Interferon Clearance Rate Decreased after Repeated Injections

(Accepted 4 May 1971)

A rapid rate of clearance of circulating interferon has been observed in mice (Baron et al. 1966; Gresser et al. 1967; Subrahmanyan & Mims, 1966) and in rabbits (Ho & Postic, 1967) after a single injection of interferon. However, in a recent study (De Clercq, Nuwer & Merigan, 1970) the rate seemed to be slower after multiple intravenous injections of interferon. These observations are extended in this paper.

Interferon was injected into the lateral tail vein of 2- to 4-week-old randomly bred Swiss–Webster albino mice. Animals were then bled at different times from the right retro-orbital plexus, and sera from two mice were pooled for each assay sample.

Mouse interferon was produced in L-929 cells as described elsewhere (Hanna, Merigan & Jawetz, 1966) with the Cassell 73-T strain of Newcastle disease virus (NDV). After inactivation of residual NDV at pH 2 for 5 days, the tissue culture fluid was concentrated tenfold by pressure dialysis in a pressure cell (Amicon Corporation, Cambridge, Massachusetts) employing a UM-10 filter. The interferon preparations contained 5,600 NIH mouse serum interferon research standard units and 0.8 mg. of protein/ml. The protein content was determined with the Lowry technique (Lowry et al. 1950 employing bovine serum albumin as a standard.

Interferon titres were measured by a plaque reduction technique in mouse L cell monolayers using bovine vesicular stomatitis virus (VSV) (Indiana strain) as the challenge virus (Merigan & Finkelstein, 1968). The interferon titre corresponded to the reciprocal of the highest dilution of the sample which reduced virus plaque formation by 50%. Interferon was characterized by its sensitivity to trypsin (1.0 mg./ml., 37°C, 1 hr), thermostability (56°C, 1 hr), non-sedimentability (100,000 g, 2 hr), and species specificity (lack of activity in human skin fibroblasts). The presence of residual activity NDV in our interferon preparations was ruled out by injection of one ml. of preparation into 9- to 11-day-old embryonated eggs, and finding no haemagglutination activity in the chorioallantoic fluid 2 days later.

Two types of controls were used to study the effects of the non-interferon material in our tissue culture preparations: First, L cell protein was obtained by freezing and thawing L cells three times, treating them with ultrasonic vibration (at power level 2 of a Sonifier 185-D generator) for 10 sec., centrifuging at 1,500 g for 10 min., and then diluting in Minimal Eagle's Medium to 0.8 mg. of protein/ml.

Second, preparations in which interferon was inactivated were obtained by heating interferon solutions at 68°C for 1 hr. This procedure reduced the interferon titre from 5,600 to 45 NIH units/ml.

Interferon preparations were injected intravenously every 2 hr up to seven times, and its clearance was measured at different times after the injections. Fig. 1 shows the serum interferon levels after the first to fifth and after the seventh injections of interferon. To correct for variation in the amount of interferon injected in different experiments (from 750 to 4,500 NIH units), our figures are expressed as a percentage of an initial concentration expected from the amount of interferon injected and the total extracellular fluid volume. The latter was calculated from the weight of each mouse. We have assumed from analysis of many individual experiments that homogeneous distribution of interferon in the extracellular...
Fig. 1. Serum interferon levels after repeated intravenous injections of interferon. Arrows indicate time of injections of interferon.

Fig. 2. Serum interferon levels at various times after the first or third injection of interferon. ●—●, interferon clearance after the first injection; ○—○, interferon clearance after the third injection.
fluid is completed and that the initial maximum concentration is reached approximately 1 min. after the injection. Thus, our values are expressed as a percentage of the extrapolated 1 min. values. Interferon clearances were significantly slower after the second to seventh injections than after only one injection; the third to seventh injections showed virtually the same clearance rates.

The interferon clearances after the first and third injections were studied in some detail. Three injections were used because the interferon clearance after the third injection was similar to that obtained with more injections. Earlier reports on interferon clearance showed a rapid disappearance of serum interferon. Ho & Postic (1967) calculated half-times of 7 to 11 min. from their rabbit data and from mouse data of Baron et al. (1966) and Gresser et al. (1967). They also noticed a tailing effect in the clearance at later times, when the rate of clearance became slower than in the earlier rate exponential phase. Our data essentially agree with and extend their observations. From Fig. 2 it appears that the points lie on a curve which is the sum of two exponentials; one, the early, and the other, the late clearance rate exponentials. The early rate half-time after one interferon injection is about 1 min., and the late rate half-time is about 50 min. After three injections of interferon, the early rate half-time increases from 1 to 1½ min., and the late rate half-time from 50 to 100 min., both indicating a slowdown in the clearance of serum interferon after multiple injections.

Preparations with identical protein content but without interferon did not influence the clearance of interferon. Control preparations of both L cell protein and heat-inactivated interferon were injected at 0.8 mg. of protein/ml. 4 and 2 hr before an interferon injection. In both cases, the interferon clearance was the same as with a single interferon injection and considerably faster than after three interferon injections.

Our findings suggest that there may be receptor sites in the body with which interferon molecules complex. When this occurs, the receptor sites would be temporarily incompetent for complexing additional interferon. A gradual saturation of these interferon receptor sites could lead to the prolonged half-life of interferon after repeated injections. This saturation would not only occur after repeated injections but also during continuous intravenous injection of interferon. The decreased clearance rate after repeated interferon injections suggests that treatment of virus infection by passive interferon might be more feasible than previously thought.

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*(Received 17 March 1971)*