by the bites of *A. quadrimaculatus* in Syrian golden hamsters (*Mesocricetus auratus*).

We are endeavoring to continue the successive cyclic transmission of these strains in an attempt to adapt *P. berghei* for regular sporozoite-induced infections under laboratory conditions.

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Interferons: Selectivity and Specificity of Action in Cell-Free Systems

Abstract. *The inhibition by interferons of viral replication was studied with cell-free preparations of the enzyme RNA synthetase derived from calf kidney cells infected with influenza virus and from chick embryo fibroblasts infected with Newcastle disease virus. Calf, mouse, and chicken interferons inhibited these enzymes with the same characteristic species specificity observed in tissue culture. A mechanism of action is proposed.*

Infection of an organism or tissue by a virus may be resisted in several ways; one of these is the production by the infected tissue of a substance that interferes with the multiplication of the virus. This substance, a protein of low molecular weight, is called interferon; it was discovered and purified by Isaacs (*1*), and other workers (*2*). Among its unusual properties is its species specificity (*3*), that is, interferon derived from a virus-infected tissue is more effective in protecting a homologous than a heterologous tissue. Accordingly there appear to be many interferons, the activity of each seeming to depend on the tissue from which it was derived rather than on the particular virus which elicits its production.

The mechanism of action of interferon has been studied to elucidate the site or sites at which viral multiplication is inhibited (*3*). Since interferon inhibits replication in cells exposed to either whole virus or infectious viral RNA (*4*), it appears that interferon does not block virus adsorption, penetration, or uncoating of the nucleic acid (*5*). That interferon does not merely inhibit the release of mature virus was demonstrated by the experiments of Ho and Enders (*6*). Infected tissue treated with interferon has been disrupted by various methods, and unlike the cases in control cultures, no excess intracellular infectious material has been found.

Cocito *et al.* (*7*) have shown that interferon can inhibit viral RNA synthesis in tissue culture cells infected with virus but does not affect the incorporation of radioactive orthophosphate into both DNA and RNA of uninfected control cells. Further, Lockart *et al.* (*8*) have shown that after treatment with interferon, the production in vivo of both virus and infectious RNA ceases abruptly, simultaneously, and to the same degree. The main action of interferon would, therefore, appear to be the inhibition of viral nucleic acid synthesis; however, it has not been possible to demonstrate without question whether interferon affects assembly of mature virus particles or virus-associated protein synthesis. Further, if there are effects, it is not known whether or not they are secondary to the nucleic acid response (*7–9*).

Infection of cells in tissue culture with an RNA-containing virus induces the formation of an RNA-synthesizing enzyme, RNA synthetase, not found in normal noninfected tissue. This enzyme has been isolated from calf kidney cells infected with influenza virus and from chick embryo fibroblasts infected with Newcastle disease virus (*10, 11*). Similar preparations have been isolated from various tissues infected with other RNA-containing viruses (*12*).

Since intracellular replication of viral nucleic acid has been implicated in the mechanism of action of interferon in tissue culture cells, it should be possible to demonstrate an effect of interferon on the synthesis of viral nucleic acid in a cell-free system. Further, if this effect is the mechanism of antiviral action of interferon, it should show the

Table 1. Properties of RNA synthetase isolated from calf kidney tissue infected with influenza virus. This incubation mixture contained: Tris buffer, *p*H 7.2, 20, μmole; $MgCl_2$, 1.5 μmole; mercaptoethylamine, 2.0 μmole; phosphoenolpyruvate, 1.0 μmole; pyruvate kinase, 35 μg; ATP, CTP, and UTP, 0.1 μmole each; GTP-8-C^{14} (specific activity, approximately 10^7 count/min μmole), 0.1 μmole; and enzyme, 350 μg in a final volume of 0.1 ml. The mixture was incubated at 37°C for 10 minutes, the reaction was terminated by the addition of 2 ml of 6 percent trichloroacetic acid, and the incorporation was determined in the usual manner (*11*). The activity is expressed as micromicromoles of GMP incorporated in 10 minutes per milligram of protein.

Prior treatment of enzyme (μg/ml)	Test system	Activity ($\mu\mu$mole/mg protein)	Inhibition (%)
	Microsomal fraction from infected cells (10)		
None	Complete	555.0	
Deoxyribonuclease, 20	Complete	392.0	29.0
Ribonuclease, 20	Complete	0.0	100.0
	Microsomal fraction from noninfected cells		
None	Complete	112.0	
Deoxyribonuclease, 20	Complete	25.2	77.5
Ribonuclease, 20	Complete	54.0	52.0
	Microsomal fraction from infected cells		
Deoxyribonuclease, 20	Complete	298.4	
	Minus Mg	236.0	20.9
	Minus ATP regenerating system	11.7	96.1
	Minus ATP	41.0	86.3
	Minus CTP	54.6	81.6
	Minus UTP	60.4	79.8
	Minus ATP, CTP, UTP	56.6	81.0

Table 2. Effect of interferon on RNA synthetase. The incubation mixture was the same as indicated for Table 1 and the activity is expressed as micromicromoles of GMP incorporated into nucleic acid in 10 minutes per milligram of protein. The activity of the various interferons were as follows: chicken interferon was active at a dilution of 1:130 against vesicular stomatitis virus on chick embryo fibroblasts and at a dilution less than 1:4 against influenza B on calf kidney cells; mouse interferon was active at a dilution of 1:100 against encephalomyocarditis virus on mouse embryo fibroblasts; and calf interferon was active at a dilution of 1:8 against parainfluenza type I virus on calf kidney cells and at a dilution less than 1:4 against Newcastle disease virus on chick embryo fibroblasts.

Interferon		Activity ($\mu\mu$mole/mg protein)	Inhibition (%)
Source	Amount (ml)		
Enzyme from calf kidney cells infected with influenza virus			
	None	286	None
Chicken	0.05	169	41
Mouse	.05	0	100
Calf	.05	9	97
Enzyme from chick embryo fibroblasts infected with Newcastle disease virus			
	None	19.4	None
Chicken	0.05	1.6	92
Mouse	.05	14.4	26
Calf	.05	17.3	11

Table 3. Effect of calf, mouse and chicken interferons on RNA nucleotidyltransferase.

Interferon*		Activity (mμmole GMP incorporated)
Source	Amount (ml)	
DNA-primed RNA nucleotidyltransferase activity†		
	None	1.70
Calf	0.04	1.73
Mouse	.04	1.60
Chicken	.04	1.58
PolyC-primed RNA nucleotidyltransferase activity‡		
	None	0.57
Calf	0.01	.57
Mouse	.01	.55
Chicken	.01	.53
PolyC-primed RNA nucleotidyltransferase activity§		
	None	1.70
Calf	0.01	1.55
Calf	.02	1.80
Calf	.05	1.72
Calf	.075	1.70
Calf	.10	1.55

* The calf interferon was active at a dilution of 1:128 against parainfluenza type I virus on calf kidney cells; the mouse and chicken interferon activities were the same as indicated in Table 2. † The incubation mixture contained: Tris buffer, *p*H 8.1, 20 μmole; $MgSO_4$, 5 μmole; mercaptoethylamine, 8.0 μmole; calf thymus DNA, 60 μg; CTP, UTP, and ATP, 0.5 μmole each; GTP-8-C^{14} (specific activity, 6×10^5 count/min μmole), 0.5 μmole; and enzyme, 1.6 μg in a final volume of 0.25 ml. The mixture was incubated at 37°C for 10 minutes, and the reaction was terminated by the addition of trichloroacetic acid. ‡ The incubation mixture contained: Tris buffer, *p*H 7.1, 20 μmole; $MgCl_2$, 2.0 μmole; $MnSO_4$, 0.5 μmole; mercaptoethylamine, 8.0 μmole; polyC, 20 μg; GTP-alpha-P^{32} (specific activity, 9.2×10^5 count/min μmole), 0.2 μmole; and enzyme, 3.3 μg in a final volume of 0.2 ml. The mixture was incubated at 37°C for 10 minutes and the reaction terminated by the addition of trichloroacetic acid. § The incubation mixture was the same as in experiment 2, except that 5.0 μg enzyme was used.

property of the species specificity observed in vivo.

Interferons isolated from several tissues (*9*), such as calf, mouse, and chicken, were tested for their capacity to inhibit the following enzymes associated with nucleic acid synthesis: (i) RNA synthetase isolated from calf kidney cells infected with influenza virus, (ii) RNA synthetase isolated from chick embryo fibroblasts infected with Newcastle disease virus, (iii) RNA synthetase isolated from *Escherichia coli* cells infected with MS-2 bacteriophage (*12, 13*), and (iv) both the DNA-primed and the polyC-primed activities of RNA nucleotidyltransferase (*14*).

Table 1 illustrates some of the properties of the RNA synthetase isolated from calf kidney cells infected with influenza virus. Similar properties were exhibited by the RNA synthetase isolated from chick embryo fibroblasts infected with Newcastle disease virus. The activity was associated with the microsomal fraction derived from infected tissues and was dependent upon all four nucleoside triphosphates, a triphosphate regenerating system, and to some extent on the presence of magnesium ions. Although the activity was not abolished by prior treatment of the microsomal material with deoxyribonuclease, all activity was lost by prior treatment with ribonuclease. This pattern of activity was absent from noninfected tissue.

The results of experiments in which the interferons obtained from tissues of three different species (*15*) were tested for their capacity to inhibit RNA synthetase activity isolated from the virus-infected tissue are given in Table 2. Chick interferon showed an inhibition of the chick-derived enzyme, whereas its effect on the heterologous calf system was significantly less. A more marked effect was seen with calf interferon (titer 1:8) which inhibited the homologous calf-derived enzyme 97 percent while having a negligible effect on the heterologous chick-derived enzyme. Mouse interferon also inhibited the calf system more than the chick system. Control preparations derived from noninfected tissue by the method employed for interferon isolation had no inhibitory effects on RNA synthetase. There was direct correlation of the effects in vitro of these interferon preparations and their capacity to protect virus-infected tissue cultures as were observed in our laboratory (see Table 2), and reported by other investigators (*16*).

Interferon did not inhibit nucleotide incorporation in similar tissue fractions derived from noninfected tissues. Since the incorporation of nucleotide was minimal, a more critical control was provided by studying the effect of interferon on isolated enzymes that take part in the synthesis of nucleic acids in normal tissue.

Table 3 shows that the interferon preparations have no effect on polyribonucleotide synthesis catalyzed by RNA nucleotidyltransferase derived from *Azotobacter vinelandii* and primed with either DNA or polyC. Increasing the concentration of calf interferon (see Table 3, experiment 3) did not produce any effect on the synthesis of polyribonucleotide.

It was of interest to study the capacity of interferon to inhibit RNA synthetase derived from nonmammalian tissue, such as *E. coli* infected with MS-2 phage (*13*). Interferon had no effect on this enzyme at a concentration some 14-fold greater than that required for complete inhibition of the mammalian enzymes.

Our data confirm the earlier reports that interferon inhibits viral RNA synthesis. We have further found that this inhibition occurs also in a cell-free system. The specific inhibition of mammalian RNA synthetase has the characteristic species specificity seen with interferon in cultured cells exposed to virus. The lack of activity in the several other nucleic acid synthesizing systems as well as in our crude preparations from noninfected tissue are in agreement with the hypothesis that interferon inhibits the synthesis of viral nucleic acid but not of normal tissue nucleic acid. The present data refer only to RNA and RNA-containing viruses. The antiviral action of interferon on DNA viruses remains to be elucidated.

While our data show that the species specificity of interferon action is present at the enzyme level, the physicochemical basis of this specificity cannot be ascertained in the absence of the knowledge of the chemical structure of the various interferons.

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Thalidomide: Effects on Ehrlich Ascites Tumor Cells in vitro

Abstract. *Thalidomide did not inhibit dehydrogenase activity or growth of Ehrlich ascites tumor cells in agar. When mixed with Ehrlich ascites tumor cells in vitro, thalidomide increased the mitotic activity. The effect of the thalidomide was not altered by the addition of nicotinic or folic acid, or by vitamin B_1 or B_6. Oxygen uptake by the tumor cells was not affected by thalidomide.*

An attempt was made to determine whether thalidomide (*1*) (DL-*N*-(2,6-dioxo-3-piperidyl)phthalimide) would produce any effect in vitro that would elucidate the metabolic pathway of this compound, which could then be validated in vivo. This study followed the demonstration that harmful effects are produced in the mouse embryo when the mother is given thalidomide prior to the formation of the limb buds (2). Our procedure is more efficient than testing in animals; because of intrauterine variability in mice, for example, several hundred pregnant females would have to be used for testing a specific pharmacological action of thalidomide.

Ehrlich carcinoma cells, which had been cultured as a cell strain for 33 months, were used. The cells were grown in a medium of Earle's salts (*3*) supplemented with 20 percent fetal calf serum and 20 percent bovine amniotic fluid. Cultured cells which had been placed in fresh medium 24 hours previously were counted, and 200,000 cells per 3 ml of fresh medium were transferred into screw-cap test tubes each containing a sterile 8 × 15-mm No. 2 coverslip. The tubes were incubated at 37°C for 6 hours at a 10-degree angle to allow attachment. The medium was removed and the cells were then exposed to 750 μg of thalidomide given in 3 ml of fresh medium. This concentration is approximately twice that which is soluble in saline at 37°C; however, after approximately 24 hours no thalidomide was visible and no change in the *p*H was noted. At 3-hour intervals, cells exposed to thalidomide and cells from the control tubes were fixed with methyl alcohol, dehydrated, and then stained with hematoxylin and eosin. The cells were still attached to the coverslips. The number of mitotic figures was recorded for the first 4000 cells counted on two coverslips with thalidomide-treated cells and two with control cells. Comparison of the incidence of mitotic figures in treated and control cells showed that thalidomide-treated cells had a maximum number of figures prior to the time required for control cells to reach their maximum. After 12 hours cells exposed to thalidomide averaged 66.75 ± 8.2 figures per 1000 cells while unexposed cells averaged 34.45 ± 5.9 figures per 1000 cells. This difference was statistically significant at the .01 level of probability. There was no change in the *p*H of the medium. At 24 hours the control cells exhibited the same incidence of mitotic figures as did the treated cells at 12 hours. No further difference was noted. This increased rate of mitosis was not reversed by the addition of 10 μg of nicotinic acid, folic acid, or pyridoxal hydrochloride per milliliter of medium, or by 2.5 μg of riboflavin per milliliter of medium.

Thalidomide was next tested for its ability to produce cytotoxicity or inhibition of dehydrogenase activity. Studies of growth inhibition were also conducted. Growth was indicated by the presence of a complete monolayer of Ehrlich cells overlayed with agar in the bottom of a petri dish, as described previously (*4*). Discs containing 25 to 100 μg of thalidomide did not inhibit the dehydrogenase activity of cells in an agar suspension, as indicated by the reduction of methylene blue. Formation of a complete monolayer of cells overlayed with agar was also uninhibited.

The effect of thalidomide on respiration and pyruvate oxidation of Ehrlich ascites tumor cells was also determined. Cells equivalent to 25 mg of dry-tissue weight were incubated for 100 minutes at 37°C in Krebs Ringer phosphate buffer free of calcium ions and containing 50 μmole of inorganic phosphate buffer, *p*H 7.4 in a total volume of 3.0 ml. Thalidomide added in amounts ranging from 30 to 6000 μg per 3 ml of medium had no inhibitory effect on oxygen uptake (determined manometrically) when pyruvate-1-C^{14} (in a final concentration of 0.01*M*) served as respiratory substrate. Nor did saturating concentrations of thalidomide produce a significant inhibition of pyruvate oxidation, as measured by the incorporation of C^{14} of pyruvate-1-C^{14} into the respiratory CO_2.

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