myosin as prepared by other workers is not extracted directly from the muscle but rather is made by ATP dissociation of previously prepared actomyosin[2,10].

Consideration was given to the possibility that the high levels of *F*-actin used were giving the same anomalous viscosity effects on artery actomyosin which have been observed for skeletal muscle actomyosin[7]. This, however, does not appear to be the case. The artery protein did not show the anomalous viscosity behaviour even when a large excess of skeletal muscle *F*-actin was added.

A different preparation of artery actomyosin, prepared according to the procedure of Laszt and Hamoir[1], did not have the actin-combining moiety associated with it. The data (Table 3) show that even large amounts of *F*-actin failed to induce an elevated $Z_\eta$ or ATP sensitivity when added to actomyosin prepared by the method of Laszt and Hamoir[1].

Table 3. EFFECT OF *F*-ACTIN ON THE RELATIVE VISCOSITY OF ARTERY ACTOMYOSIN*

| | Preparation 1 | | Preparation 2 | |
|---|---|---|---|---|
| *F*-actin/AM | $Z_\eta$ | ATP sensitivity (%) | $Z_\eta$ | ATP sensitivity (%) |
| No actin | 0·25 | 36 | 0·18 | 27 |
| 1/20 | 0·33 | 35 | 0·11 | 32 |
| 1/10 | 0·29 | 45 | 0·13 | 40 |
| 1/5 | 0·37 | 39 | 0·26 | 72 |

* The two preparations of artery actomyosin were made according to the method of Laszt and Hamoir[1].
Viscometry conditions were as described in the text. The *F*-actin was from rabbit skeletal muscle. The *F*-actin/actomyosin was on a weight basis (mg protein). The concentrations of actomyosin in the viscometer were 1·5 mg/ml. and 1·8 mg/ml. for preparations 1 and 2, respectively.

The results of this study indicate that a firm association exists between cow carotid artery actomyosin and a myosin-like component. Since the association exists even after treatment to remove myosin it is suggested that artery myosin is different in solubility properties from skeletal muscle myosin.

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## Further Purification of Chick Interferon

CHICK allantoic fluid interferon has been purified to various extents by several groups of workers. Among those who prepared interferon of high specific activity, Lampson *et al.*[1] achieved a purification factor of 1830 after treatment with perchloric acid, concentration with zinc acetate and two fractionations on '*CM*-cellulose'. Such material still contained an inactive protein component that was only removed by electrophoresis on 'Pevikon'. The final material had been purified 4,500 times. Fantes *et al.*[2] purified interferon several thousand times by adsorption on 'Doucil', elution with KSCN, precipitation of impurities in the presence of KSCN at *p*H 3·5, precipitation of interferon with methanol at *p*H 7·5 and chromatography on DEAE cellulose at *p*H 7·5. Merigan[3] purified crude interferon with perchloric acid and zinc acetate by the method of Lampson *et al.*[1], achieving a 15-fold purification. Such material, after one chromatographic fractionation on '*CM*-Sephadex', using a *p*H gradient instead of Lampson's stepwise process for eluting the active ingredient, was claimed to be purer than Lampson's material, though no evidence of electrophoretic homogeneity was presented.

We have slightly modified our original method[2]. Instead of neutralizing the acid KSCN supernatant fluid before precipitating with methanol, we lowered the *p*H further to 2·0 and added methanol (5 vol.) at this stage, precipitating some more inactive protein. The *p*H of the methanolic supernatant fluid was then adjusted to an apparent value of 7·5, causing precipitation of the active material. Chromatography on DEAE-cellulose was carried out as described before[2]. The interferon, when chromatographed on DEAE cellulose, became re-diluted and was reconcentrated on '*CM*-sephadex'[3]. Adsorption was performed at *p*H 5·5 in 0·1 M phosphate buffer, with subsequent washing with 0·1 M phosphate buffer at *p*H 6 until the eluates contained no more than about 2 μg/ml. protein, as determined by the method of Lowry *et al.*[4]. Elution was carried out with a 0·1 M phosphate buffer gradient of rising *p*H.

Three such preparations were subjected to disc electrophoresis in acrylamide gel columns by Dr. J. Williams (personal communication) at the Laboratory of Molecular Biology in Cambridge. Two of the samples, after staining with amido schwarz, exhibited only one protein band. Dissection and assay of two control columns showed that the biological activity resided in those bands. The third sample contained some minor protein contaminants, estimated to amount to less than 5 per cent of the total.

The purity of the most potent DEAE-eluate fractions was sometimes of the same order as that of the post '*CM*-Sephadex' fractions, but the '*CM*-Sephadex' process enabled the less pure off-peak DEAE cellulose fractions to be purified to the same extent, thus increasing the overall yield of highly purified material.

The post-DEAE material—a mixture of highly and moderately purified fractions—applied to '*CM*-Sephadex' had usually been purified about 500-fold. When, on the other hand, pre-DEAE material (purified about 80 times on average) was fractionated on '*CM*-Sephadex' in a similar way, it was not found possible to free the active material from an inert protein with an elution peak at a *p*H only just above that of interferon.

The best fractions were purified well over 10,000 times and contained about one million interferon units per mg protein. As the specific activities of crude interferons and also assay methods vary from one laboratory to another, these figures do not necessarily mean that interferon prepared by the process described here was of a higher degree of purity than that obtained by other workers.

The iso-electric point of partly purified interferon was determined electrophoretically in a sucrose gradient by Dr. A. Polson (personal communication) at the University of Cape Town and found to be about *p*H 6·8–6·9.

Purified interferon was, as mentioned in our previous publication[2], highly species specific. This has since also been reported by Merigan[3] and Baron *et al.*[5]. Contrary to the claims of a patent for 'non-specific interferon'[6], we were unable to overcome the species specificity of crude or purified chick interferon by treatment with carboxypeptidase.

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