differential (vacuum at exit end and hydrogen partial pressure at feed end), and the additional pressure created by zonal heating of the feed end of the column, causing palladium to release sorbed hydrogen.

Tritium exhibits the highest partial pressure over palladium and diffuses through the column faster than does either deuterium or protium. The first gas evolved, the tritium-rich fraction, is detected by a pressure increase in an evacuated proportional chamber attached to the exit end of the column. The proportional chamber is removed from the system, filled to atmospheric pressure with methane counting gas, and counted for 30 minutes under low-background conditions.

The column is regenerated by increase in the temperature of the entire system to 215°C and by evacuation of the residual hydrogen to less than 0.3 mm-Hg.

The major components of the system (Fig. 1) were designed for processing 40-ml samples. Data reported apply to this feed volume and a final collection volume of 500 cm^3 of gas in the proportional counter (2.6-liter volume).

Certain practical points should be noted regarding operation of the column. Recovery and enrichment are low if any trace of oxygen remains in the system. All leaks must be eliminated, and the columns are stored under nitrogen or other inert gas when not in use. Chemical vapors (for example, halogens) that react with palladium or catalytically combine with hydrogen must be avoided. Two and a half hours are required for the tritium to evolve from the column, but other gases inert to the column pass through in a few minutes. Gases dissolved in water or those released from magnesium upon heating may be pumped from the discharge end of the column without effect on the final result. Approximately 3 percent of the tritium remains in the hydrogen generator and may contaminate subsequent samples if they are significantly lower in tritium, unless the generator is baked at 80°C for 2 hours before reuse.

Recovery proved to be relatively independent of operating conditions. Changes in column temperature (between 20° and 40°C), in rate of feed of the sample to the hydrogen generator (0.2 to 1.0 ml/min), and in elution rate (2 to 2.5 hours) did not affect recovery. However, recovery depended on sample volume (56 percent recovery for a 50-ml sample; 80 percent for 10 ml).

Tritium can be measured by this technique at concentrations corresponding to natural levels. This relatively inexpensive, rapid analytical system facilitates the use of tritium as a tracer in hydrology, oceanography, meteorology, and biology. The principles involved may have application where tritium is to be separated from another gas or for tritium labeling of organic or inorganic compounds.

JOHN E. HOY
Savannah River Laboratory, E. I. du Pont de Nemours and Company, Aiken, South Carolina 29801

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Interferon Inducers in vitro: Difference in Sensitivity to Inhibitors of RNA and Protein Synthesis

Abstract. *Interferon can be induced by diverse agents in a variety of mammalian cell cultures through apparently two mechanisms. One results in an early (2 to 10 hours) appearance of interferon and is relatively resistant to inhibition by actinomycin, puromycin, or fluorophenylalanine. A second mechanism results in a late (18 to 24 hours) appearance of interferon and is more sensitive to inhibition by these inhibitors. The molecular basis for each mechanism is unclear. Since each interferon inducer may have multiple effects on the cell, the differences observed may not necessarily reflect a fundamental difference in the mechanism of interferon stimulation.*

Interferon induction may occur through two mechanisms (*1, 2*). One involves the synthesis *de novo* of interferon, and the other involves release of already formed interferon. This suggestion was based on the differing effects of various drugs and procedures on interferon formation by bacterial endotoxins and RNA viruses in vivo. These include: (i) different sensitivities to inhibition of RNA and protein synthesis; (ii) different kinetics of interferon appearance; (iii) different effects on interferon titers by splenectomy, adrenalectomy, change of body temperature, prior immunization with BCG vaccine (Bacille Calmette-Guérin), and cortisone administration. It was also demonstrated (*3, 4*) that interferon induction in vitro by endotoxin and virus (Newcastle disease virus) had different rates of appearance and different sensitivities to actinomycin D. Furthermore, there were different optimum temperatures for interferon induction in macrophage preparations between a fungal polysaccharide and an RNA virus (*5*).

These studies have drawbacks owing to heterogenous cell populations, secondary effects of the various drugs and agents in vivo, use of a relatively weak interferon inducer to demonstrate a mechanism of interferon induction, and little control over the metabolic changes that each inducer produced in the cells.

In our studies, in vitro we used a variety of naturally occurring and synthetic inducers of interferon in different cell lines; according to type the cell lines showed differences in reactivity to inducers. To measure their activity, inducers were incubated at 37°C with monolayers of different cell types in Eagle's minimal essential medium (MEM). After various intervals the antiviral activity of the tissue culture fluids was assayed by plaque inhibition, and interferon was characterized (*6*). Not all inducers were active in stimulating interferon in each cell type (Table 1). Only the viral inducers, namely, Newcastle disease virus (NDV) and statolon (a fungal virus preparation) induced interferon in all cell types. The synthetic double-stranded RNA consisting of polyribocytidylic acid and polyriboinosinic acid (rC/rI), described as an interferon inducer by Field *et al.* (*7*), and pyran, a polycarboxylate copolymer (*8*), failed to induce interferon in mouse L929 cells, but was effective in human skin fibroblasts and mouse peritoneal macrophages. Endotoxin induced interferon only in the mouse peritoneal macrophages. Like L929 cells, HeLa cells responded only to viral inducers; chick embryo fibroblasts, like human skin fibroblasts, responded in addition to rC/

Table 1. Interferon production in vitro. The inducer was incubated with monolayers 2 to 24 hours. Before the interferon assay, Newcastle disease virus (NDV) (Herts strain) was inactivated by maintaining samples at *p*H 2 for 5 days. Statolon (*17*) was removed by lowering the *p*H to 4.5 and centrifuging the cultures for 90 minutes at 105,000*g* (*18*). The rC/rI (*19*) was inactivated by first binding divalent cations with $10^{-3}M$ ethylenediaminetetraacetic acid, then adding an equal weight of pancreatic ribonuclease for 1 hour at 25°C (*12*); pyran (*20*) and *Salmonella enteritidis* endotoxin (*21*) were not removed from samples before assay. The previously reported time of peak response in vitro is given with a reference. HSF, human skin fibroblasts; MPM, mouse peritoneal macrophages; PFU, plaque-forming unit.

Cell type	Interferon titer (unit/4 ml)			Peak response (hr)
	2–4 hr	8–10 hr	20–24 hr	
	NDV (10 PFU/cell)			
L929	< 3	< 3	240	24–48 (*3*)
HSF	< 3	10	300	
MPM	< 3	4400	8000	5–6 (*4*)* (*11*)†
	Statolon (500 μg/ml)			
L929	< 3	< 3	7	
HSF	7	53	135	24 (*18*)‡
MPM	4	23	1150	
	rC/rI (40 μg/ml)			
L929	< 1	< 1	< 1	
HSF	53	230	260	7.5 (*7*)§
MPM	13	16	18	
	Pyran (250 μg/ml)			
L929	< 1	< 1	< 1	
HSF	2	2	3	
MPM	27	9	7	
	Endotoxin (100 μg/ml)			
L929	< 1	< 1	< 1	
HSF	< 1	< 1	< 1	
MPM	3	7	6	2.5–6 (*4*)* (*11*)†

* In bovine peripheral leukocytes. † In rabbit peritoneal macrophages. ‡ Chick embryo fibroblasts. § Rabbit embryo fibroblasts.

rI, and human peripheral leukocytes, like mouse peritoneal macrophages, responded to all inducers. Concentration of inducers was raised to cytotoxic levels or to ten times their usual amounts before the interferon inducers were designated as inactive in a given cell line (*9*). Others (*5*) have found that the yeast polysaccharide mannan, like pyran, induced interferon in mouse peritoneal macrophages but not in mouse L cells. These studies suggest several classes of cellular response to inducers, with leukocytes or macrophages being the least selective and aneuploid cell lines the most selective. Inducer penetrability appears to be a factor in cellular reactivity. For example, Dianzani *et al.* (*10*) induced significant amounts of interferon in mouse L cells with rC/rI by prior treatment of the cells with diethylaminoethyl dextran.

Our maximum titers were generally produced 18 to 24 hours after stimulation with NDV and statolon (Table 1). In contrast, peak titers to rC/rI, endotoxin, and pyran appeared earlier (2 to 10 hours). The times for peak interferon titers induced by NDV, statolon, rC/rI, and endotoxin agree in general with the results of others in different cell types. The reason for the difference in kinetics between our results with NDV in macrophages as compared with those of Kono (*3*) and Smith and Wagner (*11*) is not known, but could be related to differences in preparations.

Table 2 presents the effects on interferon production with actinomycin D at concentrations which inhibited 80 to 99 percent of cellular RNA synthesis, as judged by incorporation of radioactive uridine in each cell type studied. Interferon production induced by NDV was most sensitive to actinomycin D; induction by statolon was slightly less sensitive (especially in human skin fibroblasts). However, interferon production induced by rC/rI, pyran, and endotoxin was much more resistant to actinomycin D. The difference between the effect of 0.2 μg of actinomycin D per milliliter on induction of interferon by NDV and rC/rI was statistically highly significant ($P < .01$). Moreover, our studies have shown single-stranded polycytidylic acid to induce interferon in human skin cells by a mechanism resistant to actinomycin D (*12*). To demonstrate these differences, we used the lowest concentrations of actinomycin D which would clearly inhibit viral induction of interferon; these low concentrations are necessary to avoid possible secondary effects of the inhibitor occurring at higher concentrations. Human skin fibroblasts showed toxic effects such as shrinkage of cells, increased granularity, and detachment of the monolayer, under phase-contrast microscopy, 18 hours after a 90-minute exposure to concentrations of 1 μg of actinomycin D per milliliter or more. Since we were comparing the relative sensitivity of the various responses to actinomycin D, a viral inducer was included for direct comparison in each experiment. Interferon titers of nearly 1000 units per 4 ml were induced by 250 μg of rC/rI per milliliter in human skin fibroblasts, and were not decreased by prior treatment of cells with 0.2 μg of actinomycin D per milliliter. However, 1 μg of actinomycin D per milliliter decreased interferon production with rC/rI in these

Table 2. Effect of inhibitors of RNA and protein synthesis on in vitro interferon production. Abbreviations and assay conditions as in Table 1. Inducers incubated with monolayers 18 to 20 hours.

Cell type	Inhibitor											
	Actinomycin D* (μg/ml)					Puromycin† (μg/ml)			Fluorophenylalanine ‡ (μg/ml)			
	0	0.2	0.3	0.5	1	0	0.25	2.5§	0	2	20	200§
	NDV (10 PFU/ml)											
HSF	1000‖	100		14	7	840	220	45	630	290	150	7
MPM	8000		30		15							
	Statolon (500 μg/ml)											
HSF	208	70			10							
MPM	1150		55		3							
	rC/rI (40 μg/ml)											
HSF	190	220		100	45	100	125	150	52	55	70	140
MPM	18		24		9							
	Pyran (250 μg/ml)											
HSF	5				7							
MPM	7		7		7							
	Endotoxin (100 μg/ml)											
MPM	6		9		4							

* Prior incubation with monolayers for 90 to 120 minutes, then removed before addition of inducing agent. † Incubated with inducing agent and removed by dialysis against MEM. ‡ In MEM without phenylalanine. §At these concentrations of protein inhibitor, cytotoxicity noted at 12 hours resembled the toxicity of actinomycin D at concentrations of 1 μg/ml, as described. In the cultures incubated with these concentrations of protein inhibitors 14 hours before stimulation with rC/rI, interferon titer decreased. ‖Interferon titer in unit/4 ml.

cells (Table 2). A decrease in interferon titers after rC/rI in cells treated with 1 μg of actinomycin D per milliliter has been reported in rabbit cells *(13)* and in human amnion cells *(14)*.

The sensitivity of endotoxin-induced interferon to actinomycin D in rabbit peritoneal macrophages described by Smith and Wagner *(11)* may have been due to toxic effects of the drug because high doses of actinomycin D were used. Since interferon production after rC/rI was more rapid than that after NDV, the difference in actinomycin D sensitivity possibly reflected a late secondary effect of the drug on the cells. As a control, monolayers of human fibroblasts were first treated with 0.2 μg of actinomycin D per milliliter for 2 hours, and rC/rI was added 14 hours later. Again, no inhibition of interferon production was noted. An attempt to induce interferon with rC/rI in human skin cells previously treated with actinomycin D and NDV was unsuccessful. Cell monolayers treated with NDV or statolon showed toxic changes after 12 to 18 hours, whereas no toxicity was seen after rC/rI, endotoxin, or pyran. Furthermore, studies of C^{14}-leucine incorporation into cellular proteins showed that NDV and statolon, but not rC/rI, blocked cellular protein synthesis after 8 hours; incorporation of C^{14}-leucine 16 to 18 hours after stimulation by NDV (Herts) was only 7 percent of controls, or only 3 percent of controls if cells were already treated with actinomycin D. These findings raised the possibility that the apparent sensitivity to actinomycin D might be related to a combination of a toxic or potentiating effect of the drug and the viral interferon-inducing agent. The finding that interferon appears late after NDV induction, when protein synthesis in the host cell is markedly reduced, suggests that interferon may have been formed early after infection and released slowly.

Another strain of NDV [73-T (*15*)], did not produce visible toxicity in cell monolayers, and reduced C^{14}-leucine incorporation by only 30 percent (that is, it was tenfold less toxic than the Herts strain); its toxicity was similar to that seen with rC/rI. The interferon-inducing capacity of this strain of NDV was also markedly inhibited by 0.2 μg of actinomycin D per milliliter. We therefore could not demonstrate that the protein-inhibiting capacity of the virus was responsible for the increased sensitivity of interferon production to actinomycin D.

The role of cellular protein synthesis in interferon production was studied with the use of varying concentrations of puromycin and DL-*p*-fluorophenylalanine. In contrast to their effects on interferon induction by NDV, these inhibitors failed to affect interferon production by rC/rI in human skin fibroblasts (Table 2). Two repeat experiments with these protein inhibitors yielded results similar to those presented (Table 2). The differences in effect on interferon induction by NDV and rC/rI by the highest doses of fluorophenylalanine and puromycin were statistically significant ($P < .05$). Prior incubation of monolayers with these inhibitors for 14 hours before rC/rI was added yielded similar results. Furthermore, treatment of monolayers with actinomycin D (0.2 μg/ml) followed 8 hours later by treatment with puromycin (4 μg/ml) combined with stimulation with rC/rI did not diminish the interferon titer, despite a cytopathic effect on the cells.

These results demonstrate different sensitivities to metabolic inhibitors and different kinetics of interferon formation between NDV and statolon, compared with rC/rI, pyran, and endotoxin. Since human skin fibroblasts represent morphologically uniform cell populations, the two responses are likely to occur within the same cell. The other cell types used, mouse peritoneal macrophages, peripheral blood leukocytes, and chick embryo fibroblasts are heterogenous cell populations.

The basis for the differences between the two patterns of interferon induction is not clear. As mentioned above, it has been suggested that there may be both synthesis *de novo* of interferon and release of already formed interferon. This possibility is supported by our finding that rC/rI induced interferon was inhibited only by cytotoxic doses of drugs. Youngner *(2)* also suggested the two types may only differ in the requirement of one, the "viral" type, for synthesis of an interferon-activating enzyme, but then both result in the release of preformed interferon. The existence of early protein intermediates, essential for viral-induced interferon formation, had previously been deduced by Burke from his studies with puromycin and fluorophenylalanine *(16)*. An alternative explanation of the results is that each type of response involves the formation *de novo* of interferon, but one requires an additional step (for example, uncoating of virions) which is more sensitive to the metabolic inhibitors than interferon synthesis per se. A less likely possibility is that interferon production may involve formation *de novo* of different protein molecules with similar biological activity but different sensitivity to metabolic inhibitors and different rates of formation or release. A definitive explanation would seem to await study of the time of incorporation of radiolabeled amino acids into interferon.

MARTIN S. FINKELSTEIN*
GERALD H. BAUSEK†
THOMAS C. MERIGAN
Division of Infectious Diseases, Department of Medicine, Stanford University School of Medicine, Palo Alto, California 94304

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20. Pyran is a random copolymer of maleic acid and divinyl ether synthesized by Hercules Company, Wilmington, Delaware (NSC-46015-C).
21. *Salmonella enteritidis* endotoxin, Difco Laboratories, Detroit, Michigan.
22. Supported by PHS grant AI-05629.
* Dernham fellow of the American Cancer Society, California Division (J-116).
† PHS trainee, Biophysics Program, Stanford University (2-TO1 GM 00712-09).

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Microspectrophotometry of Photoreceptor Organelles from Eyes of the Prawn Palaemonetes

Abstract. *Microspectrophotometric measurements of individual dark-adapted rhabdoms of the prawn* Palaemonetes vulgaris *reveal the presence of two light-sensitive pigments. A pigment with maximum absorbancy at 555 nanometers is converted by light to a long-lived intermediate with wavelength of maximum absorbancy at 496 nanometers. A second pigment with wavelength of maximum absorbancy at 496 nanometers bleaches in the light, seemingly without forming detectable products at wavelengths longer than 375 nanometers. Both pigments occur in each layer of microvilli.*

The direct demonstration by microspectrophotometry of three types of cone cells maximally sensitive in different spectral regions, coupled with the observation that the outer segments of individual cones contain only one visual pigment (*1*) has established the fact that the trichromacy of human color vision has its basis in the retinal receptors. In arthropod eyes the receptor cells associate in groups of usually seven or eight where they cooperate in the formation of the rhabdom. The rhabdom, which is a compound organelle formed by masses of microvilli from several sense cells (*2*), contains the visual pigment. Each cluster of sense cells together with its rhabdom is called a retinula and comprises the functional part of a single ommatidium. Because each sense cell has its own axon, yet is closely associated or even fused with the other cells via their shared rhabdom, it is important to determine the extent to which each receptor cell represents a separate input channel to the central nervous system. Not only is it important to establish whether cells in the same retinula are electrically connected, but one would also like to know whether arthropods with color vision have more than one visual pigment in each rhabdom (*3*). What, in brief, are the individual color receptors?

As part of a study of the microspectrophotometry of single arthropod rhabdoms we have made observations on the prawn *Palaemonetes vulgaris*. Animals were dark-adapted overnight, and the preparations were mounted under near infrared light with the aid of an infrared image converter. Rhabdoms were obtained by squashing the eyes with a glass rod in the bottom of a conical centrifuge tube under several milliliters of saline solution (*4*). Detached rhabdoms and tissue debris were separated from most of the fine particles and pigment granules by light centrifugation for several minutes. A drop of suspension containing rhabdoms was mounted between cover slips on the stage of a dual-beam recording microspectrophotometer (*5*). Rhabdoms were located under infrared light, and spectra were recorded with lateral illumination and spots of light that were small with respect to the approximately 10 μm width of the rhabdom.

The rhabdoms of *Palaemonetes* contain two photosensitive pigments. Figure 1 shows the kind of experiment on which this conclusion is based. Curve 1 is the initial spectrum, relative to the base line recorded when the sample beam passed through a clear region of the slide. Although it is not shown in Fig. 1, a back scan retraces the initial spectrum, indicating that the measuring beam is not significantly actinic under the conditions of measurement. If now the preparation is illuminated for 2 minutes with a bright red light from the field illuminator of the microscope (Corning filter 2-59, wavelengths longer than 620 nm), there is a fall in absorption between 540 and about 625 nm and a rise in absorption in the region 425 to 540 nm (Fig. 1, curve 2). This represents the conversion of a pigment absorbing maximally at 555 nm (P_{555}) to a product (or products) with peak absorption at 496 nm. Controls demonstrate that the exposure to red bleaching light was sufficient to remove all of the P_{555} initially present. A yellow irradiating light (Corning filter 3-71, wavelengths longer than 470 nm) then causes the absorption to drop

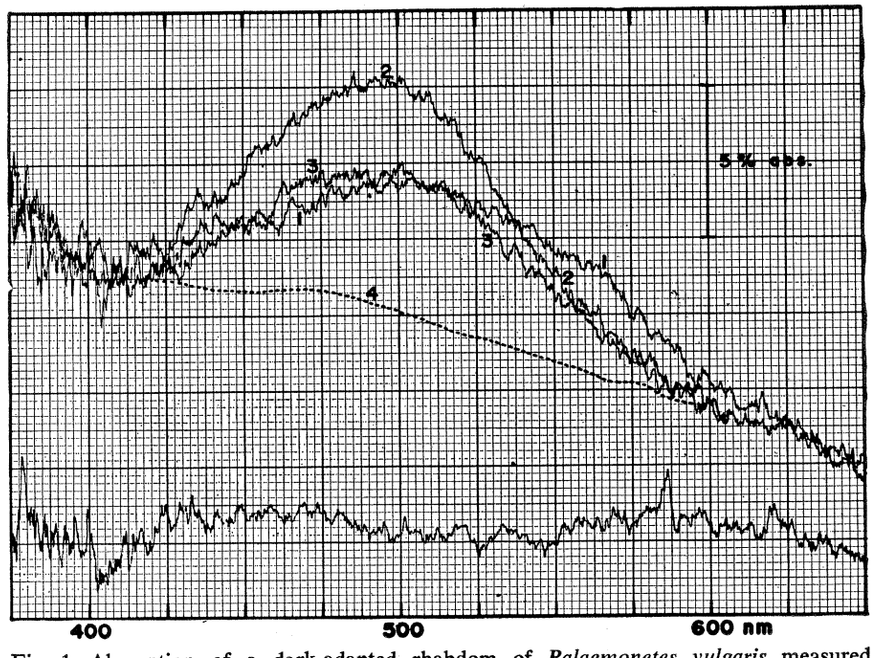

Fig. 1. Absorption of a dark-adapted rhabdom of *Palaemonetes vulgaris* measured with a 4-μm beam passing transversely through the organelle. Curve 1 is the initial spectrum; curve 2 was recorded after 2-minute exposure to bright red light (wavelengths greater than 620 nm); curve 3, after 2-minute exposure to bright yellow light (wavelengths greater than 470 nm); curve 4, 90 minutes later. Temperature, about 24°C; neutral *p*H; scanning speed, 2.1 nm/sec.