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Relationship Between the Physicochemical Nature of Human Interferon, the Cell Induced, and the Inducing Agent

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To determine the basis for differences in interferons previously noted by others, we prepared five types of human interferon, namely interferon induced in muscle skin fibroblasts using complexed polyinosinic:polycytidylic acid (IC) and Newcastle disease virus (NDV), and interferon induced in leukocytes using IC, NDV, and phytohemagglutinin. We determined the molecular weight, isoelectric point, inactivation rate at 56°C and structural activity relationships for each interferon. Significant differences between these interferons were found, many of which seem related to the cell induced rather than the inducer. We believe these findings have important pharmacological implications for the use of interferon in man.

Recent investigations have established that human fibroblast interferon induced by complexed polyinosinic:polycytidylic acid (IC) is different from human leukocyte interferon induced by viruses (6, 9, 18, 20). Dissimilarities between these two interferons have been found in their antigenicity (9), in the circumstances under which they will renature (18), and in their sensitivity to guanidine (6) and heat (20). Although many issues remain unsolved, particularly pressing questions which are still unanswered include the following: which factors dictate when a given interferon will be elaborated; is there a molecular basis for the observed dissimilarities; and how many human interferons can be expected to exist? The answers to these questions may have particular relevance for identifying a human interferon species which will be pharmacologically superior for clinical application.

We addressed these questions by preparing five varieties of human interferon: interferon prepared in human fibroblasts (muscle skin fibroblast [MSF] cells) using IC and Newcastle disease virus (NDV) as inducers and interferon prepared in leukocytes using IC, NDV, and phytohemagglutinin (PHA) as inducers. We compared these interferons in the crude state with regards to physical properties and sensitivities to chemical reagents known to attack specific bonds and groups. However, because the data presented here were determined on crude interferon, they cannot be considered absolute and could be updated when studies on purified preparations are possible. Nevertheless, since each of the interferons was studied under identical circumstances, the resulting data are clearly valid for comparing interferons and offer insight into the occasional differences among crude interferons that have been observed previously. Additionally, this information will be of value in deciding on purification schemes and ultimately in determining what alteration in native crude interferon (i.e., as it exists in its native state) are caused by a particular purification sequence.

Indeed it has been possible to determine that the cell induced exerts an important influence on the properties of the interferon secreted, that the circumstances of the induction may alter at least some of these properties, and that the observed differences appear to be associated with structural dissimilarities within the interferon molecule.

MATERIALS AND METHODS

Preparation of virus-, IC-, and PHA-induced human interferon in WBCs (WBC-NDV, WBC-IC, and WBC-PHA). After separation by low-speed centrifugation from blood acidified with ammonium chloride for 10 min, human white blood cells (WBCs) were washed twice with Leibovitz medium (L-15). The cells were subsequently suspended at a density of 10 million/ml in L-15 supplemented with 5% fetal calf serum (FCS) and rolled in test tubes for 18 h at 37°C in the presence of NDV (multiplicity of 100 median egg infectious dose [EID_{50}] per cell) for preparation of WBC-NDV interferon. The interferon-containing medium was separated by pelleting the cells at low-speed centrifugation and then sequentially dialyzed against 0.1 M citric acid at pH 2.7 for 5 days and phosphate-buffered saline (PBS) at pH 7.4 for 24 h.

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After centrifugation at 25,000 rpm for 90 min in a Beckman SW27 rotor, 2-ml aliquots of the pooled interferon-containing supernatant were frozen at −80°C. For WBC-IC, the cells, at a similar concentration, were suspended in a solution of IC (10 μg/ml) and diethylaminoethyl dextran (DEAE-D) (100 μg/ml) in PBS, rolled at 4°C for 1 h, washed three times with supplemented L-15, and rolled at 37°C for a further 18 h. The interferon-containing medium was then separated from the cells by low-speed centrifugation, aliquoted, and stored as noted above.

For WBC-PHA, fresh human leukocytes as described above were incubated with a 1:400 dilution of phytohemagglutinin P (Difco) as 2.0-ml aliquots in tissue culture tubes rolled for 18 h at 37°C. After centrifugation at 1,000 rpm for 10 min, the pooled supernatants were dialyzed in PBS (pH 7.4) for 24 h before freezing and storing at −80°C in 2.0-ml aliquots. Separate preparations of WBC-PHA were acid-dialyzed at pH 2.7 as noted above for WBC-NDV. Acid-dialyzed WBC-PHA was found to react identically to PBS-dialyzed WBC-PHA in the heat inactivation and chemical stability experiments, and was not used in the molecular weight and isoelectric focusing studies.

Preparation of IC-induced human interferon in MSF cells (MSF-IC). MSF were exposed to 10 μg of poly IC per ml and 100 μg of DEAE-D (molecular weight 2,000,000) per ml for 3 h, washed three times, and incubated overnight in L-15 supplemented with 5% FCS. The interferon-containing medium was then harvested and stored at −80°C in 2.0-ml aliquots.

Preparation of virus-induced interferon in MSF cells (MSF-NDV). Confluent MSF cells were inoculated with NDV (multiplicity of 30 EID_{50} per cell) and allowed to incubate overnight. The next morning the interferon-containing tissue culture medium was harvested and processed identically to the WBC interferons described above with sequential dialysis versus 0.1 M citric acid and PBS before centrifugation at 25,000 rpm for 90 min. Aliquots (2 ml) of the pooled interferon-containing supernatant were frozen at −80°C.

Preparation of virus-induced chicken interferon. Monolayers of primary chicken embryo fibroblasts (CEF) were exposed to Sindbis virus at a multiplicity of 1 plaque-forming unit per cell for 1 h at 37°C. Further incubation at 37°C in Geys balanced salt solution (BSS), supplemented with 5% calf serum, was carried out for 48 h. The interferon-containing medium was then sequentially dialyzed against 0.1 M citric acid at pH 2.7 for 24 h and Geys BSS at pH 7.4 for 24 h. The dialysate was distributed into 10-ml aliquots and stored at 4°C.

Interferon assays. Human interferon was assayed on MSF using vesicular stomatitis virus (VSV) as the challenge virus. The plaque reduction and microtiter techniques utilized were described previously (4, 19). In such assays, interferon-treated cell monolayers are washed free of test samples prior to virus challenge. Chicken interferon assays were performed on CEF by plaque reduction with VSV as the challenge virus (19).

Heat inactivation. Aliquots of each interferon, containing 1,000 to 2,000 interferon units per ml, were placed in a 56°C water bath. At indicated intervals (10, 20, 30, 60, and 120 min), samples were withdrawn, placed in an ice bath, and titered immediately. Comparisons of differences in the rate of heat inactivation between the fibroblast and leukocyte interferon were performed twice.

Electrofocusing. Electrofocusing was performed by the technique of Wrigley (21). Samples containing 10,000 to 80,000 interferon units were exhaustively dialyzed for 24 h against 0.05 M PBS at 4°C and then mixed with appropriate amounts of catalyst solution, acrylamide solution, and carrier ampholytes. Each interferon was focused either in both the pH 5 to 8 and 3 to 10 ampholyte ranges or twice in the pH 3 to 10 range. A current of 1 mA per tube was applied in a Buchler disc electrophoresis apparatus. The voltage was maintained constant and the amperage was allowed to diminish. Electrofocusing was performed at 4°C for periods ranging from 5 to 25 h. The gels were subsequently sliced into 12 to 15 equal segments, each of which wes homogenized in 0.5 ml of distilled water. After a measurement of pH and an overnight incubation at 4°C, 0.2 ml of a solution containing antibiotics and 4% FCS was added to each fraction prior to assay for interferon activity. Linear pH gradients were established in all experiments.

Molecular weight determinations. Estimations of molecular weight were performed on a column of Sephadex G-200 superfine, which was extensively washed with 0.05 M PBS. The void volume was marked with dextran blue, and the column was calibrated with proteins of known molecular weight. Interferon samples containing 15,000 to 40,000 interferon units in 0.8 ml were applied to the column through a flow adaptor. The specimens were eluted using inverse flow of 0.05 M PBS at pH 7.0 in general; fractions of 0.5 ml were collected at a flow rate of 0.5 ml/h. At the completion of the run, each fraction was passed through a 300-nm membrane filter (Millipore Corp.) and titered immediately. Molecular weight estimates were made by the method of Andrews (1). Each interferon was chromatographed at least twice on Sephadex G-200 superfine. MSF-IC and WBC-NDV were also run on Sepharose 6B.

Additionally, both MSF-IC and WBC-IC interferons were acidified and chromatographed at pH 2.7 using a 0.05 M citric acid eluant on both G-200 and G-75 superfine columns.

Chemical reagents. Solutions were prepared immediately prior to use with reagent-grade chemicals. The diluent for dithiothreitol and urea was PBS containing 250 μg of streptomycin per ml and 150 μg of potassium penicillin G per ml; for iodoacetamide and sodium periodate the diluent was distilled water. Solutions of urea and iodoacetamide were sterilized by membrane filtration (Millipore Corp.). The preparation of acetic anhydride solution occurred in two steps: first, saturated sodium acetate was added to an equal volume of PBS containing the interferon to be exposed; second, acetic anhydride was added with vigorous shaking until its desired final concentration was achieved.

Some preparations of 6 M urea (marked with footnote *c* in Table 3) were either rendered free of extraneous ions through passage on columns of Amberlite MB-3 or purchased "free of cyanide" contamination from Schwarz-Mann.

Exposure of interferon to reagents. One-half milliliter of each interferon was mixed with a like volume of dithiothreitol, allowed to incubate under the conditions defined in Table 1, and then diluted to the appropriate volume in L-15 medium supplemented with FCS (20 ml/100 ml), glutamine (30 μg/ml), arginine (90 μg/ml), glucose (1 μg/ml), potassium penicillin G (150 μg/ml), and streptomycin (250 μg/ml). With all other reagents, interferon was added in 0.2- to 2.0-ml quantities to the reagent solution to make the final volume of 8 ml. When the tests were performed in microtiter, the proportions of the interferon reagent solutions were maintained as above, but the final volume was only 2 ml.

As a control for each test, interferon was also exposed, in the same concentration and under the same conditions, to an identical solution which lacked the test reagents. It was deemed appropriate to make an exception for the interferon control of the iodoacetamide test. Here, to simulate the molarity of the iodoacetamide test solution, 10 mM dextrose was added to the water diluent of the interferon control. Table 1 lists the conditions under which interferon was exposed to the various reagents.

To avert cytotoxicity by test reagents upon exposure to cell culture, test and control solutions were routinely dialyzed against 250 volumes of Geys BSS for 24 h prior to assay for interferon. Dialysis was found to be unnecessary in the case of dithiothreitol and was therefore omitted. The results reported are based on interferon assays which, in every case, were devoid of cytopathic effect due to the test reagent.

To determine if observed differences between human and chicken interferons were artifactual, mixtures containing interferon from both species were also exposed to reagent and control solutions. At the completion of such a test, interferon was assayed both on chicken and human cells.

RESULTS

Thermal sensivitity. Figure 1 demonstrates the decay in biological activity of each interferon upon exposure to heat. Although every

TABLE 1. *Conditions for exposure of interferon to reagents*

Reagent	Concn (mM)	Temp (°C)	Duration of exposure prior to dialysis at 4°C (min)	pH
Dithiothreitol	5	25	30	7
Urea	6 or 8 M	25	75	7
Iodoacetamide	10	25	24 h	7
Acetic anhydride	100	4	60	5.8
Periodate	33	4	180	6.8

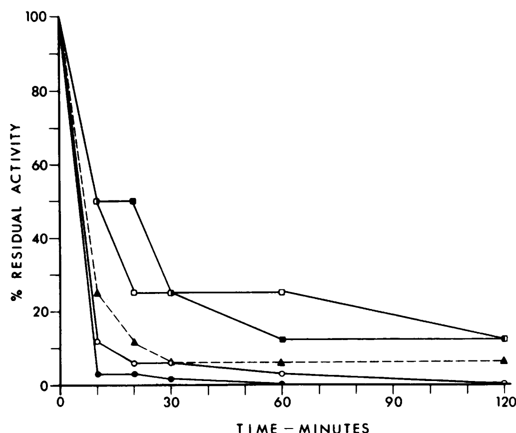

FIG. 1. *Inactivation of human interferon at 56°C. Symbols: ●, MSF-NDV; ○, MSF-IC; ■, WBC-NDV; □, WBC-IC; ▲, WBC-PHA.*

type of human interferon tested was inactivated at 56°C, WBC-NDV and WBC-IC interferons were the least susceptible, both by being slowly inactivated initially and by retaining significant activity at 2 h (Table 2). WBC-PHA was more heat labile than were the other two WBC interferons in that it resembled the fibroblast interferons in the first 30 min of exposure but differed from the latter at 2 h time by retaining a small amount of activity (whereas the fibroblast interferons were devoid of all biological activity). Repeat testing gave similar results.

Isoelectric points. The isoelectric points (pIs) determined by peaks of interferon activity after electrofocusing can be seen in Table 2, whereas Fig. 2 demonstrates the pattern of activity after this procedure for MSF-IC and WBC-NDV. Human MSF-IC had a single peak of activity with pI very close to pH 5. MSF-NDV activity resembled the MSF-IC activity, in that the majority of activity resided in a peak found close to pH 5. On the other hand, interferons derived from leukocytes had a much broader range of activity with peaks observed at both ends of the activity range. The more acid peak of the leukocyte-derived interferons induced by NDV and by IC appeared to be close to pH 5.3, whereas the more basic peak was at pH 6.9. WBC-PHA also focused over a broad pH range, with two peaks at slightly more acidic pH values than observed from the other WBC interferons. When fraction pools of WBC-NDV interferon containing the pH ranges of 6.5 to 7.0 or 5.3 to 5.7 were re-electrofocused, single peaks were then identified with pIs identical to those of the initial interferon fractions. Recovery rates for interferons during the electrofo-

TABLE 2. *Physical properties of crude human interferons*

Interferon	Stability at 56°C heat		Mol wt	pIs
	Half-life (min)	Residual activity after 2 h (%)		
Fibroblast (MSF)				
IC	3	0	300,000[a], 14,000[b]	5.1
NDV	2	0	25,000[a]	4.8
Leukocyte (WBC)				
IC	10	12	12,000[a]	5.5, 6.9
NDV	10	12	25,000[a]	5.3, 6.9
PHA	5	6	12,000[a]	4.9, 6.5

[a] As determined by chromatography at pH 7.0.
[b] As determined by chromatography at pH 2.7.

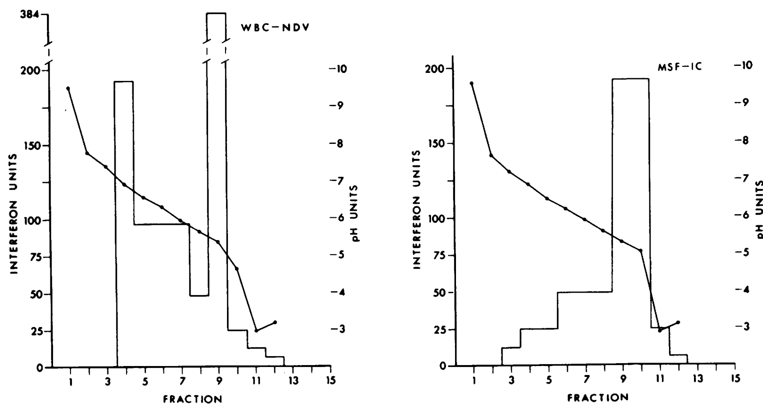

FIG. 2. *Representative patterns of interferon activity after electrofocusing. MSF-IC interferon electrofocused on pH 3 to 10 ampholytes, 9,600 units electrofocused, 38% recovery. WBC-NDV electrofocused on pH 5 to 8 ampholytes, 19,200 units applied, 100% recovery.*

cusing procedure ranged from 10 to 100%, with a mean of 46%.

Molecular weight determination. When chromatography was carried out at pH 7.0, MSF-IC interferon had an estimated molecular weight of 300,000 and MSF-NDV interferon had a molecular weight of 25,000 (Table 2). Leukocyte interferon also had a molecular weight of 25,000 when induced by NDV, but only 12,000 when induced by either IC or PHA. Both fibroblast and leukocyte interferons induced by IC were acidified to pH 2.7 and chromatographed at these lower pH ranges. Acid chromatography of MSF-IC interferon resulted in the absence of a peak at 300,000 molecular weight and in the appearance of a peak antiviral activity at 14,000 molecular weight. Occasionally under these circumstances a second activity peak, representing up to 50% of the recovered biological activity, was detected at 25,000 molecular weight. In contrast, no change occurred in the estimated molecular weight for WBC-IC interferon when chromatographed at acid pH. Molecular weight estimations performed at pH 7 for MSF-IC and WBC-NDV and at pH 2.7 for MSF-IC are shown in Fig. 3. Repeat runs of each of the interferons tested always gave identical results. Recovery rates following chromatography ranged from 5 to 30%, with a mean of 16%.

Chemical sensitivity. All five human interferons tested differed from chicken interferon in two major regards (Table 3): (i) each was considerably more resistant to disulfide bond reduction with dithiothreitol, and (ii) each was more sensitive to disruption of hydrogen bonds by urea. Among the human interferons, those derived from leukocytes were relatively less sensitive to urea than those derived from fibroblasts. Furthermore, whereas the three leukocyte interferons resembled chicken interferon in being completely resistant to attack on sulfhydryl groups by iodoacetamide, both fibroblast interferons were partially inactivated by the reagent. These differences between human leukocyte and human fibroblast interferons are

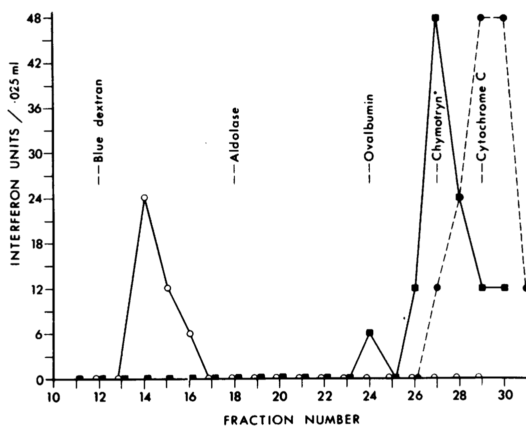

FIG. 3. *Representative molecular weight estimates confirmed from multiple determinations on G-200 superfine column (1.6 by 20 cm) at 6 to 12°C. Chromatography performed at pH 7 for WBC-NDV and at pH 7.0 and 2.7 as noted below for MSF-IC. Column calibrated with dextran blue, aldolase, ovalbumin, chymotrypsinogen, and cytochrome c. Peak fractions of the molecular weight markers were eluted and measured at 280-μm optical density. Fractions containing the peak concentrations of each marker are designated above. Symbols:* ○, *MSF-IC interferon at pH 7.0, 15,600 units applied to column, 21% recovered;* ■, *WBC-NDV at pH 7.0, 30,200 units applied, 27% recovered;* ●, *MSF-IC interferon at pH 2.7, 32,000 units applied to column, 30% recovery.*

both highly significant ($P > 0.01$, Student's t test). Like chicken interferon, all five human interferons were inactivated by acetic anhydride and sodium periodate.

DISCUSSION

The above observations permit both a comparison of interferons induced by different classes of inducer in the same cell type and a comparison of interferons induced by a single kind of inducer in cells of different types. Thus, it is possible to decide the relative role of cell and inducer in determining the nature of the interferon produced. With the exception of WBC-PHA interferon (as noted below), it is apparent that interferons derived from a given cell type tend to resemble each other in pattern of activity after electrofocusing, in heat stability at 56°C, and in chemical reactivity. Such interferons differ only in molecular weights. In contrast, interferons induced by a given inducer in cells of different type resemble each other primarily in molecular weight. Our data, thus, indicate that interferons derived from a given cell by different inducers are very similar whereas interferons stimulated by the same inducer in different cells are substantially different. These findings are not unexpected as isoenzymes have long been noted to have differing properties depending on the cell in which they are produced (5). In view of the fact that differences between interferons from different cell types involve charge, heat stability, and chemical stability (all properties dependent on molecular characteristics), we conclude that there are undoubtedly structural differences between interferons from different cells. Our hypothesis is strengthened by the recent demonstration by Havell et al. (9) of antigenic differences between fibroblast IC- and leukocyte virus-induced interferons. Nevertheless, it does appear that the inducer may exert some influence on at least one physical property, mainly molecular weight, although further investigation may show this property to be only a manifestation of dissimilar packaging. In discussing the effects of the inducer, it is important to point out that virus-induced interferons require acid dialysis to eliminate infectious particles; thus, it is necessary to ask what influence this process may have on the physicochemical properties we have reported. In considering the effects of acid dialysis on the interferon species studied here, it is important to compare the properties of the interferons induced in each cell type by viruses and by IC. In the case of virus, acid treatment was undertaken to eliminate the infectivity of the residual inducer, whereas in the case of the IC, which has no infectivity, acid treatment was unnecessary. Although differing with respect to acid treatment, interferons induced by the two inducers in a single cell strain (either leukocytes or fibroblasts) have identical electrofocusing properties, rates of heat inactivation, and chemical stabilities. If the acid had affected these properties, the interferons induced in the same cell by different inducers might well have been dissimilar rather than similar as was observed here. The reverse argument also holds; i.e., the two acid-treated interferons (i.e., WBC-NDV and MSF-NDV) are dissimilar as are the two nonacid interferons (i.e., WBC-IC and MSF-IC). If the acid treatment was responsible for all the differences observed here, these acid-dialyzed interferons should have been identical as should the nonacid-dialyzed species. In the case of molecular weights, where differences exist between interferons induced in the same cell type, acid treatment could be responsible. However, if acid artifactually creates a difference where one does not exist, it would only strengthen our thesis that the cell induced is the important determinant of the properties of native interferons.

TABLE 3. *Interferon activity after exposure to reagents*

Test	Reagent	Target	Interferon activity (%)					
			Fibroblast			Leukocyte (human)		
			Chicken virus	Human		Virus	IC	PHA
				Virus	IC			
1	Dithiothreitol	S—S	0[a]	50	100	50	50	100
2					67	70		100
3[b]			0		50			
4[b]			0		100			
5[b]			0		87			
1	Urea	H—H	72	3[c]	0[c]	24[c]	14[c]	25[c]
2				6[c]	0[c]	20[c]		50[c]
3				12	0	10		
4					1	18		
5					5			
6[b]			53		0			
1	Iodoacetamide	S—H	100	25	20	50	100	100
2				25	24	100	100	100
3					54	100		
4					35	100		
5					50	100		
6[b]			100		25			
7					50			
1	Acetic Anhydride	NH_2	0	12	6	0	6	3
2			0		1	0		
3[b]			6		6			
1	Periodate	OH OH —C—C—	0	6		0	6	3
2						0		
3[b]			0		0			
4[b]			0		0			

[a] Expressed as percentage of the activity of the same interferon stock treated simultaneously with the respective control solution.
[b] Test in which a mixture of human and chicken interferon was exposed to the treatment solutions.
[c] Test using deionized 6 M urea.

In addition to the interferons discussed above, human leukocytes may produce a really different interferon during transformation. Thus WBC-PHA, although being identical to other leukocyte interferons in most of the parameters studied here, is significantly more sensitive to thermal inactivation than either WBC-NDV or WBC-IC interferon. We interpret this difference to indicate there are probable structural dissimilarities between WBC-PHA interferon and the other two leukocyte interferons. Considering the unique role leukocytes play in the immune system, and the large number of lymphokines already described, it would not be surprising if immune interferon were structurally different from interferon induced by leukocytes in stationary phase.

It should be noted that our estimated molecular weight of 300,000 for MSF-IC interferon is larger than a previous estimate of 96,000 for the molecular weight of fibroblast IC interferon made by Carter et al. (Bacteriol. Proc., p. 213, 1971). However, in both cases it would appear likely that the large size indicates that the interferon is complexed to another protein molecule. Thus acidification and chromatography at pH 2.7 does result in a drop of molecular weight to 14,000. This lower molecular weight estimate is in close agreement with an earlier observation also made by Carter et al. (3) who reported a 12,000-molecular-weight form of IC-induced fibroblast interferon. However, they made these observations after low-salt dialysis, while we found acid chromatography was nec-

essary to reveal the smaller form. More recently, Reynolds and Pitha (16) reported MSF-IC interferon to have a molecular weight of 25,000 when estimates were performed after sodium dodecyl sulfate electrophoresis under reducing circumstances. We find that IC-induced leukocyte interferon has no large-molecular-weight form; thus, the ability of MSF-IC but not WBC-IC interferon to complex (or less likely to aggregate) can be considered a manifestation of another dissimilarity in physical properties between the two IC-induced interferons.

Our molecular weight estimates for virus-induced interferons both in fibroblasts and in leukocytes are in agreement with those reported by others. Thus, Merigan et al. (12) estimated molecular weights of fibroblast virus-induced interferons at 26,000, whereas Falcoff et al. (7) determined the molecular weight of virus-induced leukocyte interferons to also be 26,000. We are currently exploring the reasons for the differences in molecular weights between the virus-induced interferons and the other interferons.

The pIs for fibroblast interferons are in agreement with those of Stancek et al. (17) and Carter (2), who used IC and NDV, respectively, as the inducer. However, Stancek et al. found different results for virus-induced fibroblast interferon. They used interferon induced by ultraviolet-irradiated NDV (NDV-UV) in human foreskin cells and found a pattern of interferon activity that appeared to have several major activity peaks focusing over a range which began at pH 4.5 and extended to a pH at or above 8. For virus-induced leukocyte interferon using NDV-UV; this group of investigators found the same two peaks we have identified as well as a third peak with a pI at or above 9.

Our findings regarding thermal stability of fibroblast interferon are in keeping with the results of Marshall et al. (11) who found a half-life of 5 to 7 min at 56°C for interferons induced by either IC or virus. For leukocyte interferon induced by virus, our results are consistent with those of Mogensen and Cantell (15), who found the concentrated crude preparation lost 1 log of activity in 12 h, and Valle et al. (20), who reported fibroblast interferon to be more sensitive to 56°C than virus-induced leukocyte interferons after 1 h at 56°C. The finding of the latter authors that PHA-induced leukocyte interferon is more sensitive to 56°C than fibroblast interferon is at variance with our results.

Our results on the stability of chicken interferon to the chemical reagents tested are in complete accord with those published by Fantes and O'Neill (8) and others. Although there have been no comparable studies of human interferon, our findings that human interferon differs from chicken interferon in its resistance to disulfide bond reduction and sensitivity to attack on hydrogen bonds could have been anticipated from work that has been carried out on other mammalian species. Thus, Ke and Ho have found rabbit interferon to be resistant to disulfide bond reduction with 2-mercaptoethanol (10). Merigan et al., on the other hand, have demonstrated that mouse interferon is sensitive to disruption of hydrogen bonds with 8 M urea (13). Thus, the unexpected differences between human and chicken interferon reported here do have analogies in certain other mammalian species. Although Mogensen and Cantell (15) have reported human leukocyte interferon to be susceptible to the effects of mercaptoethanol, another disulfide bond-reducing agent, their conditions were more harsh than those used here and hence are not comparable to ours. We find certain differences in stability between leukocyte and fibroblast interferons. Thus, all three leukocyte-derived interferons are more resistant to hydrogen bond attack and to sulfhydryl oxidation than the fibroblast interferon.

It is clear from the information reported here that different forms of human interferon exist. Furthermore, as we have noted above, the cell type appears to be more significant than the inducing agent in determining the characteristics of the interferon produced. It is important to add that interferons derived from cells other than fibroblasts or leukocytes may be found to differ from those already described. Since human interferons do differ in size, charge, and stability, it is very likely that they will be found to differ in absorption, distribution, metabolism, and elimination in man. Furthermore, the procedures for purification and the relative yield of biologically active products will also necessarily vary. Finally, it is entirely possible that there may be found differences in biological function among these interferons. It is likely that efforts to provide a therapeutic preparation of interferon will be most productive if these differences among the interferons are clearly recognized.

ACKNOWLEDGMENTS

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LITERATURE CITED

1. Andrews, P. 1964. Estimation of the molecular weight of proteins by Sephadex gel-filtration. Biochem. J. 91:222-223.

2. **Carter, W. A.** 1970. Interferon: evidence for subunit structure. Proc. Natl. Acad. Sci. U.S.A. 67:620-628.
3. **Carter, W. A., and P. M. Pitha.** 1971. Structural requirements of ribopolymers for induction of human interferon: evidence for interferon subunits, p. 89-105. *In* R. F. Beers, Jr., and W. Brann (ed.), Biological effects of polynucleotides. Springer-Verlag, New York.
4. **Cesario, T. C., and J. G. Tilles.** 1973. Inactivation of human interferon by urine. J. Infect. Dis. 127:311-314.
5. **Dixon, M., and E. Webb.** 1964. Isoenzymes, p. 656-659. *In* enzymes, p. 656-659. Academic Press Inc., New York.
6. **Edy, V. G., A. Billiau, M. Joniau, and P. DeSomer.** 1974. Stabilization of mouse and human interferons by acid pH against inactivation due to shaking and guanidine hydrochloride. Proc. Soc. Exp. Biol. Med. 146:249-253.
7. **Falcoff, E., F. Fournier, and C. Chany.** 1966. Etude comparative des poids molecularres de deux interferons humans produits in vitro. Ann. Inst. Pasteur (Paris) 111:242-248.
8. **Fantes, K. H., and C. F. O'Neill.** 1964. Some similarities between a viral inhibitor of plant origin and chick interferon. Nature (London) 203:1048-1050.
9. **Havell, E. A., B. Berman, C. A. Ogburn, K. Berg, K. Paucker, and J. Vilcek.** 1975. Two antigenically distinct species of human interferon. Proc. Natl. Acad. Sci. U.S.A. 72:2185-2187.
10. **Ke, Y., and M. Ho.** 1968. Studies of the physico-chemical inactivation of rabbit interferon. Proc. Soc. Exp. Biol. Med. 129:433-438.
11. **Marshall, L. W., P. M. Pitha, and W. A. Carter.** 1972. Inactivation of interferon: the effects of protonation. Virology 48:607-611.
12. **Merigan, T. C., D. F. Gregory, and J. K. Petralli.** 1966. Physical properties of human interferon prepared in vitro and in vivo. Virology 29:515-522.
13. **Merigan, T. C., C. A. Winget, and C. B. Dixon.** 1975. Purification and characterization of vertebrate interferon. J. Mol. Biol. 13:679-691.
14. **Mogensen, K. E., and K. Cantell.** 1973. Stability of human leukocyte interferon toward heat. Acta Pathol. Microbiol. Scand. Sec. B 80:382.
15. **Mogensen, K. E., and K. Cantell.** 1974. Human leukocyte interferon: a role for disulphide bonds. J. Gen. Virol. 22:95-103.
16. **Reynolds, F., and P. M. Pitha.** 1975. Molecular weight study of human fibroblast interferon. Biochem. Biophys. Res. Commun. 65:107-112.
17. **Stancek, D., M. Gressnerove, and K. Paucker.** 1970. Isoelectric components of mouse, human and rabbit interferons. Virology 41:740-750.
18. **Stewart, W. E., P. DeSomer, V. Edy, K. Paucker, K. Berg, and C. Ogburn.** 1975. Distinct molecular species of human interferons: requirements for stabilization and reactivation of human leukocytes and fibroblast interferons. J. Gen. Virol. 26:327-331.
19. **Tilles, J. G., and M. Finland.** 1968. Microassay for human and chick cell interferons. Appl. Microbiol. 16:1706-1707.
20. **Valle, M., G. Jordan, S. Haahr, and T. Merigan.** 1975. Characteristics of immune interferon produced in human lymphocyte cultures compared to other human interferons. J. Immunol. 115:230-233.
21. **Wrigley, C.** 1968. Gel electrofocusing—a technique for analyzing multiple protein samples by isoelectric focusing. Sci. Tools 15:18-32.