

## Inactivation of Human Interferon by Body Fluids (37735)

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Although native interferon can be readily demonstrated in human serum during the course of viral diseases, the pattern of its distribution and the means of its elimination from the body have yet to be determined. To fully appreciate the significance of any observed distribution for interferon, it will be necessary to be aware of any loss of bioactivity resulting from local inactivation. It has already been demonstrated that human urine is capable of inactivating interferon *in vitro* and presumably *in vivo* (1). The present report describes the effect of human feces, bile, saliva, serum, and cerebrospinal fluid on interferon activity.

*Materials and Methods. Tissue culture.* Human muscle skin fibroblasts (MSF) were grown with supplemented Leibovitz medium (L-15) in tissue culture flasks, dishes, and microtiter plates as described previously (1, 2).

*Interferon induction and assay.* Interferon was induced in MSF with a solution of Poly I-Poly C and diethylaminoethyl dextran in phosphate-buffered saline (1, 2). Assay of interferon was carried out by plaque reduction and microtiter techniques using MSF and vesicular stomatitis virus (1, 3).

*Exposure of interferon to clinical speci-*

*mens.* One part interferon was mixed with three parts of a clinical specimen. Controls were prepared by adding one part interferon to three parts diluent (supplemented L-15 lacking serum). The test and control mixtures were assayed for interferon activity immediately or after incubation at 37° for 4 or 24 hr.

*Clinical specimens. Serum.* Serum was obtained from eight healthy adults and one adult convalescing from aseptic meningitis. Prior to testing, the sera were stored at 4° for periods ranging from 30 min to 3 months.

*Saliva.* Six specimens of saliva, obtained from four healthy adults, were each filtered through a 300 nm Millipore filter and stored at 4° for up to 5 days prior to use.

*Cerebrospinal fluid.* Sterile acellular fluid with normal sugar and protein was obtained from each of five patients, stored at 4°, and tested within 48 hr (specimens No. 1 and 4) or at 5 days (specimen No. 5).

*Pleural fluid.* A transudate from a patient in congestive heart failure was tested after storage for 8 days at 4°.

*Bile.* Bile was collected from the T tube of a patient who had undergone surgery for relief of biliary obstruction. After the bile had been stored for 1 month at 4°, it was passed through a 300 nm Millipore filter, diluted in L-15, and tested. A 1:6 dilution of bile was found necessary to avert toxicity for MSF in the interferon assay system.

*Feces.* Stool specimens from three individuals were diluted 1:4 with L-15 diluent and homogenized. After centrifugation at 2000 rpm for 20 min, the supernatant was further diluted to give a final dilution of 1:10, passed through a 300 nm filter, and stored at 4° for up to 1 week prior to use. One extract was subjected to ultracentrifuga-

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TABLE I. Interferon Activity After Exposure to Human Body Fluids.

Specimens		Titer (units/0.025 ml)					
		0-hr Exposure <sup>a</sup>		4-hr Exposure		24-hr Exposure	
		Test	Control	Test	Control	Test	Control
Serum	1	—	—	48	192	—	—
	2	—	—	96	192	—	—
	3	—	—	200 <sup>b</sup>	500 <sup>b</sup>	—	—
	4	—	—	210 <sup>b</sup>	500 <sup>b</sup>	—	—
	5	1300 <sup>b</sup>	1000 <sup>b</sup>	—	—	100 <sup>b</sup>	510 <sup>b</sup>
	6	5632	5632	768	>1536	384	>1536
	7	5632	5632	768	>1536	768	>1536
	8	960	960	480	960	240	960
	9	960	960	240	960	60	960
Cerebrospinal fluid	1	—	—	24	48		
	2	—	—	24	48		
	3	192	192	48	96		
	4	192	192	24	96		
	5	96	96	—	—	24	96
Pleural fluid	1	—	—	48	48		
Bile	1	—	—	<24	96		
Stool extract	1	<24	192	<12	192		
	2	>1000 <sup>b</sup>	1000 <sup>b</sup>	<1 <sup>b</sup>	500 <sup>b</sup>		
	3	<12	48	<12	24		
Saliva	1	—	—	24	48		
	2	—	—	<24	48		
	3	48	192	24	48		
	4	384	768	96	768		
	5	960	960	960	960	960	960
	6	384	384	96	384	<96	192

<sup>a</sup> Exposed and immediately titered.

<sup>b</sup> Titer in units/2 ml by plaque reduction assay.

tion at 40,000 rpm for 50 min before filtration. A portion of a second stool extract was dialyzed against 4000 vol of Gey's medium for 72 hr before both dialyzed and undialyzed aliquots were tested.

*Stability of interferon in the presence of bovine fetal serum.* Samples of interferon were assayed for activity after incubation at 37°, multiple freeze-thaw cycles, or filtration through a Millipore filter with 300 nm size.

*Results. Stability of interferon in L-15 with 20% FCS.* After 24 hr incubation at 37°, the titer of interferon was 50% of the nonincubated controls. The fall in titer generally occurred within the first 4 hr of incubation. Interferon samples subjected to up to five

freeze-thaw cycles or filtered through a 300 nm Millipore filter<sup>3</sup> had the same titer as untreated controls.

*Stability of interferon in prepared clinical specimens.* The titers of interferon samples were assayed following incubation with and without various prepared clinical specimens. The results are recorded in Table I, where the relative bio-activity for body-fluid-treated interferon is expressed as a percent of the activity of the diluent-treated control. For interferon assayed immediately after mixing with a clinical specimen (0 hr), no consistent effect on titer was observed. However, after

<sup>3</sup> Final concentration of bovine fetal serum just prior to Millipore filtration was 7.5%.

incubation of interferon for 4 hr (4 hr) at 37° with serum, spinal fluid, bile, feces, or saliva, titers of exposed samples were, with one exception, invariably lower than controls. Prior dialysis of the stool extract did not prevent inactivation. Similar exposure of interferon to a sample of pleural fluid was not associated with loss of activity. Interferon incubated for 24 hr with serum, cerebrospinal fluid, or saliva usually lost even more activity. It is notable, however, that one saliva specimen (No. 5) failed to diminish the relative interferon activity with up to 24 hr of exposure. Saliva from this same individual collected on other occasions (specimens No. 1 and 4) was associated with apparent inactivation of interferon at four hr.

*Discussion.* In a previous report, interferon was noted to be inactivated following incubation with urine for 4 hr *in vitro* (1). This time period was considered to be the interval during which interferon might be exposed to bladder urine *in vivo*. In the present study, although interferon was usually 50% inactivated when incubated in the presence of control medium, incubation of interferon in body fluids resulted in significantly lower titers. Thus, it was shown that interferon exposed to serum or cerebrospinal fluid for 4 hr had 50% less activity than the controls. When the period of exposure was lengthened to 24 hr, which is a conservative estimate of the interval interferon might be exposed to these fluids *in vivo*, almost all bio-activity was lost. Of two specimens of saliva similarly tested, one behaved identically to the samples of sera and spinal fluid discussed above, and the other failed to inactivate the inter-

feron.<sup>4</sup> It is of interest that interferon may have been totally inactivated when exposed to bile or feces for 4 hr.

The component of urine which inactivates interferon has been shown to be dialyzable (this is not true for the component of stool which inactivates interferon). Since phenolic substances occur in the urine in concentrations which are capable of inactivating interferon, it is believed that they contribute to the inactivation demonstrated (1). Components of other body fluids which may inactivate interferon are currently being evaluated.

*Summary.* The biological activity of interferon was determined after exposure to human secretions and excretions *in vitro*. Crude interferon lost some activity when incubated for 4 hr with control medium, but was usually inactivated by at least 50% more when incubated in the presence of serum, saliva, or cerebrospinal fluid, and by close to 100% when incubated with stool extract or bile. Interferon incubated for 24 hr with either serum or cerebrospinal fluid lost almost all activity.

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<sup>4</sup> Such variability in behavior of saliva might be expected when one considers the different conditions that might exist in the mouth from moment to moment, particularly in regard to the effect of both neural stimuli and food on the amount and character of saliva.

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