Interferon and Uninfected Cells.* (30695)

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Several reports have appeared in the literature stating that interferon exerts an effect on either growth or on a major synthetic pathway in uninfected cells. Sonnabend working with a crude preparation of interferon, finds that the late labelling of RNA is inhibited by interferon(1). He also finds similar results with partially purified interferon (personal communication). These results are similar to those found by Levy *et al* as the only detected effect of a crude interferon preparation on uninfected cells(2,3). At that time Levy *et al* cautioned that these effects might be due to impurities in the preparation. Cocito *et al*(4, also personal communication) find that both crude and partially purified preparations of interferon inhibit P^{32} incorporation into both RNA and DNA. However, they find similar if not greater inhibition by comparable preparations from noninterferon containing materials made from uninfected cells. It is, therefore, difficult to maintain that these biochemical effects are interferon-associated. The question is of obvious importance in any attempt to understand the mechanism of the antiviral action of interferon.

The availability of interferon of a high degree of purity(5) makes it possible to examine the question more exactingly. The present paper deals with experiments to study possible effects of interferon on the growth rate of chick embryo (CE) cells, as measured by the increase in protein, DNA and RNA contents, and the rates of synthesis of RNA and protein.

Experimental. Materials and methods. General methods of cell culture, RNA, DNA and protein determination are as described previously(3). H^3 uridine and C^{14} valine analysis were done simultaneously on filters in a Packard Tri Carb liquid scintillation counter. Interferon was assayed by its capacity to reduce the number of plaques produced by vesicular stomatitis virus (VSV) on CE cells. One unit of interferon is defined as that amount which reduces by one-half the number of plaques of a challenge of approximately 50 pfu of VSV on CE cells. Purified interferon was made from the allantoic fluid of chick embryos infected with the WS strain of influenza virus, as described by Merigan(5), and had a specific activity of 33 units/μg protein. This represented a purification of about 2000-fold over the starting material.

Procedures and results. Five ml of primary CE cells were plated out on plastic Petri dishes at a concentration of 2×10^6 cells per ml. After several hours, when ⅓ to ½ of the surface of the dish was covered with freshly attached cells, the remainder of the cell suspension was removed and replaced with either 5 ml of new medium containing 15 units of purified chick interferon, or 5 ml of medium without interferon. Fifteen units of interferon will inhibit VSV plaque formation by 99%. Five dishes were removed at this time to determine starting values of RNA, DNA and protein. The remaining dishes were incubated at 36° in 4% CO_2-96% air. Fifteen and 39 hours after addition of interferon, 5 interferon-treated and 5 control plates were exposed to 5 μc H^3 uridine (0.5 μg) and 1 μc of C^{14} valine (0.1 μg) for 20 minutes. An additional 5 dishes of each type were exposed to the isotopes for 20 minutes followed by a "cold chase" for an additional 3 hours with 50 μg of uridine and 50 μg of valine in Eagle's Basal Medium (BME) 5% calf serum. At these times the cells were washed twice in the dishes with phosphate buffered saline (PBS), scraped into PBS, extracted twice with cold 10% perchloric acid, washed once with alcohol, and dissolved in 0.1 N NaOH. Aliquots were taken to determine RNA, DNA and protein content as well

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as the isotope content. Results are given in Tables I and II, the former giving the content of the macromolecular constituent, and the latter the rate of isotope incorporation.

It will be seen that within the variation that is to be expected in the measurement of the metabolism of different cultures, there is no effect of interferon on any of the functions measured. Since the DNA content may be taken as a measure of cell number, the continued rise in DNA content indicates that the cell number continued to increase during the observation period. It might be pointed out that as the cells age, the protein content per cell and RNA per cell both increase, the protein content increasing more than the RNA. The declining *rate* of RNA synthesis with age of cells, as contrasted with the lack of decline in rate of protein synthesis (Table II) is probably the explanation for the fact that the protein *content* per cell increases more than the RNA content.

TABLE I. Effect of Purified Interferon on Increase of RNA, DNA and Protein Contents of CE Cells. Data expressed as μg/petri dish (A), or as indicated ratios (B).

Part A.

	Protein		RNA		DNA	
Time	Int	Control	Int	Control	Int	Control
Zero time	20		4.3		5.5	
15 hr	39	35	7.1	5.5	7.5	7.3
18 "	49	44	6.9	6.9	7.2	7.3
39 "	76	87	10.3	12.3	8.2	9.1
42 "	102	118	13.5	14.0	9.8	9.4

Part B.

	Protein/DNA		RNA/DNA		Protein/RNA	
Time	Int	Control	Int	Control	Int	Control
Zero time	3.6		.78		4.7	
15 hr	5.2	4.8	.94	.75	5.5	6.4
18 "	6.8	6.0	.96	.95	7.1	6.3
39 "	9.3	9.6	1.26	1.35	7.4	7.0
42 "	10.4	12.5	1.38	1.49	7.6	8.4

TABLE II. Effect of Purified Interferon on Incorporation of H^3 Uridine and C^{14} Valine into CE Cells.

	S.A. RNA cpm/mg × 10^{-3}		S.A. protein cpm/mg × 10^{-3}	
Hrs	Interferon	Control	Interferon	Control
15	122	142	28	29
18	526	555	190	237
39	70	54	38	40
42	231	189	252	197

Ratio RNA synthesis/protein synthesis

Hrs	Interferon	Control
15	4.37	4.87
18	2.78	2.34
39	1.84	1.34
42	.92	.96

Those samples labelled 18 and 42 hours had isotope exposure beginning at 15 and 39 hours, respectively, but had an additional 3 hour exposure in the presence of cold chase and 1.0 ml more medium.

TABLE III. Effect of Interferon on Prolonged Incorporation of H^3 Uridine into CE Cells.

Spec. act. RNA, cpm × 10^{-2}/μg RNA		Spec. act. protein, cpm × 10^{-2}/μg protein	
Interferon	Control	Interferon	Control
132	133	374	352

Sonnabend(1) found that interferon caused an inhibition of incorporation of RNA precursors into RNA only when the precursor was left in contact with the cells for 4 hours or more. To determine if our preparations of interferon acted similarly the following experiment was performed. Seventy-five units of interferon were added to each of 5 Petri dishes, and 5 dishes served as controls. After 21 hours of exposure, 5 μc of H^3 uridine and 1 μc of C^{14} valine was added to each dish. The isotopes were left in contact with the cells for 6 hours with no cold chase, and the specific activity determined as before. The data are summarized in Table III.

Even after prolonged labelling, there was no effect of interferon on the rates of synthesis of RNA or protein.

Control experiments demonstrated that the antiviral effect of the interferon could still be seen after 48 hours.

Discussion. The preceding experiments demonstrate that interferon does not necessarily exert an effect on several aspects of the metabolism of uninfected cells, under conditions when a strong antiviral effect may be demonstrated. Of course, these experiments do not rule out a minor or more subtle effect, nor do they rule out the possibility that different sets of conditions might demonstrate an effect. However, until satisfactory demonstration of such effects is made, it is reasonable

to conclude that the antiviral action of interferon is not through an effect on one of the major synthetic pathways of normal cells. It is also probable that previous results showing effects on normal cells are attributable to impurities in the preparations. While the interferon used in the present experiments is not completely pure, it can be stated that those impurities responsible for effects on normal cells have been eliminated. This explanation is consistent with the results of Baron, Merigan and McKerlie(6) on the effect of crude and purified chick and mouse interferons on growth of cells in tissue culture. These authors clearly distinguish the effects on virus inhibition from the cell growth inhibiting effects of these preparations.

Conclusions. The effect of purified chicken interferon on growth of uninfected chick cells in tissue culture was studied. The criteria for growth used were the increase in amounts of protein, DNA and RNA, and the synthetic rates of protein and RNA. Doses of interferon that were strongly inhibitory to viral growth caused no changes in these parameters.

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Serum Insulin Concentration and Birth Weight in Human Infants.* (30696)

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Transitory hyperglycemia and glycosuria (1), impaired glucose tolerance(2,3) and hyperlipemia(3,4) are signs of the added metabolic burden which pregnancy imposes upon the islands of Langerhans. In predisposed individuals and especially in women who show tendency to bear large infants, this added burden may precipitate the appearance of "metagestational" clinical diabetes(1,3,5). The reasons for the apparent relationship between diabetes and tendency to bear large infants are not clear: a commonly accepted hypothesis states that, in diabetic women, high maternal blood glucose results in fetal hyperglycemia, stimulation of the fetal pancreas, hyperinsulinism and, consequently, increased fetal size. The following observations lend support to this hypothesis: a. the placenta is not an effective barrier against the transfer of glucose and, at least in rabbits, the fetal blood glucose fluctuates with that of the mother, although at slightly lower levels(6); b. the pancreata of large infants and of "normal" infants of diabetic mothers show hypertrophy and hyperplasia of the islets of Langerhans(7-9) and increased insulin content(10); c. diabetic rats tend to produce larger than normal pups with insular hypertrophy and hyperplasia(11-13); d. marked hypoglycemia often occurs in infants of diabetic mothers shortly after birth, when they are separated from the maternal sources of glucose(7); e. increased body fat and carbohydrate content(14,15) and accelerated glucose disposal rate(16) are prevalent in infants of diabetic mothers. Although these observations bear witness to fetal hyperinsulinism, direct measurements of insulin in the blood of the newborn have been few. One group of investigators(17) noted a rise in fetal serum immuno-reactive insulin (IRI) con-

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