Effect of Crude and Purified Interferons on the Growth of Uninfected Cells in Culture.* (30694)

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One of the possible explanations of the antiviral action of interferon is that it inhibits some part of normal cellular metabolism which is required for virus multiplication. Inhibition of an important cellular metabolic event might be manifested as an inhibition of growth or biosynthesis of uninfected cells. Homologous interferon has not been observed to decrease the number of chick embryo cells in mitosis(1), nor to affect growth of human thyroid cells in culture(2), nor affect growth rate, DNA, RNA and protein synthesis of cultured chicken embryo cells(3). In contrast, other laboratories have reported that interferon preparations inhibit growth of cultures of mouse L cells(4) and decrease cellular synthesis of RNA in cultured rat and chicken cells(5,6).

Since all these studies employed crude or partially purified interferons, it is possible that the effects were due to noninterferon components. The present study was undertaken to help determine whether noninterferon inhibitors of cell growth were responsible for the reported inhibition of cell growth. For this purpose highly purified chicken interferon(7) and crude preparations of mouse serum interferon containing different quantities of interferon were used in studies of cell growth.

Materials and methods. Cells. Mouse L cells were cultured in Eagle's medium containing 5% calf serum and antibiotics. Primary mouse embryo cells were prepared by trypsinization of 17-day-old embryos and were grown in Eagle's medium containing 10% calf serum and antibiotics. Secondary chick embryo cells were obtained by trypsinization of primary chick embryo cell cultures originally prepared from 9-day-old embryos. Growth medium for secondary chick embryo cells was Eagle's medium with 10% fetal calf serum and antibiotics. For experiments, 1.0 ml of cell suspension in growth medium was added to each of a group of culture tubes. In experiments using mouse cells, interferon was added to the cell suspension before plating. In experiments using chick embryo cells, interferon was added 3 or 4 hours after plating of cells. After incubation at 37°C for various times, groups of 3 or 4 tubes were trypsinized and the cells counted in a hemocytometer. All counts were repeated 1 or 2 times. All experiments described were repeated at least once with similar results.

Interferon. Mouse serum interferon was prepared by injecting mice intravenously with Newcastle disease virus, as described(8). To destroy residual virus, the interferon preparations were acidified to pH 2 for at least 5 days at 4°C before neutralization. Normal mouse sera were similarly treated. Chicken interferon was purified by methods previously described(7). Interferon was assayed by vesicular stomatitis virus (VSV) plaque reduction on mouse embryo or chick embryo cell cultures, as previously described(9), or assayed by inhibition of cytopathic effect (CPE) in tube cultures challenged with 1000 pfu of VSV. The interferon assay in tube cultures was more sensitive than the assay in plates in proportion to the decreased cell number in tubes. Interferon titers are expressed as the dilution calculated to give 50% protection. In all experiments the antiviral action of homologous interferon was confirmed by protection of tube cultures against the CPE of 1000 pfu of VSV.

Results. Detection of cell growth inhibitor in preparations of interferon. The effect of crude mouse serum interferon, chicken allantoic fluid interferon and appropriate control fluids on the growth of cultured mouse L cells is shown in Table I. L cells were mixed

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TABLE I. Growth of Mouse L Cells in the Presence or Absence of Mouse or Chicken Interferon.

Growth medium containing	Dilution	Protective units of interferon/ tube	Cell count × 10^3/tube after 24 hr	48 hr	% inhibition at 48 hr
None	—	—	380	710	0
Mouse serum interferon	1/25	350	410	420	41
Normal mouse serum	"	—	540	610	14
Mouse serum interferon	1/250	35	450	700	1
Normal mouse serum	"	—	300	690	3
Chicken interferon	1/2.5	350	190	150	79
Normal allantoic fluid	"	—	64	175	76
Chicken interferon	1/25	35	390	280	61
Normal allantoic fluid	"	—	530	760	0

TABLE II. Growth of Mouse L Cells in the Presence or Absence of Varying Amounts of Mouse Interferon.

Growth medium containing	Dilution	Protective units of interferon/ tube	Cell count × 10^3/tube after 24 hr	48 hr	72 hr	% inhibition at 72 hr
None	—	—	150	185	260	0
Mouse serum interferon	1/50	4000	150	220	265	0
Normal mouse serum	"	—	125	180	300	0
Mouse serum interferon	1/500	400	135	205	250	4
Normal mouse serum	"	—	80	170	190	27
Mouse serum interferon	1/5000	40	100	240	275	0
Normal mouse serum	"	—	85	165	250	4

with growth medium containing one of the preparations and dispensed so that each culture tube received 5×10^5 cells in 1.0 ml. The experiments were generally continued until maximum cell growth occurred in control tubes. The mouse serum interferon in higher concentration protected the L cells against VSV and also inhibited cell growth by 41%. However, the 10-fold higher dilution of mouse serum interferon was also antiviral but did not inhibit cell growth. Neither the control fluids nor the heterologous chicken interferon protected L cells against VSV; however, these preparations inhibited cell growth to varying degrees. The inhibition of cell growth or plating was observed to decrease with increased dilution of the mouse serum interferon and the normal allantoic fluid. It should be noted that the inhibition of plating of cells at 24 hours by the low dilutions of chicken interferon and normal allantoic fluid can account for the final percent inhibition at 48 hours. Since the higher dilutions of the chicken preparations and all the mouse preparations showed no inhibition of plating, the final percent inhibition at 48 hours represents inhibition of cell growth by these preparations. Similar results were obtained in an experiment using primary mouse embryo cells. The finding that inhibition of cell growth was not always correlated with the antiviral action of interferon suggests the presence of a noninterferon growth inhibitor.

Growth inhibition by preparations containing increased amounts of mouse interferon. Preparations of mouse serum containing increased amounts of interferon would not necessarily contain proportionately increased amounts of noninterferon inhibitors of cell growth. In the experiment shown in Table II, a mouse serum interferon was used which contained 20 times more interferon than the serum shown in Table I. In this way the more potent interferon could be diluted 20 times more and still have the same antiviral activity. It may be seen in Table II that the lower dilutions of mouse serum interferon did not inhibit cell growth, although they contained more interferon than the serum dilution which inhibited growth in Table I. This finding is consistent with the presence of a noninterferon inhibitor of cell growth. The 27% inhibition of cell growth by a 1/500 dilution of normal serum is of borderline signi-

TABLE III. Growth of Chick Embryo Cells in Presence or Absence of Chicken Interferon.

Exp	Hr after adding cells to tubes	Cells per tube in presence of Interferon	Control
1	3 hours	80,000*	80,000
	27 "	160,000*	150,000
	51 "	270,000	325,000
2	4 hours	140,000†	140,000
	28 "	150,000†	140,000
	52 "	240,000	250,000
	76 "	330,000	300,000

* 100 units interferon added at 3 hr.
100 " " " " 27 "
† 20 units interferon added at 4 hr.
20 " " " " 28 "

ficance and was not studied further.

Effect of highly purified chicken interferon on growth of secondary chick embryo cells in culture. Chicken interferons of increased purity have become available(7,10). The effect of purified chicken interferon (containing 0.18 microgram protein per unit of interferon) on the growth of chick embryo cells in tube cultures is shown in Table III. To observe effects on growth rate in the absence of possible effects on plating efficiency, interferon was added after the cells had attached to the culture dish. There was no significant effect of interferon on cell growth as compared to control cultures in growth medium.

A second experiment was done using another similarly purified preparation of interferon. As may be seen in Table III there was again no inhibition of cell growth by purified chicken interferon.

Discussion. The present findings indicate that amounts of interferon up to 4000 protective units do not inhibit the growth of cells in culture when the experiments were suitably controlled for impurities. The results are in agreement with the observations by Levy and Merigan(11) of the absence of inhibition of cell growth and rates of synthesis of RNA and protein by highly purified chicken interferon. Also it is commonly observed that cells *in vivo* grow rapidly during recovery from virus infection when the antiviral effect of interferon is at a maximum.

There are other examples of noninterferon components of interferon preparations and control materials which are associated with biological and biochemical activity. The antiviral action of interferon preparations on heterologous cells is often the result of noninterferon components(7,9,12). Cocito, Schonne, and DeSomer, in unpublished observations, have found that the inhibition of rat cell DNA and RNA synthesis by interferon preparations can also be caused by extracts from uninfected cells. Also increased production of acid by cells treated with interferon has been shown to be due to noninterferon contaminants(3,10).

These results reemphasize the need for purification of interferon and thorough characterization of biological and biochemical activities attributed to interferon.

Summary. The antiviral activity of crude interferon preparations was not always correlated with inhibition of cell growth suggesting the presence of a noninterferon growth inhibitor in many preparations of interferon. This interpretation is consistent with the further observations that: 1) growth inhibition was not increased as was the antiviral action of interferon in a second preparation of crude mouse interferon, and 2) purified chicken interferons did not inhibit cell growth.

1. Wagner, R. R., Levy, A. H., Ann. N. Y. Acad. Sci., 1960, v88, 1308.
2. Baron, S., Isaacs, A., Brit. Med. J., 1962, v1, 18.
3. Levy, H. B., Snellbaker, L. F., Baron, S., Virology, 1963, v21, 48.
4. Pauker, K., Cantell, K., Henle, W., ibid., 1962, v17, 324.
5. Cocito, C., DeMaeyer, E., DeSomer, P., Life Sci., 1962, v12, 753.
6. Sonnabend, J. A., Nature, 1964, v203, 496.
7. Merigan, T. C., Winget, C. A., Dixon, C. B., J. Mol. Biol., 1965, v13, 679.
8. Baron, S., Buckler, C. E., Science, 1963, v141, 1061.
9. Baron, S., Barban, S., Buckler, C. E., ibid., 1964, v145, 814.
10. Lampson, G. P., Tytell, A. A., Nemes, M. M., Hilleman, M. R., Proc. Soc. Exp. Biol. and Med., 1963, v112, 468.
11. Levy, H. B., Merigan, T. C., Proc. Soc. Exp. Biol. and Med., 1966, v121, 53.
12. Buckler, C. E., Baron, S., Fed. Proc., 1965, v24, 318.

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