PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **180**, 137–143 (1985)

The Physiological Interferon Response: IV. Production of Interferon by the Perfused Human Placenta at Term (42155)

V. BOCCI,* L. PAULESU,* AND M. G. RICCI†

Institute of General Physiology and †Department of Obstetrics and Gynecology of the University of Siena, Italy

Abstract. Human placentas at term, free of bacterial and viral diseases, have been perfused and maintained sterile for up to 13 hr. Several parameters indicate that the organs remained viable and released interferon into the perfusate in a progressive fashion. The amount of interferon was small and the individual variations indicate that there are "poor" and "good" placenta producers. Both interferons -α and -β were produced with a prevalence of the latter type. The partial acid lability and the type heterogeneity suggest that under physiological conditions the placentas produce unusual interferons, the function of which remains speculative.

Interferon (IFN) or IFN-like components have been identified in murine placentas (1, 2). An obvious follow-up of this research was to investigate if IFN was detectable in the human amniotic fluid (AF) and, in fact, Lebon *et al.* (3) measured IFN-α in most of the their AF samples. However, Tan and Inoue (4) detected IFN only in about 25% of the fluids and Cesario *et al.* (5) could not find any IFN at all and actually detected inactivators against IFN-β. The reason for this discrepancy may lie in the different sensitivities of the assay systems used as well as in the variable presence of IFN inhibitors. Although transplacental transfer of IFN in humans is limited (6, 7), IFN present in AF may come from the mother's blood. On the other hand, the placenta itself or its annexes could be the primary site of production and, as IFN displays immunomodulatory properties (8), it could be involved in the immunoregulation of fetal acceptance.

It appeared to us that the most direct approach to clarify the physiological role of the placenta in the production of IFN will be to isolate and perfuse the human placenta and the results so far obtained are described herein.

Materials and Methods. *Perfusion system.* The apparatus adapted to perfuse the human placenta is similar to those used in our laboratory and previously described (9, 10). It was sterilized by ethylene oxide and housed in a horizontal laminar flow cabinet to prevent contamination.

Perfusion technique. A number of women who would potentially be placenta donors were followed throughout pregnancy. They were periodically checked for any bacterial, viral, or fungal infections until term delivery was performed by cesarean section. If the placenta was free of visible defects, it was cannulated under aseptic conditions: two 8 Fr umbilical catheter (Pharmaseal S.p.A., Trieste, Italy) were secured into the umbilical arteries while the vein drained through a large glass cannula. Flow rates were controlled to make sure that arterial inflow equaled venous outflow. The perfusion, using a gravity flow with a pressure of about 70 mm Hg, was carried out with about 1 liter of warm Hanks' balanced salt solution containing 10 IU/ml heparin so that venous effluent rapidly became almost bloodless. The flow rate was about 70 ml/min. The placenta was then transferred in a sterile container to our laboratory and perfusion was resumed within 15 min. Perfusion medium (2.2 liter for each phase) was composed of RPMI 1640 without phenol red, containing 5 μg/ml insulin (Wellcome), 1 IU/ml heparin (Liquemin Roche), and 100 μg/ml piperacillin (Lederle). Donors were not hypersensitive to this antibiotic.

The perfusion medium was continuously recirculated by the use of a peristaltic pump (Type 501S Watson Marlow Co., Bucks, U.K.) between the reservoir and the organ chamber and the perfusion schedule was as follows: the equilibration period during which organ temperature (37°C), arterial pressure (59 ± 15 mm Hg), flow rate (176 ± 6 ml/min), and pH (7.3) became constant lasted about 25 min (Fig. 1). The first perfusion cycle was continued for 7 hr when, owing to

0037-9727/85 $1.50

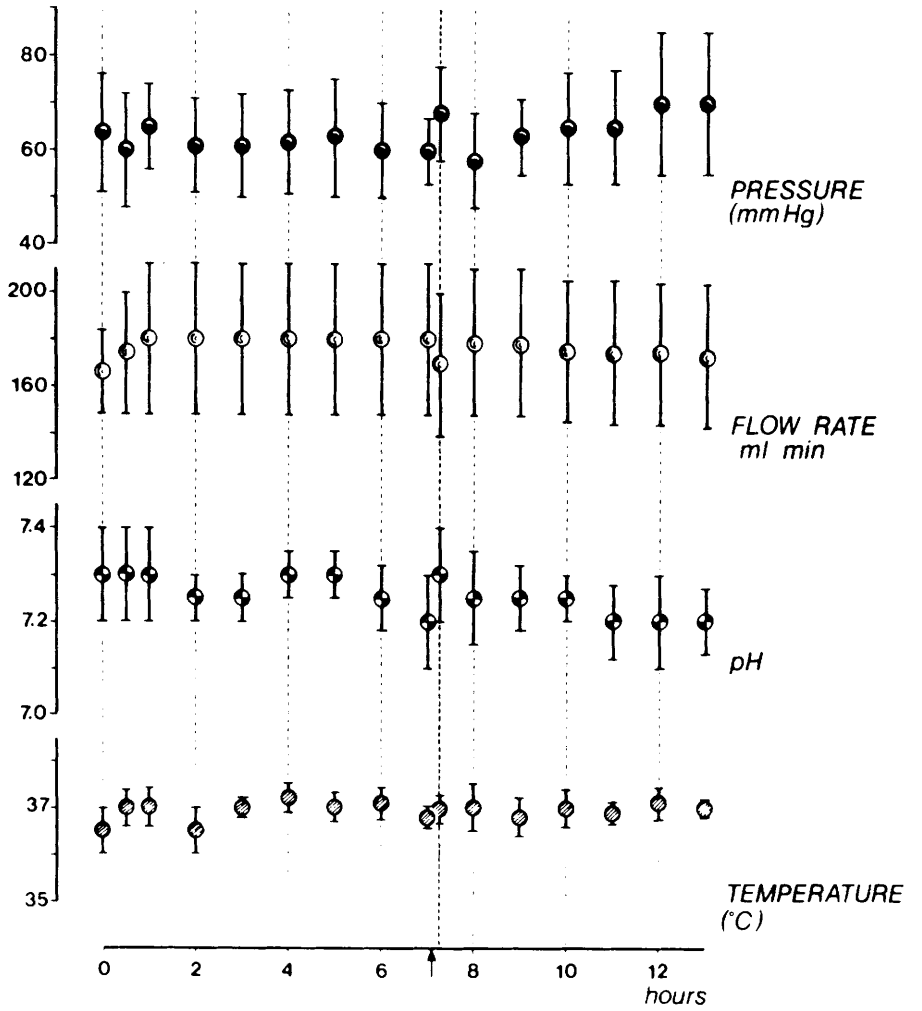

FIG. 1. Variations of the umbilical arterial pressure, flow rate, pH, and temperature during the first (0–7 hr) and the second (7–13 hr) period of perfusion. The arrow indicates the time of change of the medium. Average ± standard deviation of five placental perfusions.

pH instability, it appeared useful to change the medium. The second period usually lasted 6 hr, after which perfusion was stopped for practical reasons. During the second part of the perfusion the medium did not contain heparin, and human albumin (Farma Biagini) was added to a final concentration of 5 mg/ml to prevent any possible inactivation or absorption of IFN. Oxygenation and pH adjustment at 7.3 were ensured by a mixture of 95% O_2–5% CO_2.

Sample collection. Perfusate samples (10 ml) were collected in a calibrated cylinder at a given time and replaced with a fresh medium addition of small volumes of 25% glucose and 12% bicarbonate solutions so that the pH did not go lower than 7.2 and glucose remained at a steady-state concentration. At the end of perfusion the placenta was drained of excess liquid and was weighed. Segments of placenta were also weighed on an analytical balance and were dried at 95°C until the dry weight, 24 hr apart, was constant. Segments of placenta (1 g) were collected for extracting and measuring total tissue LDH (5 ml distilled water) and IFN (5 ml phos-

phate-buffered saline). The homogenates frozen at −20°C were thawed and clarified by centrifugation at 105,000*g* for 1 hr.

Chemical and enzymatic determinations. Perfusate samples were centrifuged to remove a small amount of blood cells and the following determinations were performed on the supernatant: glucose, lactate, and lactate dehydrogenase were measured by using the glucose-oxidase method and the optimized enzymatic uv methods, respectively, as defined by Boehringer-Mannheim GmbH. Na^+ and K^+ were determined by flame photometry. Plasma proteins were measured according to the method of Gornall *et al.* (11) using bovine serum albumin as a standard.

Experimental values of five successful placental perfusions are given as means ± standard deviation.

IFN assay and characterization. The microplaque reduction assay described by Langford *et al.* (12) was used throughout for the titration of IFN using human amnion cells (Wish), epitheloid human carcinoma (HEp 2), and vesicular stomatitis virus (VSV) as a challenge virus. Cell monolayers were infected with about 50 plaque-forming units of VSV and samples were tested at least twice in duplicates. Titrations were always made employing the international reference preparation (IRP) for human IFN-α and -β (obtained from NIAID; NIH, Bethesda, Md.). However, because the antiviral activity (A.A.) present in the placental perfusate was heterogenous, we could attribute the A.A. to neither the standard IFN-α nor -β, and therefore, all titers were reported as laboratory Units/milliliter or Units/gram dry tissue. Titers were very similar using both Wish or HEp-2 cells. All biological samples were sterile according to standard bacteriological tests; most of the samples were tested also for the possible presence of endotoxin by the Limulus amoebocyte lysate (LAL) test. Detection levels ranged among different kits between 0.025 and 0.05 ng/ml. Perfusate samples were concentrated 50-fold by using Minicon concentrators B 15.

Characterization of interferon was carried out according to the classical procedure using acidification of samples at pH 2.0 with 0.1 *M* HCl, followed by neutralization at pH 7.0 prior to the assay, heating at 56°C for 1 hr, dialysis for 1 day at 2°C, ultracentrifugation at 105,000*g*, and proteolytic (trypsin: 0.25% for 30 min at 37°C) and other enzymatic treatments (DNase and RNase: 0.3% for 1 hr at 37°C). Neutralization of IFN was carried out as follows: concentrated perfusate samples (50 μl, 150 units/ml) as well as IFN international standards were incubated (60 min at 37°C) with an equal volume of individual and pooled anti-IFN antisera and the residual antiviral activity was then assayed. Anti-IFN-α and anti-IFN-β antisera (G-026-502-568 and G-028-501-568, obtained from NIAID-NIH) were used at a dilution of 1:2000 and 1:40, respectively. Anti-IFN-γ antiserum (kindly provided by Professor J. Vilcek and Dr. M. P. Langford, with a neutralizing titer of about 1:2500 and 1:10,000 against 10 units/ml, respectively) (13) was used at a dilution of 1:20. For each experiment the amounts of antihuman IFN-α, -β, and -γ employed were sufficient to completely neutralize the corresponding IFNs. Control sera (control antisera sheep to human leukocyte, G-027-501-568 and to human fibroblast, G-029-501-568 interferon, obtained from NIAID-NIH) were used in parallel with all samples.

Results. Some physical characteristics of the perfusion are reported in Fig. 1 and the metabolic performance of the placenta is summarized in Fig. 2. In spite of considerable consumption of glucose (0.9 ± 0.2 mg/g placenta wet wt/hr) normal levels were maintained throughout. Lactate production increased about fivefold from the initial level and was more rapid in the final phase of perfusion. Na^+ and K^+ ions varied little, indicating that cells were not leaking K^+ into the medium. Plasma proteins increased progressively from about 2 up to 6 mg/ml owing to diffusion of proteins present in the extravascular space and in the maternal circulation. During the second phase of perfusion, because of addition of albumin to the medium, protein levels remained constant throughout. Lactic dehydrogenase levels rose more rapidly during the final perfusion cycle. However, if LDH levels are taken as a marker to express organ viability, present results indicate that enzyme levels increased about fivefold which corresponds to only 0.6–2.5% of the total LDH content. On the whole,

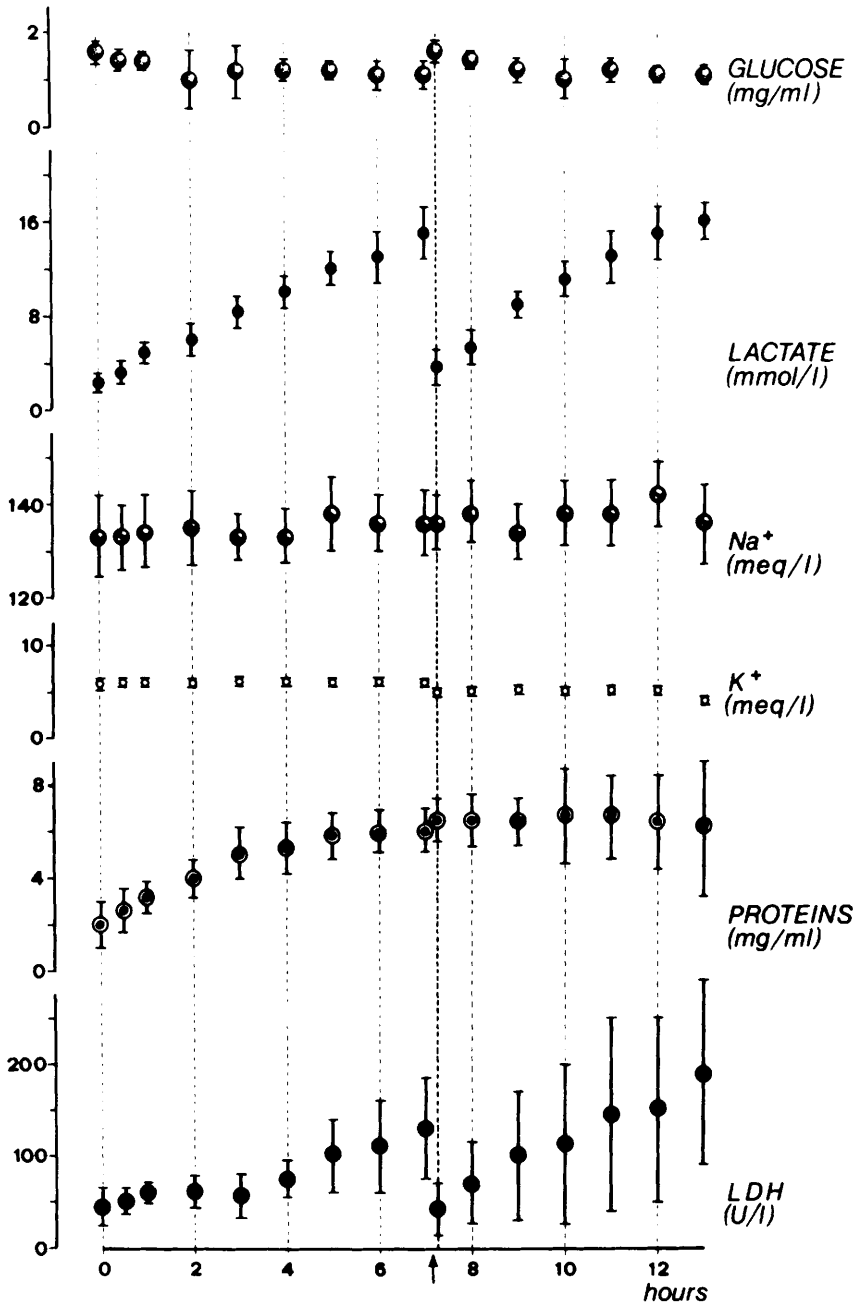

FIG. 2. Glucose, lactate, Na^+, K^+, plasma proteins, and lactate dehydrogenase levels in the medium perfusing the placenta during the first (0–7 hr) and the second (7–13 hr) period. The arrow indicates the time of change of the medium. Average ± standard deviation of five placental perfusions.

therefore, placental viability appeared very satisfactory.

Figure 3 shows that A.A. increased progressively during both perfusion periods: from below 4 ± 2 U/ml it rose to a maximum of 25 ± 5 U/ml. After the medium was changed, the A.A. fell again to below 3 U/ml but increased once more as during the first per-

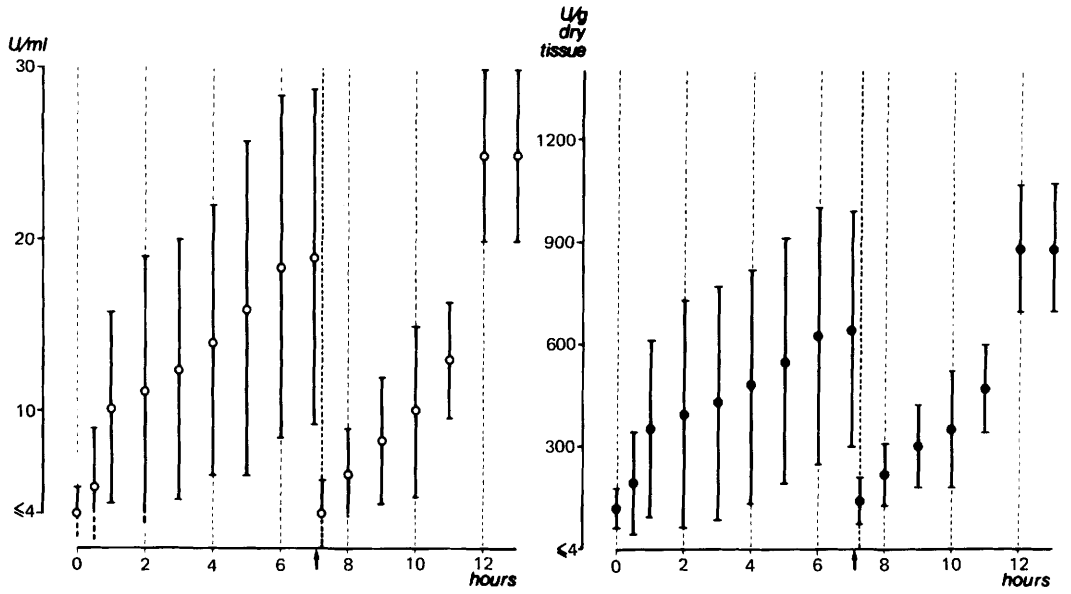

FIG. 3. Interferons levels as U/ml (left panel) and U/g dry tissue (right panel) in the medium perfusing the placenta during the first (0–7 hr) and the second (7–13 hr) period. The arrow indicates the time of change of the medium. Average ± standard deviation of five placental perfusions.

fusion period. As it was expected in the case of normal placentas the A.A. was low but, even though it became diluted in the large perfusion volume, total units were $5.5 \pm 1.1 \times 10^4$. A.A. reported as Units/gram dry tissue was $8.7 \pm 1.8 \times 10^2$ U/g. Placental extracts contained 198 ± 40 U/g of dry tissue. As mentioned, these placentas can be considered normal as far as they were, and remained sterile and endotoxin-free throughout perfusion and were free from toxoplasmosis, syphilis, and viral (CMV, rubella, HVH) infections. The finding that A.A. increased during the second phase of the perfusion with a kinetic similar to the one observed initially indicated that placentas went on synthesizing proteins with A.A.

Characterization of IFN has been greatly hindered, on one hand, by the very low titer in the perfusate and, on the other hand, by the loss of IFN during the concentration procedure. Unfortunately, as is known to happen (14), the amount of IFN did not increase proportionately probably because of adsorption of some of the IFN-β into the filter. Thus Table 1 reports a preliminary characterization of the residual IFN activity. Assuming to have lost about 70% of the IFN-β and as the majority of the residual activity still reacts with anti IFN-β the most likely provisional conclusion seems that most of the A.A. produced by the placenta belongs to IFN-β. Owing to the weak activity we have been unable to characterize further the IFN species and to define their molecular weights.

TABLE I. CHARACTERIZATION OF HUMAN PLACENTAL INTERFERONS OBTAINED BY CONCENTRATING PLACENTAL PERFUSATES

Treatment	IFN (U/ml)	Degree of inactivation (%)
None	150	0
Heating	150	0
Dialysis	150	0
Ultracentrifugation	150	0
pH 2.0	62	59
DNase and RNase	150	0
Trypsin	<3	100
Anti IFN-α serum	96	36
Anti IFN-β serum	83	45
Anti IFN-γ serum	150	0
HEp-2	150	0
MDBK	120	20
Chick embryo fibroblasts	<3	100

Discussion. The presence of IFN in the human AF is a controversial subject and, owing to transplacental transfer of protein molecules, IFN present in AF might be the result of an exchange between maternal and fetal circulations. However, it has been postulated (15) that a potential site of the physiological IFN response could be the placenta, particularly at the interface among the semiallogeneic conceptus and maternal tissue. The most direct way of defining the role of the placenta and its annexes was to investigate if the isolated and perfused human placenta was able to release any A.A. into the perfusate. The results show that human placenta at term can be satisfactorily perfused for many hours and remain viable. The novel result is that human placenta does produce and release into the perfusate small amounts of A.A. referable to IFN. On this basis the previously demonstrated presence of IFN in AF *in vivo* (3) can be unequivocally attributed to the placenta and/or annexes. Thus, while we can exclude other maternal sources, it remains uncertain which part(s) of the placenta and annexes is the main IFN contributor(s). We plan to clarify this issue when we can dispose of anti-IFN antibodies labeled with fluorescein.

An interesting feature of the placental IFNs is their heterogeneity: so far, by the use of antisera, we have detected both IFN-α and -β while antiserum against IFN-γ failed to neutralize any A.A. Most of IFN is very likely of β type. The amount of acid-labile IFN (16) (clearly not due to IFN-γ) and the relative amount of IFN-α and -β present in the concentrated perfusates showed individual variations. This point deserves a more detailed investigation devoted to evaluating molecular species and their antigenic structures. This is interesting because Goiran *et al.* (17) have found a number of unusual IFN-α and -β produced by virus-infected amniotic membranes.

On the other hand, normal AF contained exclusively IFN of α type (3) and it may well be that expression of IFN-β becomes evident only after viral stimulation, or it is produced in such a low amount that it is undetectable *in vivo* but is measured *in vitro* because the perfusate pool does not exchange with maternal body fluids. It is worthwhile recalling that IFN is catabolized so rapidly *in vivo* that its persistence in the plasma is very short (18).

In conclusion, our present results indicate that normal human placentas at term produce and release IFN-α and -β in the perfusate. It would be of great interest to know when IFN production begins and if it varies during normal pregnancy. At this stage we can only speculate that IFN may have a role in embryonic development and differentiation as well as checking maternal immunity against the fetus, but clearly more experimental data are needed to support these suggestions.

This work was supported by contract No. 84.0183452 from CNR, Rome, Progetto finalizzato Controllo delle malattie da infezione, and by the Ministero Pubblica Istruzione. We are grateful to several members of the Istituto S.V.T. "A. Sclavo" Siena, namely, Dr. C. Cioni, Dr. L. Vanni, and Dr. A. Semboloni for providing chicken embryo fibroblasts, to Dr. F. Lattanzi for the many LAL tests, and to Mr. A. Lorenzetti and Mr. L. Palazzi for sterilization of the apparatus. We are very grateful to Mr. A. Vanni for constructing the perfusion system and assisting during the initial perfusions, to Mr. M. Fedeli for chemical and enzymatic determinations, and to Mr. S. Focardi for technical help in the tissue culture laboratory. We are indebted to Professor J. Vilcek and Dr. M. P. Langford for the gift of interferon-γ antisera.

1. Fowler AK, Reed CD, Giron JD. Identification of an interferon in murine placentas. Nature (London) **286**:266–267, 1980.
2. Weislow OS, Kiser R, Allen PT, Fowler AK. Partial purification of a placental interferon with atypical characteristics. J IFN Res **3**: 291–298, 1983.
3. Lebon P, Girard S, Thepot F, Chany C. The presence of α-interferon in human amniotic fluid. J Gen Virol **59**:393–396, 1982.
4. Tan YH, Inoue M. The detection of interferon activity in human amniotic fluid. In: Merigan TC, Friedman RM, eds. Interferon. New York, Academic Press, p249, 1982.
5. Cesario T, Goldstein A, Lindsey M, Dumars K, Tilles J. Antiviral activities of amniotic fluid. Proc Soc Exp Biol Med 168:403–407, 1981.
6. Overall JC Jr, Glasgow LA. Fetal response to viral infection: Interferon production in sheep. Science (Washington, DC) **167**:1139–1141, 1970.
7. Bocci V. Distribution of interferon in body fluids and tissues. Tex Rep Biol Med **35**:436–442, 1977.
8. De Maeyer E, De Maeyer-Guignard J. Effect of interferon on cell mediates immunity. Tex Rep Biol Med **41**:420–426, 1982.
9. Bocci V, Pacini A, Pessina GP, Bargigli V, Russi M.

Metabolism of interferon: Hepatic clearance of native and desialylated interferon. J Gen Virol **35**:525–534, 1977.

10. Brandani Pacini A, Bocci A. An analysis of the optimal conditions for perfusing an isolated rabbit kidney with homologous blood. Renal Physiol **6**:72–79, 1983.
11. Gornall AG, Bardawill CJ, David MM. Determination of serum proteins by means of the biuret reaction. J Biol Chem **177**:751–756, 1949.
12. Langford MP, Weigent DA, Stanton GJ, Baron S. Virus plaque—reduction assay for interferon: Microplaque and regular macroplaque reduction assays. In: Pestka S, ed. Methods in Enzymology. New York, Academic Press, Vol 78:p339, 1981.
13. Langford MP, Weigent DA, Georgiades JA, Johnson HM, Stanton GJ. Antibody to staphylococcal enterotoxin A. Induced human immune interferon (IFNγ). J Immunol **126**:1620–1621, 1981.
14. Sulkowski E, Goeddel DV. Hydrophobicity of human interferons. In: Munk K, Kirchner H, eds. Interferon. Vol 11: pp106–117, 1982.
15. Bocci V. The physiological interferon response: The site and the meaning. In: Khan A, Hill NO, eds. Human Lymphokines: Biological Immune Response Modifiers. New York, Academic Press, p241, 1982.
16. Preble OT, Black RJ, Friedman RM, Klippel JH, Vilcek J. Systemic lupus erythematosus: Presence in human serum of an unusual acidlabile leukocyte Interferon. Science (Washington, DC) **216**:429–431, 1982.
17. Goiran PD, Galliot BR, Chudzio T, Chany C. Unusual human interferons produced by virus infected amniotic membranes. Proc Natl Acad Sci USA **80**:2628–2631, 1983.
18. Bocci V. Pharmacokinetic studies of interferons. Pharmacol. Ther. **13**:421–440, 1981.

Received January 22, 1985. P.S.E.B.M. 1985, Vol. 180.
Accepted May 10, 1985.