molecule must be different, since there is no way for nonpolar molecules to produce a polar band pattern. Molecular polarity has already been demonstrated for myosin (*14*) and tropomyosin (*15*). One cannot decide at present, however, whether the two chains in the molecule run in the same or opposite directions. If the two chains are identical in sequence, then the polarity of the molecule requires that they run in the same direction. Although the three-dimensional packing of the molecules cannot be deduced from these observations, it is possible to build a filament where the molecules are related by parallel twofold screw axes to produce the band pattern shown in the two-dimensional plan of Fig. 2. Evidence regarding the three-dimensional packing arrangement of paramyosin in native filaments under certain conditions has been obtained (*16*). It is important to note that although the axial periodicity is very regular in fibrous protein paracrystals, there is no long-range order in the lateral packing (*15*).

The assembly of paramyosin filaments in vitro gives some insight into the structure of the filaments containing paramyosin in muscle. The antiparallel relations between molecules lead to the assembly of a filament with opposite polarity at either end. This kind of packing had been demonstrated for myosin from vertebrate striated muscle (*14*) and is an important aspect of the design requirements of the sliding filament mechanism of contraction. It is possible that, at the center of the native paramyosin filament, the molecules have antiparallel dimer relations similar to those occurring in either the type DI or type DII paracrystals. Growth would proceed at either end of the filament by polar assembly. Another fundamental aspect of the aggregates in vitro is the presence of "gaps" and "overlaps" in the structure. These occur necessarily in forming a filament with an axial repeat of 725 Å from molecules 1275 Å long, and there must therefore be gaps in the molecular packing of the native filament. A pattern of "gaps" which resembles the lattice seen in the native paramyosin filament can, in fact, be produced by a specific staggering of the type PI arrays. Myosin may be located at the surface of the native paramyosin filament (*17*). Myosin and paramyosin have α-helical coiled-coil rod regions very similar in length and aggregation properties (*18*). The interaction of these proteins may be crucial in accounting for the structure of the filament. A paramyosin aggregate could provide a bonding area with the appropriate geometry and polarity for positioning a surface array of myosin molecules.

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Chick Interferon: Heterogeneity of Electric Charge

Abstract. *Chick interferon from allantoic fluid, virus-induced and partly purified, consists of several active components of different charge. The components are separable by elution at different* pH *values from a carboxymethyl-Sephadex column; they also occupy different* pH *zones in electrofocusing gradients. Most of the interferon is, however, isoelectric near neutrality.*

The active moiety in purified preparations of chick interferon is apparently not homogeneous (*1–3*). The purpose of our work was to prove that heterogeneity was not a result of partial adsorption to a solid matrix and to learn something about the relative abundance of factors in the interferon concentrates.

Interferon from allantoic fluid was prepared, partly purified, and concentrated by methods described (*3*). The concentrate in our experiments contained 25,600 interferon units and 780 μg of protein per milliliter; this represented 70-fold purification and an overall recovery of activity of 40 percent. The concentrate (100 ml in 0.1*M* phosphate buffer *p*H 5.9) was applied to a carboxymethyl-Sephadex (C50, medium grade) column (2 g; 190 × 15 mm), that was previously equilibrated with the same buffer. The column was washed with 0.1*M* phosphate buffer (*p*H 6), and the fractions were eluted by means of a steep, rising *p*H-gradient. The gradient was formed by running 0.1*M* trisodium phosphate into 300 ml of 0.1*M* phosphate buffer (*p*H 6.0). The effluent was collected in 10 ml portions in polypropylene tubes. The fractions were assayed for interferon content (*3*), and their *p*H values were determined (Table 1).

In order to obtain fairly concentrated eluate fractions, a steep *p*H-gradient had been chosen. Therefore, elution did not proceed at equilibrium, and the *p*H of a fraction was not directly related to the isoelectric point of its interferon

component (*3*). In order to get a direct correlation (*4*), portions of fractions Nos. 6 to 22, each containing 6400 to 12,800 interferon units, were applied (in 0.1*M* phosphate buffer, *p*H 5.9) to fresh carboxymethyl-Sephadex columns (65 mg; 40 × 6 mm). These were eluted with consecutive portions (2 × 5 ml each) of 0.1*M* phosphate buffers of *p*H 6.0, 6.1, up to *p*H 8.0 (Table 2). The interferon content of each eluate fraction was again determined. Under these conditions, the bulk of a sample of the unfractionated concentrate eluted at *p*H 6.6, and this suggested, according to Lampson *et al.* (*4*), an isoelectric point of 7.0.

To confirm the charge-heterogeneity in another system, several of the original eluate fractions (those shown in Table 1) were also subjected to isoelectric focusing in 0.8 percent ampholyte solution (*p*H 5 to 8) (*5*). With this method, substances with different charges are separated in a liquid milieu, thus avoiding complications that may be caused by adsorption to solid matrices. Prior dialysis of the fractions against glycine solution seemed unnecessary, and the fractions were used without further adjustment. During the first 3 hours of every experiment, the potential was gradually raised from 300 to 700 volts; it was kept at this level for the next 27 hours and was raised to 900 volts for the last 16 hours. The liquid was then withdrawn from the column through the outlet capillary and was collected in 3-ml portions. Interferon content and *p*H of each fraction was determined (Table 2). A sample of the unfractionated interferon concentrate was investigated by the same method. The results of this experiment clearly suggested heterogeneity, with most of the activity lying between *p*H 6.6 and 7.1, but a more detailed interpretation was difficult. (Some of the fractions referred to in columns 2 and 3 of Table 2 also contained a second minor peak, but these were not included in the table.)

Table 1. Elution from a carboxymethyl Sephadex column of partly purified, concentrated chick interferon by means of a rising *p*H gradient in 0.1*M* phosphate buffer.

Fraction code	Volume (ml)	*p*H	Interferon (unit/ml)	Recovery (%)
		Column input		
A	100	5.9	25,600	100
		*0.1M Phosphate wash p*H *6*		
B	500	6.0	<20	
C	400	6.0	<20	
D	100	6.0	<20	
		*0.1M Phosphate rising p*H *eluate fractions*		
1	10	6.0	<20	
2	10	6.0	<20	
3	10	6.01	<20	
4	10	6.02	<20	
5	10	6.10	640	0.3
6	10	6.29	3,200	1.3
7	10	6.53	6,400	2.5
8	10	6.66	6,400	2.5
9	10	6.79	12,800	5
10	10	6.88	12,800	5
11	10	6.96	25,600	10
12	10	7.02	12,800	5
13	10	7.11	12,800	5
14	10	7.20	12,800	5
15	10	7.28	12,800	5
16	10	7.35	6,400	2.5
17	10	7.50	4,800	1.9
18	10	7.62	3,200	1.3
19	10	7.81	3,200	1.3
20	10	8.11	6,400	2.5
21	10	8.49	5,400	2.5
22	10	9.78	3,200	1.3
23	10	10.27	640	0.3
24	10	10.38	160	0.1
25	10	10.59	<80	
26	10	10.80	<20	

Table 2. Rechromatography on carboxymethyl Sephadex column and electrofocusing of interferon solutions obtained from first carboxymethyl-sephadex fractionation.

Original eluate fraction	*p*H of buffer eluting peak activity	*p*H of peak ampholyte fraction
6	6.1	
7	6.4	6.40
8	6.4	6.59
9	6.4	
10	6.4	
11	6.4	6.58
12	6.6	
13	6.6	6.69
14	6.8	
15	6.8	6.86
16	6.8	
17	6.9	7.23
18	6.9	
19	6.9	
20	6.9	
21	7.2	7.34
22	7.2	

The experiments confirm that the active material in chick interferon, as purified by our methods, is not one entity, but that it consists of several (maybe five or more) components or even of a near-continuous series. The purification steps themselves are unlikely to lead to the formation of artifacts (*2*), but as the recovery of activity was only 40 percent, the presence of additional factors in crude interferon cannot be excluded. The results do not answer the question whether one homogeneous interferon is bound to a series of differently charged substances or whether distinct interferon moieties with different charges exist. However, in view of the great number of biologically important proteins known to exist as a series of closely related substances (for example, numerous enzymes, immunoglobulins, serum albumins, hemoglobins, serum lipoproteins, cytochromes, γ-casein, and complement) (*6*), it would not be surprising if interferons also exhibited polymorphism. Interferons from several animal species fall, very roughly, into two groups according to their molecular weights (about 20,000 to 40,000 and 100,000 or more) (*7*); these size differences are sometimes accompanied by gross differences in charge. More subtle charge variations, as those described in this communication, have only recently been noted. This is perhaps not surprising, as the greatest proportion of the interferon used in these experiments was isoelectric near the center of the *p*H range. A superficial investigation of the unfractionated material would suggest, as shown, an isoelectric point of about *p*H 7.0, that is, in the generally accepted region.

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