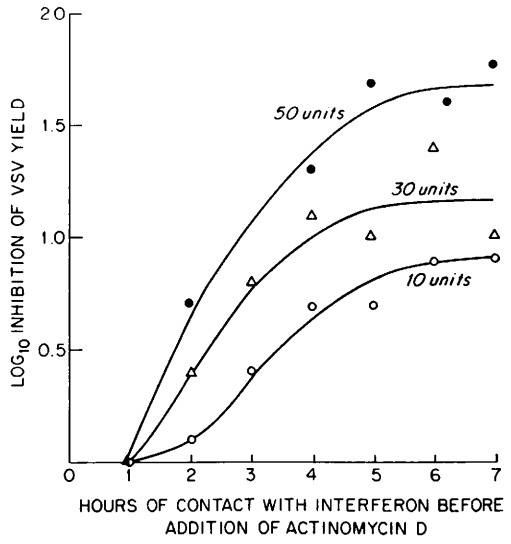

FIG. 1. Timed addition of actinomycin D to mouse embryo cells pretreated with interferon.

the presence of actinomycin D, which was added to stop any further production of mRNA. The degree of resistance at each challenge time would be expected to indicate the amount of the proposed AVP produced before virus challenge plus the amount of the hypothesized mRNA for AVP which was translated in time to inhibit virus replication. It has previously been determined that the resistance (AVP) is stable over the time intervals used in the present study (7). As shown in Fig. 1 antiviral resistance begins by 1–2 hr in the presence of 30 or more units of IF, implying the prior production of mRNA for AVP. It becomes detectable only after 3 hr in the presence of 10 units of IF. The maximum rate of development of resistance appears between 2 hr and 4–5 hr. After 5–7 hr maximum resistance has developed, indicating that the amount of mRNA required for production of the highest concentration of AVP at each dose level has been synthesized by 5–7 hr.

Is new mRNA produced and translated after removal of IF and challenge with VSV? The ME cells were treated with IF and at the times shown in Fig. 2 were washed free of IF and challenged with VSV in the presence or absence of actinomycin D. As shown the rate of development of resistance was the same whether actinomycin D was present or absent. Since production of mRNA is inhibited in the presence of actinomycin D this result indicates that no new mRNA for AVP is transcribed and then translated in ME cells after removal of IF and simultaneous challenge with VSV. The cessation of transcription and translation may be due to removal of interferon or to the reported inhibition by VSV of cellular macromolecular synthesis (8).

Estimation of time between transcription and production of antiviral state *(antiviral protein).* To estimate the time of translation, mRNA production was stopped with actinomycin D, and the subsequent time required for development of maximum resistance was determined. Specifically, ME cells were treated with IF, 200 units/ml, and after different intervals of time IF was removed and actinomycin D was added. Then the tubes were divided in groups, one of which was challanged immediately in the presence of actinomycin D and the other groups were challenged after 3 or 6 hr of further incubation at 37°. The results are plotted in Fig. 3.

As shown 3 and 6 hr after the removal of IF and the addition of actinomycin D the level of resistance remained unchanged. This finding indicates that the translation of the proposed mRNA for AVP is largely com-

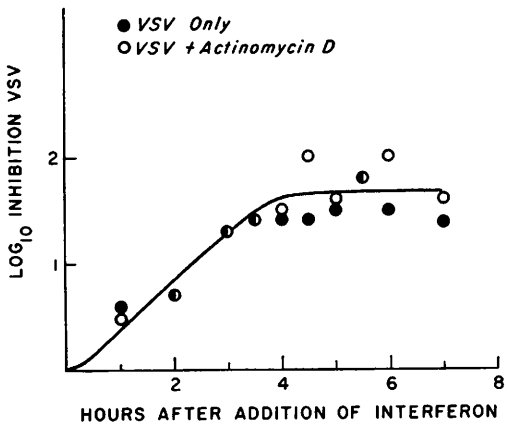

FIG. 2. Effect of actinomycin D on the development of resistance in mouse embryo cells pretreated with interferon. (●) VSV only. (○), VSV + actinomycin D.

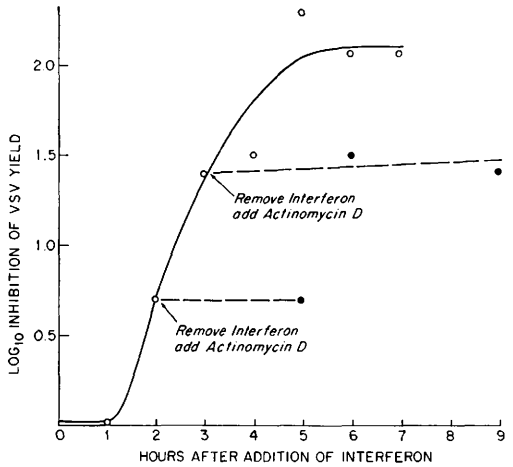

FIG. 3. Effect of actinomycin D added with challenge virus on the development of resistance in mouse embryo cells treated with interferon.

pleted within a short time after removal of IF because slower translation would have resulted in increasing resistance with time after addition of actinomycin D. It also follows that the mRNA is relatively unstable because otherwise it would continue to be translated, thereby causing a rising level of resistance.

Effect of removal of interferon or inhibition of protein and RNA synthesis. To help confirm the previous deduction, an experiment was designed to compare the degree of resistance following alteration of development of resistance in three ways: (a) removal of IF; (b) inhibition of RNA synthesis with actinomycin D; and (c) inhibition of both RNA and protein synthesis with actinomycin D and cycloheximide. Specifically, ME cultures were treated with IF, 30 units/ml. After 3 and 5 hr all the tubes were washed carefully to remove IF and were divided into four groups. One group was challenged at once without any treatment; a second group was challenged at the same time in the presence of actinomycin D, 1 μg/ml. A third group was refed with plain medium and a fourth was refed with medium containing actinomycin D and cycloheximide. After 3 hr of further incubation, the third and fourth groups were washed and challenged. The results are reported in Table I.

As shown, all three procedures, (a) simple removal of IF, (b) the removal of IF followed by the repression of RNA synthesis, or (c) removal of IF followed by the suppression of RNA and protein syntheses, arrest the rise of the resistance level. As in the preceding experiments, control cultures treated with actinomycin D and IF did not develop resistance and these treated cultures manifested 95% inhibition of RNA synthesis within 1 hr. The same results have been obtained when fluorophenylalanine was used

TABLE I. Effect on Development of Resistance of Removal of Interferon and Inhibition of Protein and RNA Synthesis.

Interferon[a] (hr)	Treatment after removal of interferon	Treatment before challenge (hr)	VSV yield ($\log_{10}$/ml)	Reduction of VSV yield ($\log_{10}$)	Reduction of VSV yield (%)
0	None	—	6.6	—	—
0	Cycloheximide[b] + actinomycin D[c]	3 or 5	6.5	—	—
3	None	0	5.8	0.8	84
3	Actinomycin D	0	5.7	0.8	84
3	None	3	5.7	0.9	87
3	Cycloheximide + actinomycin D	3	5.6	0.9	87
5	None	0	5.3	1.3	95
5	Actinomycin D	0	5.2	1.3	95
5	None	3	5.4	1.2	94
5	Cycloheximide + actinomycin D	3	4.9	1.6	98

[a] 30 units/ml.
[b] 10 μg/ml.
[c] 1 μg/ml.

as the inhibitor of protein synthesis in place of cycloheximide.

These findings support two conclusions. The unchanging resistance following inhibition of both protein and RNA synthesis for 3 hr confirms the relative stability of AVP (7) since no new AVP is synthesized under these conditions (2). Failure of resistance to continue to increase over the 3 hr after simple removal of IF suggests that removal of IF rapidly results in the cessation of production of the hypothesized mRNA for AVP since the level of the relatively stable AVP seems to be determined by the production of its mRNA.

It is probable that the factors which govern the development and maintenance of the antiviral state in interferon-treated cells may interrelate somewhat differently in different cell systems. In contrast to the 6-hr stability of resistance in ME cells after removal of interferon, chick embryo cells have been reported to continue to develop resistance for a period of time after removal of IF (9–11). Under certain experimental conditions this has been interpreted to be due to a relatively stable mRNA for AVP which continues to be translated after removal of IF (12). Under other conditions the continued development of resistance after removal of interferon from chick embryo cells has been interpreted to be due to continued transcription of mRNA after removal of IF from extracellular fluids (13).

Summary and Conclusions. The present study was performed to help determine the interaction of some of the factors which determine the development and maintenance of the antiviral state in IF-treated mouse embryo (ME) cell culture. Actinomycin D timing experiments were used to estimate the rate of formation of the hypothesized mRNA for the antiviral protein (AVP) of the IF system. Earliest formation is detectable by 1–2 hr and the amount of mRNA required for the production of the maximum resistance at each dose level is synthesized by 5–7 hr. Inhibition by actinomycin D of RNA production at the time of viral challenge of IF-treated ME cells, indicated that no new mRNA for AVP is transcribed and then translated after removal of IF and simultaneous challenge. Arrest of RNA synthesis in ME cells pretreated with IF was followed by an unchanged level of resistance over 6 hr, thereby suggesting that translation of the hypothesized mRNA into AVP is completed very rapidly and that the mRNA is unstable relative to AVP. Removal of IF from cultures in the presence or absence of inhibition of protein synthesis was also followed by an unchanged level of resistance. This finding was interpreted to mean that removal of IF from ME cultures rapidly results in the cessation of production of the hypothesized mRNA for the AVP.

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Received June 21, 1968. P.S.E.B.M., 1968, Vol. 129.