JOURNAL OF INTERFERON RESEARCH 5:129-136 (1985)
Mary Ann Liebert, Inc., Publishers

Antiviral Effect of Bacterially Produced Human Interferon (Hu-IFNα_2) Against Experimental Vaccinia Infection in Calves

JOHN WERENNE,[1] CATHERINE VANDEN BROECKE,[1] ANNE SCHWERS,[2] ANNE GOOSSENS,[1] LOUIS BUGYAKI,[3] MARIA MAENHOUDT,[2] and PAUL-PIERRE PASTORET[2]

ABSTRACT

The heterologous antiviral efficiency of bacterially produced human interferon (Hu-IFNα_2) in the bovine species was studied, using vaccinia infection as experimental model. In a double blind experiment, young calves were intramuscularly injected daily for seven consecutive days with different doses of Hu-IFNα_2 or placebo, the treatment starting 24 h before intradermal inoculation of vaccinia virus. A clear protection by interferon was observed in all the IFN treated animals, although individual variations in the sensitivity to IFN were recorded. The efficiency of treatment varied according to the dose of IFN used: With the highest dose (10^6 IU/kg), complete protection could be obtained.

The only side-effect observed was hyperthermia. Circulating antiviral activity appeared quite early after each IFN injection, presented a more or less biphasic kinetics, and was completely cleared after 24 h, justifying the daily treatment schedule. The first evidence of an in vivo antiviral effect of human interferon in the bovine species opens broad perspectives for a future use of interferon in veterinary medicine.

INTRODUCTION

In veterinary medicine, viral diseases cause important economic losses, especially in cattle. Therefore, a broad spectrum antiviral compound would be of high interest to improve the overall efficiency of bovine production. Interferon seems a very promising candidate for such purposes.[1] However, bovine interferon was not yet available in sufficient amounts to test its antiviral activity in vivo.

Since the recombinant DNA technology allowed large scale production of different types of human interferon (for review, see Ref. 2), numerous clinical studies using these molecules as antitumor or antiviral agents in man have been undertaken.

Bacterially produced human α_2 interferon (HuIFNα_2),[3] one of the subtypes of human leukocyte interferon, has been shown for the first time to exert an in vivo antiviral activity against experimental

[1]Department of General Chemistry, Faculty of Sciences, Free University of Brussels, CP 160, Av. F. Roosevelt 50, B.1050 Brussels, Belgium; [2]Department of Virology, Faculty of Veterinary Medicine, University of Liège, Rue des Vétérinaires 45, B.1070 Brussels, Belgium; [3]Institute of Hygiene and Epidemiology (IHE), Rue A. Delporte 27, B.1050 Brussels, Belgium.

vaccinia infection in rhesus monkeys.[4] Furthermore, different human interferons,[5,6] including Hu-IFNα_2,[7] are highly active in bovine cell cultures.

Therefore, the in vivo antiviral effect of Hu-IFNα_2 in the bovine species was tested using intradermal inoculation of vaccinia virus. Indeed, this experimental system produces in calves skin lesions allowing quantification of interferon activity.

MATERIALS AND METHODS

a) Interferon

Bacterially produced human alpha interferon Hu-IFNα_2 was kindly supplied by Dr. C. Weissmann and Dr. M. Fountoulakis from Zürich University. Its specific activity was 10^8 IU/mg of protein and it only contained 5.5 ng of pyrogen/10^8 IU.

b) Virus

Vaccinia virus, strain ELSTREE (used for the production of human vaccine in Belgium), was titrated on chorio-allantoic membrane of embryonated hen's eggs and suspended in a glycerin containing buffer to a final concentration of 10^8 PFU/ml.

c) Experimental procedure

A double blind experiment was performed (Table 1). Twelve two- to three-week-old calves, from different origins, were maintained in the IHE quarantine stable, to avoid spreading of vaccinia virus. Hu-IFNα_2 at different doses, or phosphate buffer saline for control animals, in a volume of 0.5 ml, was intramuscularly injected daily for seven consecutive days, starting on day −1.

TABLE 1. DATA ON EXPERIMENTAL ANIMALS AND THEIR TREATMENT.

Calf number	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*
Weight (kg)	30	43	42	41	25	35	28	35	27	30	40	40
Breed	BW	BW	BW	BW	BW	BW	BW	BW	BW	BW	BB	R
Sex	M	F	M	F	M	F	F	F	F	M	F	F
Treatment												
Vaccinia virus inoculation	+	+	+	+	+	+	+	+	+	+	−	−
Hu-IFNα_2 (IU/kg)	0	0	10^6	10^6	10^6	0.2 × 10^6	0	0.5 × 10^6	0.2 × 10^6	0.5 × 10^6	0	10^6
											not included in the double blind test	

BW: Black and White.
BB: Belgian Blue.
R: Red.
F: Female.
M: Male.

At day 0, all the animals except two (11 and 12) were intradermally inoculated with vaccinia virus preparation, on a shaved area of the flank, using a bifurcated needle; the two uninfected calves were inoculated by the same way, but with glycerin buffer only.

Plasma samples for the determination of circulating antiviral activity were collected at 0, 30 min, 2 h, and thereafter every 3 h until 24 h after the first administration of interferon, and 0, 30 min, 2, 5, 8, and 24 h after each of the following injections. Body temperature was measured just before each blood sampling. Skin lesions were daily scored for their size, number, appearance, and degree of evolution and photographed. Serum samples from days 0 and 15 were taken for anti-vaccinia antibodies determination.

d) Interferon titration

Circulating antiviral activity was determined by the classical technique of reduction of cytopathic effect of VSV in human HEp-2 cells.[8]

e) Seroneutralization test

Sera from days 0 and 15 were tested for the presence of antibodies against vaccinia virus by inhibition of pock formation induced by this virus on chorio-allantoic membrane of embryonated hen's eggs.

RESULTS

Two calves (5 and 12) were in poor condition at the beginning of the experiment. Both of them died on day 0: the necropsy revealed cachexia, anemia, and chronic lesions of arthritis and pneumonia, reflecting a severe bacterial systemic infection prior to interferon treatment. The experiment was therefore performed with ten calves only.

a) Vaccinia lesions

The three untreated control calves (Fig. 1) showed a very similar evolution of vaccinia lesions. A clear protection by interferon was observed in all the treated animals: lesions developed more slowly and remained significantly smaller. Figure 2 shows the aspect of skin lesions on day 9, in two control and two IFN-treated animals.

The diameter of skin lesions appeared to be a good criteria to evaluate the efficiency of IFN treatment. As shown in Fig. 1, the inhibition of vaccinia lesions development depended on the IFN dose injected. However, important individual differences appeared in the sensitivity to IFN treatment. For example, in calf 3, no skin lesions at all could be observed, while calf 4, treated with the same IFN dose (10^6 IU/kg) developed small vesicles (Fig. 3).

b) Side effects

IFN treatment was well tolerated by all the animals. However, hyperthermia was recorded in the treated calves following each IFN injection.

c) Pharmacokinetics of plasma interferon

Figure 4 gives the evolution of circulating antiviral activity in five treated calves during the 24 h following the first administration of IFN. No antiviral activity was detected before the beginning of

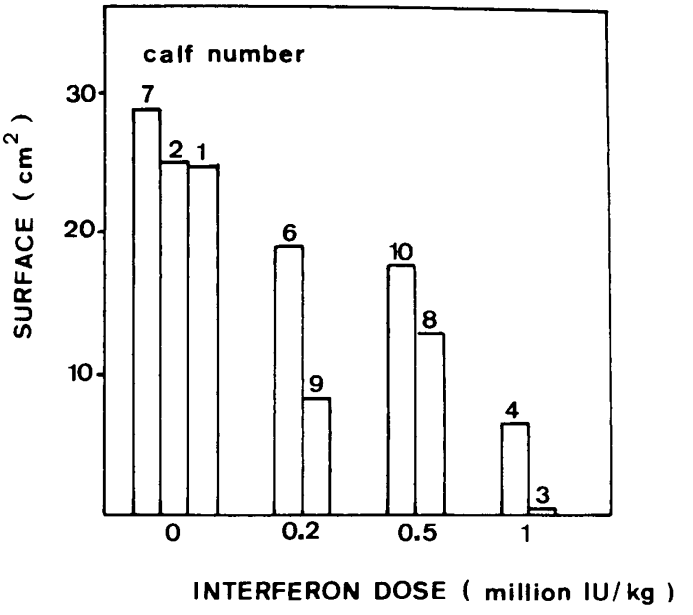

FIG. 1. Vaccinal lesions size. The total surface of the vaccinia lesions (34 virus inoculation sites in each case) was measured by planimetry eight days after infection.

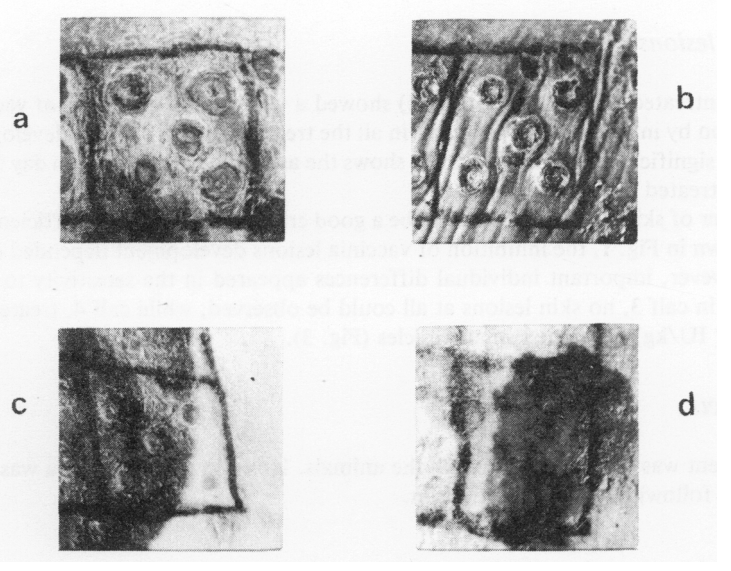

FIG. 2. Skin lesions nine days after infection. a,b: control calves (a = calf number 7, b = calf number 1); c,d: interferon treated calves (c = calf number 9, 0.2 × 10^6 U/kg, d = calf number 3, 10^6 U/kg).

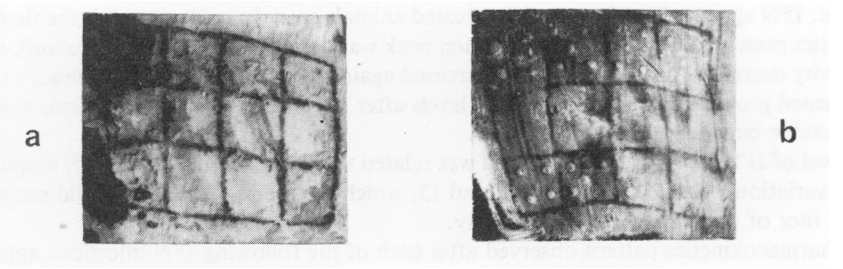

FIG. 3. Effect of 10^6 U/kg IFN treatment on two different calves. a: calf number 3, b: calf number 4.

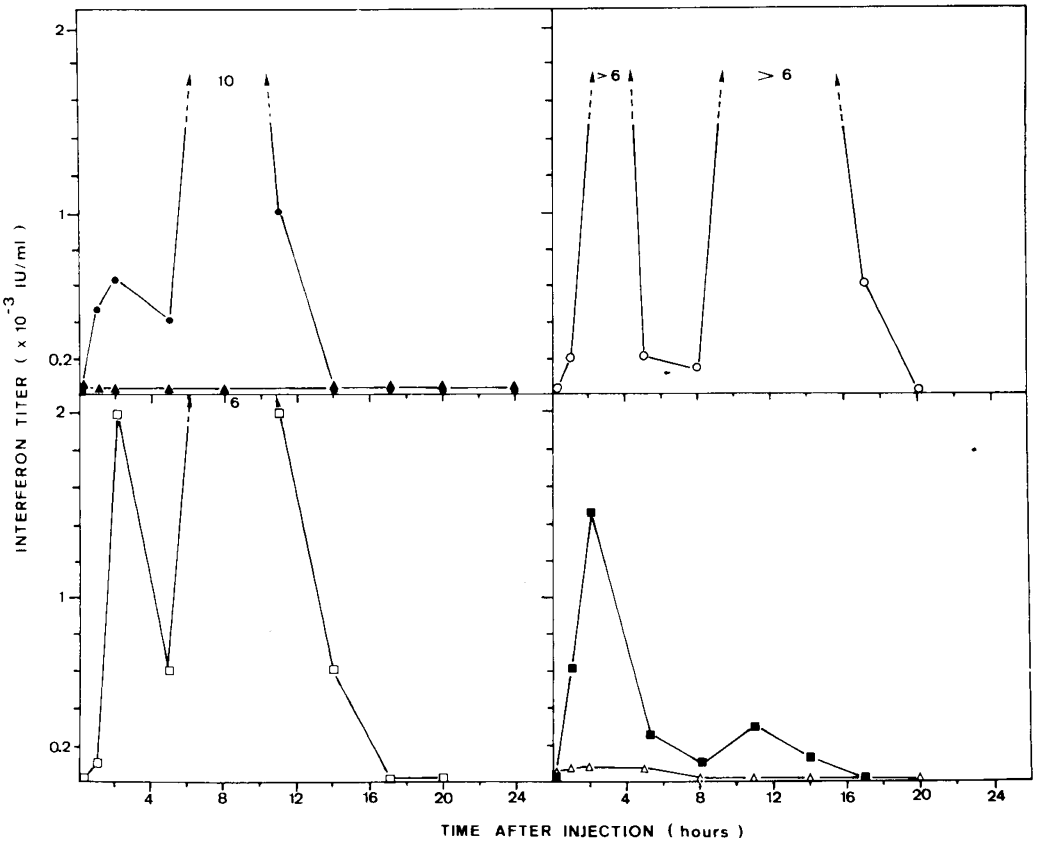

FIG. 4. Pharmacokinetics of interferon. The circulating antiviral activity was titrated in HEp2 cells. ▲ calf 7 (control), ● calf 4 (10^6 IU IFN/kg), ○ calf 8 (0.5 10^6 IU IFN/kg), □ calf 6 (0.2 10^6 IU IFN/kg), ■ calf 12 (10^6 IU IFN/kg), △ calf 5 (10^6 IU/kg). The last animals died on day 0 from pre-existing lesions; in these cases, only a minor fraction of the injected interferon was appearing in the circulation.

treatment; IFN appeared in plasma of the treated animals as early as 30 min after the first injection. The kinetics presented a biphasic aspect: a first peak was observed 2 h PI (postinjection); the level of IFN activity decreased until 5 to 8 h PI, increased again to reach a second peak about 11 h PI, and then returned progressively to undetectable levels after 14 to 17 h. Untreated controls never showed any detectable circulating antiviral activity.

The level of IFN recovered from plasma was related to the dose initially injected, despite some individual variations. Particularly, calves 5 and 12, which died the following day, did not present the expected titer of circulating antiviral activity.

The pharmacokinetics pattern observed after each of the following IFN injections appeared very similar, although blood samples were collected only for 8 h PI (data not shown).

d) Seroconversion

None of the twelve calves possessed anti-vaccinia antibodies at the beginning of the experiment. Neutralizing antibodies against vaccinia virus appeared on day 15 in the sera of all the inoculated animals, except calf 3, the only one which showed no skin lesions.

DISCUSSION

Bacterially produced Hu-IFNα_2 is clearly active against experimental vaccinia infection in calves (Figs. 1 and 2). Moreover, the absence of seroconversion in calf 3 confirms the evidence of a strong inhibition of virus multiplication by interferon. This confirms the results obtained in a similar preliminary experiment performed on two calves.[9,10]

However, there are important individual variations in the sensitivity to IFN treatment (Figs. 2 and 3). Although a significant reduction of skin lesions was observed for each dose tested (Fig. 1), 10^6 IU/kg seems the minimal dose able to confer a complete protection. After the end of the seven day treatment, no further development of lesions was seen contrary to what is described after a shorter (two days) treatment in the previously reported study.[10,11]

IFN treatment is well tolerated by all the animals: the only side effect is the classical hyperthermia described in most studies with exogenous interferon,[12,13] except that realized in rhesus monkeys by Schellekens and coworkers.[4] As the interferon preparation used was highly purified and only contained 5.5 ng of pyrogen per 10^8 IU, this transient hyperthermia seems to be provided by interferon itself.

Circulating interferon activity appeared quite early: a first peak was observed 2 to 5 h after injection. This agrees with the numerous pharmacokinetics studies realized following intramuscular injection of human alpha interferon in different species.[14-17] After a transient decrease, a second peak is observed 8 to 11 h PI (Fig. 4). In most previous studies, data are only reported for 5 to 8 h after injection. However, Breinig and coworkers[17] followed the level of circulating antiviral activity during 24 h in a human patient repeatedly treated with conventionally prepared human α interferon: These authors observed a very similar biphasic kinetics of circulating interferon, with a first peak 4–5 h and a second one 9–11 h after each IM injection.

No further circulating antiviral activity is detected 24 h PI. This confirms the results of our preliminary experiment in calves[9,18] and of most previous studies.[14-16] Breinig et al.,[17] however, still observed low levels of plasma interferon 24 h PI, but no more at 48 h PI. Similarly, Jordan and coworkers[19] have shown that leukocyte interferon injected intramuscularly in patients with malignancy complicated by disseminated herpes zoster, is not completely eliminated in 24 h. They have also shown that repeated injections cause a progressive rise of the average peak level indicative of an accumulation of interferon in tissues. Whether this is indicative of a slower degradation of interferon in the homologous species or of different pharmacokinetics properties for the different interferon species is not yet known. In favor of the last interpretation we should mention the recent

observation made in mice by Sarkar[20] showing that Hu-IFNα_1 is cleared from the circulation more rapidly than the mixture of human interferons produced in leukocytes.

As, in our case, interferon was completely cleared from the circulation after 24 h, the daily treatment schedule adopted seems convenient[9] and is recommended for further studies using exogenous interferon in the bovine species.

Although the level of circulating interferon recovered in treated animals varies according to the dose injected, as already reported by Schellekens et al.,[4] some individual variations were observed. Particularly, in the two ill animals (5 and 12), who died the following day, the expected level of plasma antiviral activity was not recovered. This raises the interesting possibility that trapping of circulating interferon can occur at the site of the already developed lesions. Further investigations are needed to substantiate such an interpretation.

In conclusion, this is the first complete report of an efficient protection against a viral infection in cattle obtained by a way other than classical vaccination. Interferon appears therefore very promising as a broad spectrum antiviral compound in veterinary medicine. Some recent findings support this opinion: The evidence of a role of endogenous interferon in the control of experimental rotavirus diarrhea in newborn calves[21,22] and the curative effect of orally administered bovine interferon in cats suffering from feline leukemia virus-induced non-regenerative anemia.[23] Moreover, Hu-IFNα_2 is active in vitro against several viruses responsible for important economical losses in animal production: infectious bovine rhinotracheitis virus (BHV1), pseudorabies virus (SHV1),[24] and bovine rotavirus (unpublished data).

On the other hand, the calf may represent a convenient model for the in vivo study of the action of interferon against natural viral infections.

ACKNOWLEDGMENTS

We wish to thank Dr. C. Weissmann and Dr. M. Fountoulakis from Zürich University, Switzerland, for giving us bacterially produced Hu-IFNα_2.

We also thank Dr. J. Content from Pasteur Institute, Brussels, for coding the double blind study, and E. Van Kerckhove for serological tests.

We thank Dr. B. Brochier for his occasional but very kind help during this study.

This work was partially supported by grants from the "Institut pour l'encouragement de la Recherche scientifique dans l'industrie et l'agriculture" (IRSIA) from which C. Vanden Broecke was a student fellow.

Part of these data were presented at the Third Annual International Congress for Interferon, held in Miami (USA), November 1-3, 1982.

REFERENCES

1. WERENNE, J. (1982). L'interféron: perspectives actuelles. Ann. Méd. Vét. **126**, 93-122.
2. WEISSMANN, C. (1981). The cloning of interferon and other mistakes. Interferon **3**, 101-134.
3. NAGATA, S., TAIRA, H., HALL, A., JOHNSRUD, L., STREULI, M., ECSODI, J., BOLL, W., CANTELL, K., and WEISSMANN, C. (1980). Synthesis in *E. coli* of a polypeptide with human leukocyte interferon activity. Nature **284**, 316-320.
4. SCHELLEKENS, H., DE REUS, A., BOLHUIS, R., FOUNTOULAKIS, M., SCHEIN, C., ECSODI, J., NAGATA, S., and WEISSMANN, C. (1981). Comparative antiviral efficiency of leukocyte and bacterially produced human α interferon in rhesus monkeys. Nature **292**, 775-776.
5. GRESSER, I., BANDU, M.T., BROUTY-BOYE, D., and TOVEY, M. (1974). Pronounced antiviral activity of human interferon on bovine and porcine cells. Nature **251**, 543-545.
6. DAGENAIS, L., PASTORET, P.-P., VANDEN BROECKE, C., and WERENNE, J. (1981). Susceptibility of bovine rotavirus to interferon. Arch. Virol. **70**, 377-379.
7. STREULI, M., HALL, A., BOLL, W., STEWART, W.E., II, NAGATA, S., and WEISSMANN, C.

(1981). Target cell specificity of two species of human interferon produced in *E. coli* and of hybrid molecules derived from them. Proc. Natl. Acad. Sci. USA **78,** 2848–2852.

8. STEWART W.E., II (1979). *The Interferon System.* Springer-Verlag, Vienna, New York.
9. WERENNE, J., PASTORET, P.-P., VANDEN BROECKE, C., SCHWERS, A., GOOSSENS, A., BUGYAKI, L., and MAENHOUDT, M. (1983). Bacterially produced interferon as an antiviral in the bovine species, in: *The Biology of the Interferon System.* E. De Maeyer and H. Schellekens (eds.). Elsevier Science Publishers, New York. pp. 419–424.
10. GOOSSENS, A., SCHWERS, A., VANDEN BROECKE, C., MAENHOUDT, M., BUGYAKI, L., PASTORET, P.-P., and WERENNE, J. (1982). Antiviral efficiency of interferon in the bovine species: preliminary data on the activity in calves of bacterially produced interferon. Arch. Int. Physiol. Biochimie **90(4),** B193.
11. SCHWERS, A., GOOSSENS, A., VANDEN BROECKE, C., MAENHOUDT, M., BUGYAKI, L., PASTORET, P.-P., and WERENNE, J. (1983). Antiviral efficiency of bacterially produced human interferon in the bovine species. EEC workshop on interferon and non-specific immunity (in press).
12. INGIMARSSON, S., CANTELL, K., and STRANDER, S. (1979). Side effects of long term treatment with human leukocyte interferon. J. Infect. Dis. **140,** 560–563.
13. SCOTT, G.M., SECHER, D.S., FLOWERS, D., BATE, J., CANTELL, K., and TYRELL, D.A.J. (1981). The toxicity of interferon, in: *Antiviral Research Abstr. 1,* No. 1, Special Abstract issue, **63,** p. 46.
14. CANTELL, K. and PYHALA, L., (1976). Pharmacokinetics of human leukocyte interferon. J. Infect. Dis. **133,** Suppl., A6–A12.
15. EDY, V.G., BILLIAU, A., and DE SOMER, P. (1976). Comparison of rates of clearance of human fibroblast and leukocyte interferon from the circulatory system of rabbits. J. Infect. Dis. **133,** Suppl., A18–A21.
16. BILLIAU, A., HEREMANS, H., VERVERKEN, D., VAN DAMME, J., CARTON, H., and DE SOMER, P. (1981). Tissue distribution of human interferons after exogenous administration in rabbits, monkeys and mice. Arch. Virol. **68,** 19–25.
17. BREINIG, M.C., HO, M., WHITE, L., ARMSTRONG, J.A., PAZIN, G.J., HAVERKOS, H.W., and CANTELL, K. (1982). Effect of prolonged administration of interferon-alpha on pharmacokinetics, fever, lymphocyte proliferative response and NK cells activity. J. IFN Res. **2,** 195–207.
18. SCHWERS, A., VANDEN BROECKE, C., GOOSSENS, A., MAENHOUDT, M., BUGYAKI, L., PASTORET, P.-P., and WERENNE, J. (1982). Administration of human interferon to young calves: circulating antiviral activity and biological response of the host. Arch. Int. Physiol. Biochimie **90(4),** B214.
19. JORDAN, G.W., FRIED, R.P., and MERIGAN, T. (1974). Administration of human leukocyte interferon in herpes Zoster. I. Safety, circulating antiviral activity and host responses to infection. J. Infect. Dis. **130(1),** 56–62.
20. SARKAR, F.H. (1982). Pharmacokinetic comparison of leukocyte and *E. coli* derived human interferon type alpha. Antiviral Res. **2,** 103–106.
21. SCHWERS, A., PASTORET, P.-P., MAENHOUDT, M., DAGENAIS, L., VANDEN BROECKE, C., GOOSSENS, A., and WERENNE, J. (1983). Tentative de reproduction expérimentale de la diarrhée à rotavirus chez des veaux nouveau-nés privés de colostrum. Ann. Rech. Vét. **14,** 265–270.
22. VANDEN BROECKE, C., SCHWERS, A., DAGENAIS, L., GOOSSENS, A., MAENHOUDT, M., PASTORET, P.-P., and WERENNE, J. (1984). Interferon response in colostrum-deprived newborn calves infected with bovine rotavirus: its possible role in the control of the pathogenicity. Ann Rech. Vét. **15(1),** 29–34.
23. TOMPKINS, M.B. and CUMMINS J.M. (1982). Response of feline leukemia virus-induced non-regenerative anemia to oral administration of an interferon-containing preparation. Feline Practice **12(3),** 6–15.
24. GOOSSENS, A., SCHWERS, A., VANDEN BROECKE, C., DAGENAIS, L., MAENHOUDT, M., DUWYN, R., VAN CAMP, B., PASTORET, P.-P., and WERENNE, J. (1983). Sensibilité des virus de la rhinotrachéite infectieuse bovine (*Bovine herpesvirus 1*) et de la maladie d'Aujeszky (*Suid herpesvirus 1*) à l'interféron humain produit par des bactéries (Hu-IFNα_2). Ann Méd. Vét. **127,** 135–139.

Address reprint requests to:
Dr. John Werenne
Department of General Chemistry
Faculty of Sciences
Free University of Brussels
CP 160, Av. F. Roosevelt 50
B. 1050 Brussels, Belgium

Received 6 August 1984/Accepted 4 October 1984