sorption of HCO_3^- ions *per se,* but rather is the secretion of H^+ ions which react with filtered HCO_3^- to generate H_2CO_3. Second, the accumulation of excess H_2CO_3 in tubular fluid disproves the previously suggested hypothesis(3,7) that endogenous carbonic anhydrase might be located in the luminal membrane of renal tubular cells, thereby exerting a catalytic action on dehydration of excess H_2CO_3 in tubular fluid.

Finally, the calculated pH gradient (pH_B-pH_{TF}) between blood and tubular fluid was 1.07. For a pH gradient of this magnitude to result from passive distribution of H^+ ions along an electrochemical gradient(8,9), it can be calculated from the Nernst equation [$E_T = -61.5\ (pH_B\text{-}pH_{TF})$] that a transtubular potential difference (E_T) greater than 66 mV, lumen negative to blood, would be required. Previous measurement of proximal E_T under conditions of $NaHCO_3$ diuresis gave an average value of only −20 mV(10). Therefore, it is concluded that H^+ ions are secreted into the lumen against an electrochemical gradient by an active transport process.

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Purification and Characterization of Chick Embryo Interferon. (28080)

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Various virus-host animal or virus-cell culture systems have been shown to elaborate a "soluble" substance called interferon(1), which interferes with the propagation of viruses in related host or host cell systems. Interferon appears to be an active principle in the virus interference phenomenon. Interferon, as first described by Isaacs and Lindenmann(1), was prepared in fragments of chick chorioallantoic membrane incubated with heat-inactivated influenza virus. Wagner(2) demonstrated the presence of an interferon in the allantoic fluid of embryonated eggs infected with influenza virus and other workers have demonstrated interferons in other systems. Several groups of workers (2-6) have described the biological and biophysical properties of crude or semi-purified interferons. The present report describes the biological and biophysical qualities of a highly purified interferon derived from the allantoic fluid of embryonated hens' eggs infected with the WS strain of influenza virus.

Materials and methods. Preparation of crude interferon. Nine- or 10-day-old embryonated eggs were inoculated by the allantoic route with $10^{4.5}$ ID_{50} of WS influenza strain RIE 5372 virus obtained from Dr. I. Tamm. The eggs were incubated at 36°C for 84-96 hours after which they were chilled at 4°C and the allantoic fluid containing the interferon was harvested. The crude fluids were centrifuged in the cold for 20 minutes at 1500 rpm and the supernates were stored at 4°C for periods up to 14 days prior to processing for purification. In vitro *assay for interferon activity.* Serial 2-fold dilutions of the interferon sample were diluted in mixture 199 containing 1% calf serum and antibiotics, and one ml amounts were added in

quadruplicate to monolayer primary cell cultures of whole chick embryo. After 6 or 7 hours incubation at 36°C, the interferon was removed and was replaced with 1 ml of the same culture medium containing 100 $TCID_{50}$ of Enders' strain 823 of Eastern equine encephalomyelitis virus. The cultures were incubated at 36°C for 3 days, at which time the controls showed total destruction of cells. The titer was the highest initial dilution of interferon sample which gave total suppression of viral cytopathic effect. The assay error was found to be $\pm$ 60% ($p<0.05$). One unit of interferon was 1 ml of the least amount of active substance which effected total suppression of Eastern equine encephalomyelitis virus in the assay. In vivo *assay for interferon activity*. One-day-old White Leghorn chicks from a proved Rous and Newcastle Disease (NDV)-susceptible flock were injected with crude or purified interferon in 0.5 ml volume dose and with Rous or Newcastle Disease Virus (NDV) according to the regimens shown in Tables IV and V in the text. *For Rous assay,* the interferon and Rous virus were given into the subcutaneous tissue of the wing web according to Groupe *et al.*(7). The Rous virus used was Bryan's strain CBr-H564-B obtained from Dr. Vincent Groupe and was propagated in chick brain. Approximately 30 wing web tumor doses, as read 21 days after inoculation, were used. The wing webs were examined daily for presence of tumors employing both direct and transmitted illumination. The experiments were terminated on the 12th to 18th day following virus inoculation, depending on the interferon regimen, at which time the tumors were removed and the length, width and thickness were measured. Interferon activity was evaluated in terms of tumor exclusion, tumor size (index = length $\times$ width $\times$ thickness in mm), and time of gross tumor appearance. *For NDV assay,* the interferon was given intraperitoneally in 0.5 ml dose, and challenge consisted of 10 LD_{50} of virus administered by the peritoneal route. Interferon activity was expressed in terms of duration and percentage of survival of chicks. The experiments were terminated on the twenty-first day following NDV virus.

Results. Purification of interferon. Interferon was purified according to the procedure outlined in Fig. 1. Purification consisted in removal of virus and most of the non-active protein by perchloric acid followed by concentration and purification by precipitation with zinc acetate. Two cycles of chromatography on carboxymethyl cellulose (CM-cellulose, Schleicher and Schuell) were employed, each followed by a zinc precipitation step to effect concentration and purification. The interferon was adsorbed quantitatively to the CM-cellulose and the bulk of inactive protein passed through the column at pH 6.0. Only peak fractions showing highest specific activity were saved. A second minor peak having interferon activity (Fig. 2 and 3) was observed in both chromatograms but there was insufficient material to investigate its properties. The twice chromatographed interferon was purified further by zone electrophoresis on pevikon*(8). For this purpose, a 2 cm $\times$ 17 cm tray of pevikon was used and 0.3 ml of the interferon concentrate was applied in a well located 8 cm from the anode. One hundred seventy volts (10 volts/cm) was applied for 7 hours at 5°C in a Shandon zone electrophoresis apparatus using 0.03M borate buffer, pH 8.9, in the electrode vessels. Sections 0.5 cm wide were made of the pevikon and these were extracted with 0.05N acetic acid in 0.9% sodium chloride solution. The findings (Fig. 4) indicated considerable separation of interferon activity from inactive protein material. Substance from the peak fraction (Table IV) had a specific activity of 236,000 interferon units/mg of protein. Over-all purification was 4500-fold and 0.0042 μg of protein had one unit of interferon activity. Protein was measured by the method of Lowry *et al.*(9). Storage of purified interferon in glass containers reduced activity by adsorption and this was prevented by use of polypropylene vessels. Bovine serum albumin(10) or bovine serum albumin (0.05%) with Tween 80 (20 γ/ml) prevented adsorption to glass and effected desorption of adsorbed interferon.

A summary of data relating to a typical

* Obtained from Stockholm Superfostat Fabriks A.-B., Stockholm, Sweden.

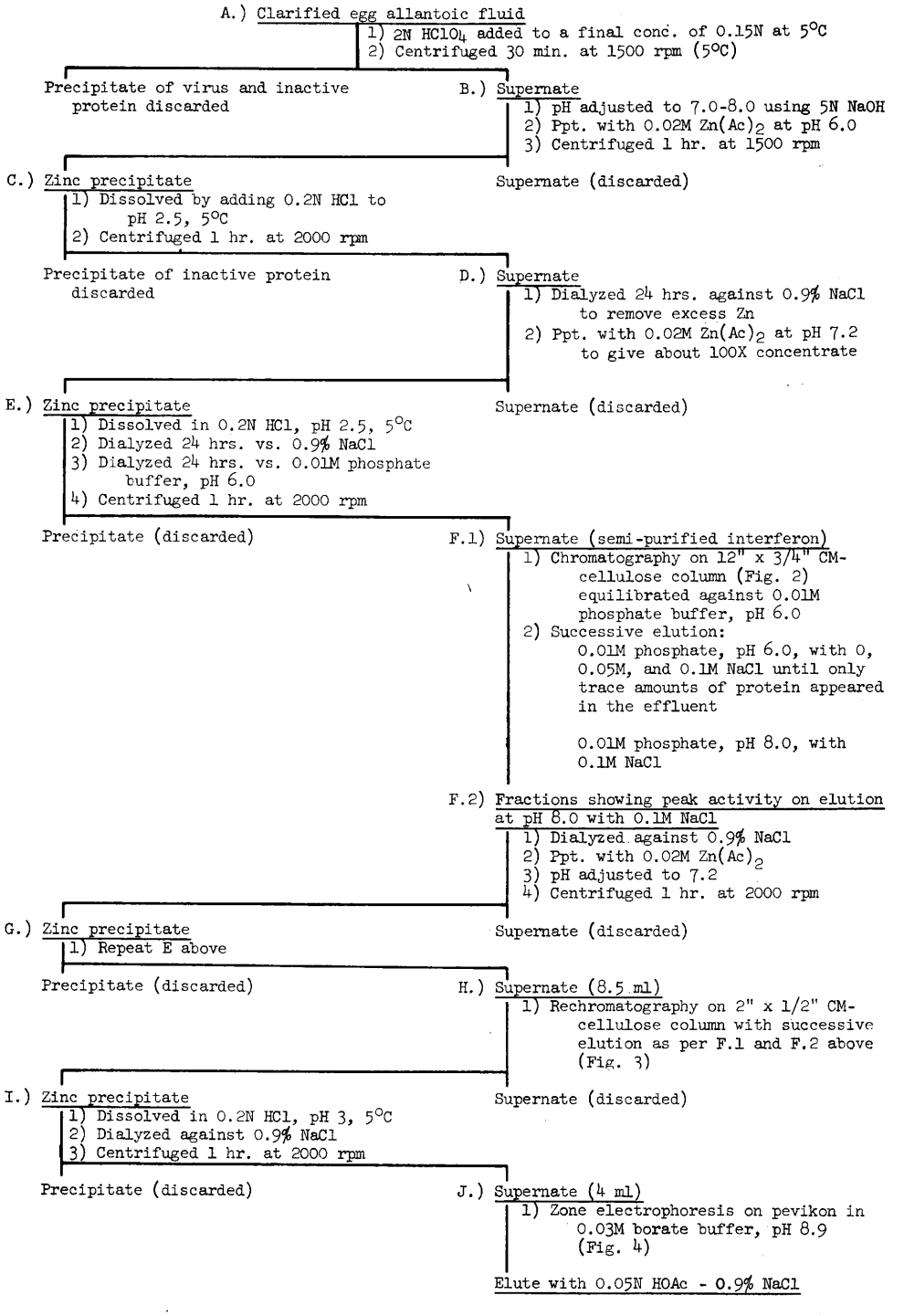

FIG. 1. Flow sheet for purification of interferon.

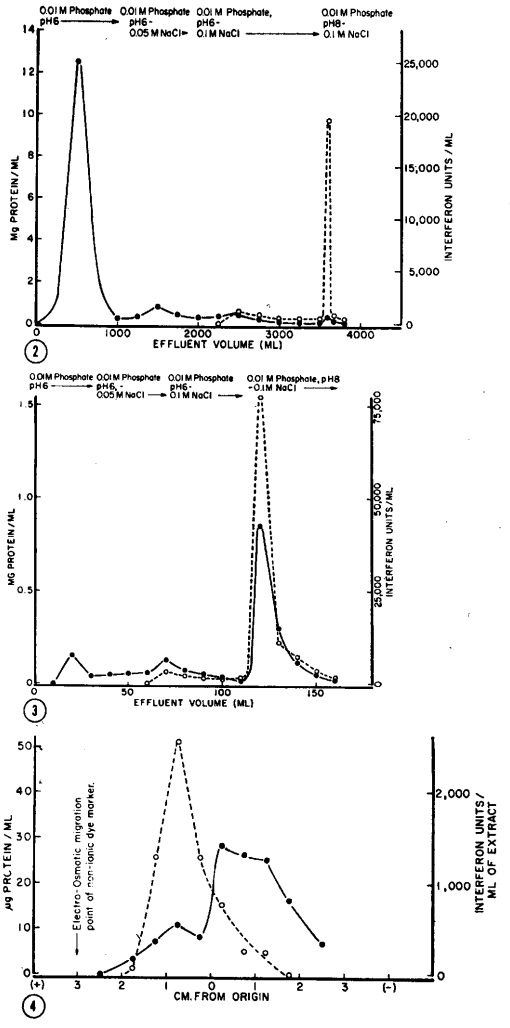

FIG. 2. Chromatography on CM-cellulose of interferon concentrated 100-fold with zinc acetate. ●——● mg protein (Lowry) per ml of effluent. O- - -O interferon units/ml of effluent.

FIG. 3. Rechromatography on CM-cellulose of main interferon peak fraction obtained from the first chromatogram. ●——● mg protein/ml of effluent. O- - -O interferon units/ml of effluent.

FIG. 4. Zone electrophoresis of twice-chromatographed interferon on pevikon. ●——● mg protein (Lowry) per ml of extract. O- - -O interferon units/ml.

purification is shown in Table I. Low yield of interferon was obtained because only the material in the peak fractions with highest specific activity in the chromatographic purification was retained. Homogeneity of the twice-chromatographed interferon was tested by ionophoresis on cellulose acetate strips using 0.03M borate buffer at pH 8.9. These studies indicated that the active fraction was still contaminated with a fast moving component which travelled toward the cathode. No attempt was made to determine precisely the iso-electric point of interferon. However, comparison of its mobility on cellulose acetate to pure proteins of known iso-electric point such as bovine albumin, chymotrypsin, lysozyme, and ribonuclease indicated that the interferon is a slightly basic protein with iso-electric point around 8. It is recognized that mobility could have been impeded by adsorption, hence the finding is only approximate. Tween 80 (20 γ)-0.05% bovine albumin solution was required to elute the interferon from the cellulose acetate paper for assay of biological activity.

For the assays presented below, only "peak activity" purified fractions were used.

Biochemical-biophysical characterization of purified interferon. Enzyme sensitivity. Twice-chromatographed interferon was incubated with various enzymes in appropriate buffer at optimal pH. Enzyme preparations of proved activity for homologous substrate were used and all experiments were controlled in tests of interferon incubated in the identical system but without enzyme. Table II shows that interferon activity was destroyed by proteolytic enzymes but not by peptidases, α amylase, lipase or DNA'se. *Effect of heat, pH and ultraviolet light on activity of purified interferon.* Table III shows that there was significant loss of interferon activity on heating at 76°C or higher temperature. When tested at 25°C for 1 hour, there was more than 67% loss in activity at pH 1 and partial loss at pH 11. The activity was stable at pH 2-10 under these conditions. When tested at 5°C, interferon activity was stable for at least 1 hour at pH 1. To measure ultraviolet inactivation, interferon in 2 mm depth in a 10 cm diameter petri dish was placed 7 inches from a Westinghouse sterilamp. Activity of the interferon was rapidly destroyed by this treatment. *Ultraviolet absorption data.* Twice-chromatographed, pevikon-purified interferon gave an ultraviolet absorption spectrum characteristic for protein with a maximum at 278 mμ and a minimum at 253 mμ. Assuming a value of 16% N for protein, the

TABLE I. Summary of Typical Purification of Interferon.

Purification step (Fig 1)	Vol	Total interferon units	Interferon units per mg protein	%* recovery of interferon	Fold-purification
A) Clarified egg allantoic fluid	50 liters	12,800,000	52	—	—
B) Supernate from allantoic fluid treated with perchloric acid	55 "	12,800,000	116	100	2.25
C) First zinc precipitate	2.6 "	10,000,000	520	78	10
E) Second " "	490 ml	6,300,000	820	49	16
F1-2) First chromatography on CM-cellulose	120 "	2,300,000	50,500	18	970
G) Zinc precipitate of most active CM-cellulose fractions	8.5 "	1,300,000	57,000	10.2	1100
H) Second chromatography on CM-cellulose	10 "	1,025,000	92,000	8.0	1750
I) Zinc precipitate of most active CM-cellulose fractions	4 "	920,000	95,000	7.2	1830
J) Eluate following zone electrophoresis on pevikon	—	—	236,000	—	4500

* With reference to total initial activity.

extinction coefficient at 280 mμ for interferon ($E^{1\%}_{1\,cm}$) was 8.6. Nitrogen was determined by the micro-Kjeldahl method. The content of tyrosine and tryptophan was calculated according to the Bencze and Schmid formula (11) from the ultraviolet absorption spectrum measured in 0.1N NaOH. A value of 2.3% tyrosine and 2.6% tryptophan was calculated. From these data, a minimum molecular weight of 7900 was calculated. The ultraviolet absorption ratio of $E\frac{260}{280} = 0.84$ precluded the possible presence of nucleic acid in greater than trace amount. *Other analyses.* The arginine content of twice-chromatographed, pevikon-purified interferon was 7.3% as determined by a modified Sakaguchi method(12). Lysine content was 11.1% as measured by the modified ninhydrin method of Slobodian *et al.*(13). A trace amount of carbohydrate was detected by the orcinol method(14). *Biological activity-sedimentation coefficient.* The mass of purified interferon was insufficient to permit measurement of sedimentation coefficient by optical methods. Instead, twice-chromatographed interferon was spun at 39,000 rpm (120,000 × *g*) in the swinging bucket rotor of a Spinco

TABLE II. Effect of Enzymes on Activity of Twice-Chromatographed Interferon.

Enzyme	Incubation Time (hr)	Incubation Temp	Interferon activity (units/ml) Untreated	Interferon activity (units/ml) Treated with enzyme	Loss in activity (%)
*Trypsin	4	37°	640	160	75
*Chymotrypsin	4	37°	640	80	88
*Pepsin	1	37°	96	8	92
*Papain	2	37°	96	6	94
Leucine amino peptidase	24	25°	6400	6400	0
*Carboxy peptidase	24	40°	3840	3840	0
α-amylase	3	25°	128	128	0
Lipase (microbial)	3	38°	96	96	0
*DNA'se (alkaline)	1	38°	96	96	0

* Crystalline enzymes of commercial origin. Other enzymes were semi-purified and were of commercial origin.

TABLE III. Effect of Heat, pH and Ultraviolet Light on Activity of Purified Interferon.

Treatment	Interferon activity (units/ml) Untreated	Treated	Loss in activity (%)
Temp* (1 hr)	256		
25°C		256	0
37 "		256	0
47 "		192	25
56 "		192	25
66 "		192	25
76 "		96	63
86 "		64	75
100 "		16	94
pH† (1 hr at 25°C)	120		
1		<40	>67
2		120	0
7		120	0
10		120	0
11		80	33
Ultraviolet light* (2537 Å)	128		
1 min		32	75
2 "		16	88
4 "		0	100

* In 0.006M (phosphate) 0.15M NaCl, pH 7.0.
† In appropriate buffer solution at 20 μg protein/ml.
The pH and temperature samples of interferon were twice chromatographed while ultraviolet samples were twice chromatographed and pevikon-purified.

model L centrifuge. Samples were removed from the upper and lower layers of the centrifuged material and assayed for content of interferon activity. The approximate activity sedimentation coefficient and molecular weight were calculated according to Hogeboom and Kuff(15). The average activity sedimentation coefficient was 2.6×10^{-13} and the approximate molecular weight calculated from the Svedberg equation ranged from 20,000 to 34,000.†

Biological activity of interferon. Rous assay. Table IV shows that crude interferon and interferon at the pre-chromatography (E) and twice-chromatographed (H) stages of purification were active in preventing development of tumors, in delaying the time of first appearance of tumors, and in suppressing the extent of tumor development. There was such considerable variation in results in the individual groups as to preclude definitive appraisal of the effect of dose of interferon and of the influence of administration of multiple doses of the substance after the virus was given. However, in separate experiments not shown in the table, it was found that interferon treatment started after the virus was given was without measurable effect, even when initiated as early as 6 hours after virus. Similarly, interferon administration begun after first appearance of pin-point size tumors was of no consequence in terms of size of resulting tumor and did not effect tumor regression. *NDV assay.* Crude and purified interferons, as shown in Table V, effected a small but detectable suppression of death and increased survival time in baby chicks. No apparent advantage was shown when interferon was given after NDV virus. The degree of individual variation in the experimental groups was so great as to preclude judgment of the relationship between interferon amount and biological effect within the ranges tested.

Discussion. A high degree of purification of influenza-chick embryo interferon was achieved. The preparation recorded in this report was purified 4500-fold based on activity:protein ratios for the crude and final materials. In the chick cell-Eastern equine encephalomyelitis virus assay system used, one unit of interferon activity was 0.0042 μg of protein. Considering the limits of error in the assay, the unit was between 0.0017 μg and 0.0067 μg of protein. Such activity is well within the range of *in vitro* potency of antibiotic substances such as penicillin (0.05 μg) and tetracycline (0.2 μg) assayed with highly sensitive organisms(16). Table VI provides a summary of the physicochemical and biological properties of chick interferon as measured in the present study. Essentially, chick interferon is a slightly basic protein of low molecular weight, is free of nucleic acid and constituents yielding absorption in the visible spectrum. A trace amount of carbohydrate was found.

Certain of the basic properties of chick embryo interferon reported here are in marked variance with those recorded by Burke, Isaacs and others(1,3-5,17-21) and by Wagner(2). Isaacs and Burke(17) and Wagner(2) have both reported destruction of

† The authors are indebted to D. S. Spicer for aid in the sedimentation studies.

interferon by proteolytic enzymes but Wagner noted failure of destruction by papain. All the proteolytic enzymes tested in our study including papain destroyed purified interferon. In agreement with others(2,17), the activity was not affected by DNA'se and, in addition, was not destroyed by peptidases, by α amylase or by lipase. The heat and pH sensitivity properties were essentially as recorded by the earlier workers. One striking

TABLE IV. Effect of Interferon Administration on Induction and Growth of Rous Tumors in Chicks.

Exp No.	Interferon fraction	Interferon regimen* Pre-Rous virus, dose	Interferon regimen* Post-Rous virus, dose	Results: Time of tumor appearance (% positive, day) 7	9	11	Results: No. with tumor/total animals	Results: Avg tumor size (index)†
1	A-Crude	2 × 128 units†	9 × 128 units	0	50	50	5/10	.8
	E-Zn^{++} ppt., pre-chromat.	2 × 256 "	9 × 256 "	0	10	80	8/10	2.0
	Control	—	—	30	100	100	20/20	3.6
2	A-Crude	2 × 128 "	8 × 128 "	0	0	30	3/10	1.9
	"	2 × 128 "	18 × 128 "	0	0	10	1/10	2.7
	E-Zn^{++} ppt., pre-chromat.	2 × 1042 "	8 × 1024 "	0	0	20	2/10	2.0
	"	2 × 1042 "	18 × 1024 "	0	0	10	1/10	2.2
	Control	—	—	75	90	95	19/20	3.0
3	A-Crude	2 × 64 "	—	0	40	40	4/10	2.0
	"	2 × 64 "	8 × 64 "	0	0	60	6/10	2.0
	"	2 × 64 "	13 × 64 "	0	0	50	5/10	1.1
	E-Zn^{++} ppt., pre-chromat.	2 × 3200 "	—	0	30	30	3/10	2.0
	"	2 × 3200 "	8 × 3200 "	0	0	50	5/10	1.8
	"	8 × 3200 "	13 × 3200 "	0	0	50	5/10	2.0
	Control	—	—	25	100	100	20/20	2.6
4	A-Crude	2 × 256 "	5 × 256 "	0	0	50	5/10	.3
	H-Twice-chromat.	2 × 96 "	5 × 96 "	0	0	40	4/10	.1
	Control	—	—	60	90	95	19/20	10.8

* Pre-inoculation doses were given at 24 hr and at 6 hr before virus; post-inoculation doses were given at 24 hr intervals after virus.

† Index = product of dimensions of 3 axes. Tumors were collected on day 12 to 18 post inoculation of virus in the different experiments.

TABLE V. Effect of Interferon Administration on NDV Virus Infection in Chicks.

Exp No.	Interferon fraction	Interferon regimen* Pre-Rous virus, dose	Interferon regimen* Post-Rous virus, dose	Results: Total survival† No. survived/ Total animals	%	Results: Length of survival Avg (days)	Excess avg (days)
1	A-Crude	2 × 96 units	—	6/18	33	10.7	3.7
	E-Zn^{++} ppt. pre-chromat.	2 × 96 "	5 × 96 units	7/19	37	9.5	2.5
	Control	—	—	7/30	23	7.0	—
2	A-Crude	2 × 64 "	—	3/10	30	9.9	2.2
	"	2 × 64 "	5 × 64 "	4/15	27	10.0	2.3
	E-Zn^{++} ppt. pre-chromat.	2 × 3200 "	—	6/10	60	13.8	6.1
	"	2 × 3200 "	5 × 3200 "	5/15	33	10.5	2.8
	Control	—	—	2/30	7	7.7	—
3	A-Crude	2 × 256 "	—	4/10	40	8.1	4.0
	H-Twice-chromat.	2 × 96 "	—	1/9	11	6.2	2.1
	Control	—	—	0/17	0	4.1	—

* Pre-inoculation doses were given at 24 hr and at 6 hr before virus; post-inoculation doses were given at 24 hr intervals after virus.

† Experiments terminated on day 21 post virus.

TABLE VI. Summary of Properties of Egg Interferon Measured in Present Study.

Biochemical and biophysical properties

Stability

Proteolytic enzyme sensitivity: trypsin, chymotrypsin, pepsin, papain.
Non-proteolytic enzyme insensitivity: peptidases, α amylase, lipase, DNA'se.
Heat sensitivity: 76°C, 1 hr.
pH stability: pH 2-10, 25°C, 1 hr; pH 1, 4°C, 1 hr.
Ultraviolet light stability: rapidly destroyed.
Adsorbs to: glass, starch, agar, paper, cellulose acetate, pevikon.
Eluted from glass and cellulose acetate by: Tween 80, bovine albumin.

Size

Non dialyzable through Visking tubing.
Approximate molecular wt:
Calculated average activity sedimentation coefficient: 2.6×10^{-13}.
Measured molecular wt (approximate): range 20,000-34,000.
Calculated minimum molecular wt for the basic unit: 7900 based on tyrosine-tryptophan content. Complete molecule likely consists of a multiple of this.

Composition

Ultraviolet extinction coefficient, 280 mμ: $E^{1\%}_{1\ cm} = 8.6$.
Ultraviolet absorption spectrum: characteristic of protein. Max 278 mμ, min 253 mμ.
Slightly basic protein: iso-electric point around pH 8.0.
Amino acid composition:
Tyrosine (calculated): 2.3%.
Tryptophan (calculated): 2.6%.
Arginine: 7.2% (less than in lysozyme).
Lysine: 11.1% (more than in lysozyme).
Other:
Trace of carbohydrate present.
Nucleic acid absent.
No absorption in the visible spectrum.

Biological activities

Interferes with propagation of Eastern equine encephalomyelities virus in chick embryo cell culture system.
Unit activity in above system = 0.0017-0.0067 μg/ml.
Prophylactically suppresses number, size, and time of appearance of virus-induced Rous tumors. Ineffective therapeutically.
Prophylactically prevents or delays death from Newcastle disease virus infection.
Does not uncouple oxidative phosphorylation in chick liver mitochondria system.
Does not stimulate aerobic or anaerobic glycolysis of Ehrlich ascites cell system.
Does not affect glucose utilization or lactic acid production by chick embryo cells in culture.

property of purified chick interferon was the extreme sensitivity to destruction by ultraviolet radiation. The sensitivity to such radiation was found to be increased as extraneous proteins were removed. The apparent protective effect of contaminating protein may account for the considerable stability to ultraviolet irradiation reported by Zemla and Vilcek(22) and by Isaacs and Burke(17).

During these studies, it became apparent that partially purified chick interferon adsorbed strongly to glass, paper, starch, agar and the like but was not adsorbed to polypropylene vessels. Zone electrophoresis on paper or starch was not useful, therefore, in the further purification of interferon following chromatography. It was found, however, that the interferon did migrate by ionophoresis on cellulose acetate paper. Ionophoretic analysis revealed the presence of a fast migrating basic material, protein in nature, which had certain properties so similar to interferon that it was adsorbed and eluted along with the active substance on the CM-cellulose column. Even though interferon migrated on cellulose acetate paper, drastic measures were required for quantitative elution of the substance. Tween 80-bovine serum albumin solution was excellent for purpose of elution while saline and buffer at neutral pH were without effect. All highly purified samples of interferon were stored in polypropylene containers to preclude loss by adsorption.

It was found subsequently that chick interferon migrated as a slightly basic protein and could be separated from the contaminating basic protein by zone electrophoresis on

a bed of pevikon. This process was employed as the final step for purification of interferon for chemical analyses. The activity was eluted from the pevikon using 0.05N acetic acid and material from the peak fraction was analyzed. Elution could also be effected using Tween 80-bovine serum albumin. The weakly basic property of the interferon as observed on pevikon zone electrophoresis was in agreement with the findings on cellulose acetate ionophoresis in which a number of purified proteins of known iso-electric points were compared and the slightly basic nature of interferon was indicated. These findings are in marked contrast to those of Burke(4) who suggested that chick interferon is an acidic protein and is in agreement with Wagner(2) who reported interferon to be a basic protein. The finding of significant amounts of tyrosine-tryptophan in the present material does not agree with the reported absence of these amino acids as recorded by Wagner.

The small amount of interferon recovered from the 50 liters of crude allantoic fluid was insufficient to permit a precise molecular weight determination by direct optical measurement in the sedimenting boundary. It was necessary, therefore, to determine the approximate molecular weight based on determination of the biological activity-sedimentation coefficient. From such studies, the approximate molecular weight was calculated to be in the range of 20,000 to 34,000. A minimum possible molecular weight, based on the assumption that each interferon molecule must contain at least 1 molecule of tyrosine and 1 of tryptophan, was calculated to be 7900 and the entire molecule may be comprised of multiples of this basic unit.

The 20,000 to 34,000 molecular weight range obtained in these studies for chick interferon is significantly lower than the 63,000 reported by Burke(4) based on ultracentrifugal analysis by the Archibald method(23). The molecular weight estimated by Porterfield *et al.*(5) to be less than 80,000, based on agar diffusion methods, is hardly of significance in view of the strong adsorptive quality of agar for interferon.

Isaacs *et al.*(21) have suggested that the biological activity of interferon may be due to uncoupling of oxidative phosphorylation. Experiments in this laboratory using twice-chromatographed interferon at 3000 units per ml indicated no uncoupling of oxidative phosphorylation and no detectable effect on the respiration of isolated mitochondria from chicken liver. It was also found that this purified interferon had no observed effect on aerobic or anaerobic glycolysis of Ehrlich ascites cells *in vitro*. Crude interferon did, however, stimulate glycolysis of chick embryo cells and it is clear that such activity was due to an extraneous substance.‡ In addition, purified interferon revealed no effect on glucose utilization or lactic acid production by chick embryo cells in culture. These findings are in disagreement with those of Isaacs *et al.*(21) but support the data of others who conclude that interferon does not uncouple oxidative phosphorylation. Thus, interferon is active even when oxidative phosphorylation cannot function owing to anaerobiosis(24), dinitrophenol does not affect growth of foot-and-mouth disease or Western equine encephalomyelitis virus(24,25), and stimulation of aerobic glycolysis in chick embryo cells was due to extraneous substance other than interferon(26).

The extreme divergence in properties of chick interferon as purified by Burke, Isaacs and their coworkers from that described here reasonably requires explanation. One answer might be that Burke, Isaacs interferon was prepared using isolated chick chorioallantois and inactivated influenza virus whereas the present interferon was prepared in the infected whole chick embryo. Such explanation, while convenient, is lacking in substance and it is evident that the real difference lies in the degree of purity of the materials prepared in the two laboratories. It is impossible from the published data to define the purity of Burke's(4) interferon since the activity was not assayed with precision and since the activity:protein ratios were not given. Instead the average purification factor was stated to be 17-18-fold with an average recovery of 7-15% of activity. By use of this basic information and data collected

‡ These experiments were conducted by Dr. T. M. Devlin.

in our laboratory, it is possible to appraise roughly the quality of the Burke's material. Crude interferon prepared in this laboratory by the Burke, Isaacs method in isolated chick chorioallantois usually contained 1.5 mg protein and 6 interferon units per ml or 4 units of activity per mg of protein while the crude interferon in allantoic fluid used in the present study contained 5 mg protein and 256 units of interferon per ml or 51 units per mg of protein. Accepting the 18-fold purification factor, the Burke purified interferon would contain 72 units of activity per mg of protein. The purified interferon prepared in the present study was purified 4500 times and contained 236,000 units of activity per mg of protein. It appears likely, therefore, that Burke's purified material consisted largely of an extraneous material which contained some interferon. Demonstration of a single symmetrical peak on chromatography, of a single band on starch gel electrophoresis, and of a single sedimenting boundary, as employed by Burke, Isaacs, is judged inadequate proof of homogeneity. As shown here, the extraneous fast-moving component was not separable from interferon by chromatography and could be resolved only by ionophoresis on cellulose acetate or on pevikon. Starch gel electrophoresis was inadequate for separating the two components because of the strong adsorptive quality of starch(4). Ultracentrifugal analysis could not be expected to demonstrate the presence of a second component unless it were present in great enough amount to render it optically visible.

The biological data collected in the present investigation showed that the highly purified as well as the crude interferon was active and that this activity was demonstrable both in *in vitro* and *in vivo* systems using oncogenic or necrosis producing viruses. The demonstrated activity against Rous virus was consistent with the earlier findings of Strandstrom *et al.*(27) who showed suppression by interferon of pock and tumor production by Rous virus in the chick embryo. As amply shown by de Somer *et al.*(28), interferon functioned principally as a prophylactic agent and was without significant effect once infection was established.

Summary. Chick embryo interferon was purified from allantoic fluid of embryonated eggs infected with influenza A virus. Purification consisted essentially of acid precipitation to remove virus and extraneous protein, concentration and purification by precipitation with Zn^{++}, column chromatography on CM-cellulose, and zone ionophoresis on pevikon. The interferon was purified 4500 times with respect to initial protein and one unit of interferon activity was 0.0042 μg protein in the chick embryo cell-EEE virus assay system used. Interferon was found to be a slightly basic protein of low molecular weight and free of nucleic acid as well as constituents giving absorption in the visible spectrum. A trace amount of carbohydrate was found. Detailed findings in the analyses are presented. The striking difference in properties of interferon from those described heretofore by other workers appears to lie in previous failure of purification. Both crude and purified chick interferon were active in suppressing Rous sarcoma development and NDV virus infection in susceptible chicks but were without therapeutic effect.

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Alkaline Phosphatase, Feathering and Bone Ash in Chicks As Affected by Hemin.* (28081)

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Previous work(1,2) showed that parenteral injections of hemin, protoporphyrin and hematoporphyrin produced severe growth depression and varying degrees of mortality in chicks. Also, injections of hemin and protoporphyrin resulted in moderate to severe bowing of legs in a large percentage of chicks similar to nutrient deficiencies. Incidence of bowing was directly related to dose.

Present experiments were designed to correlate certain physical and biochemical aspects of the deformity with those of known nutrient deficiencies. Among these nutrients are zinc(3), manganese(4), choline(5), biotin(6,7), niacin(8) and folic acid(9).

Materials and methods. Day-old White Rock male chicks (Arbor Acres) were used in all experiments. Weighing, distributing, housing and feeding of chicks and preparation and administration of solutions were essentially as previously reported(2). Solutions were prepared fresh daily. In these trials, 5 mg level of hemin (Eastman Kodak Co.) was used and treatment was started on 8th day.

Serum alkaline phosphatase activity (SAP) and bone alkaline phosphatase activity (BAP) were determned by the method of Bessey, Lowry and Brock(10). SAP determinations were made at 2, 6 and 10 days after treatment in Trial 1. SAP and BAP were determined at 2 and 6 days in Trial 2. Bone enzyme was extracted from cleaned, weighed tibiae by grinding in cleaned sea sand and adding sufficient distilled water to make a 2% solution (w/v). This mixture was incubated for 3 days at 34°F, filtered, and a 0.1 cc sample used for activity determination.

Per cent bone ash was determined on fat-free, dry femurs according to the method described by the Assn. of Official Agricultural Chemists(11).

Results. The effect of hemin on SAP is shown in Table I. In Trial 1, SAP is significantly depressed for 6 days following the last injection and for 2 days in Trial 2. At 10 days in Trial 1 and at 6 days in Trial 2, average activity and range of activity are still below those of the controls; however, the dif-

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