the method of Lowry *et al.* (*9*). The mean index of precision (λ) of ten consecutive recent assays is 0.23 ± 0.025.

The arbitrary unit is 10 μg of nitrogen of a standard preparation (THV-44-D) of supernatant from ultracentrifuged hog thyroid extract. One unit produces a statistically significant fall in serum calcium (about 1 mg/100 ml) in test rats.

Additional purification of thyrocalcitonin was achieved by gel filtration. In a typical preparation, 5 g of lyophilized ultracentrifuged supernatant fluid containing 300 mg of nitrogen (obtained from 170 g of thyroid tissue) was applied to a column of Sephadex G-50 (3.8 by 90 cm), equilibrated with 0.05*M* sodium acetate buffer (*p*H 3.8). On elution with the same buffer, a large inactive protein peak first emerged, then a smaller protein peak, and then a nucleotide fraction (*10*). The hypocalcemic activity was associated with the descending limb of the smaller protein peak and the ascending limb of the nucleotide fraction. When the most active fractions (containing 15 mg of nitrogen) were pooled, they represented a tenfold increase in specific activity with a yield of 40 to 60 percent. This product was applied to a column of carboxymethyl-Sephadex G-25 (1.9 by 14 cm), equilibrated with the same buffer as before. On elution with the same buffer, increased stepwise from 0.05*M* to 0.2*M*, only nucleotides were eluted. Protein was eluted on changing to a gradient of sodium chloride increasing to 2.0*M*, in 0.2*M* sodium acetate (*p*H 3.8). The hypocalcemic activity was associated with the descending portion of the protein peak. The pooled active fractions showed an additional fivefold improvement in specific activity with a yield of 70 percent in this step.

Although the site of action of thyrocalcitonin is not yet known, that there may be a direct effect on bone, important physiologically, is an intriguing possibility. The kidney is not essential for the hypocalcemic effect, since thyrocalcitonin is active in nephrectomized rats. The activity of thyrocalcitonin in rats fed a low calcium diet suggests that decreased absorption of calcium from the gut is not responsible for the hypocalcemic effect. Since thyrocalcitonin is active in parathyroidectomized rats (*2*), it does not produce its effect by an action on the parathyroid gland or on parathyroid hormone. The parallel decreases in serum inorganic phosphate (*11*) and serum calcium (Table 1), also noted by Kenny (*12*), are consistent with an effect on the deposition of bone salt.

Elegant experiments on the perfusion of the thyroid and parathyroid glands of dogs (*13*), later extended to sheep (*14*), led Copp *et al.* to introduce the concept of a hypocalcemic hormone, named calcitonin, working antagonistically and in concert with the parathyroid hormone in the normal regulation of the calcium concentration in plasma. This work was confirmed independently by Kumar *et al.* (*15*). Although many of the experiments by Copp *et al.* did not discriminate between the thyroid gland and the parathyroid gland as the site of origin of calcitonin, some experiments pointed to the parathyroid gland as the source of calcitonin. On the other hand, studies in the goat (*4*) and in the rat (*16*) support the conclusion that the newly recognized hypocalcemic principle originates in the thyroid rather than the parathyroid gland. Whether or not thyrocalcitonin is the same as calcitonin, its marked activity and easy availability encourage further investigation of its chemistry, pharmacology, and therapeutic potentialities.

Note added in proof: Since this report was submitted, Baghdiantz *et al.* (*17*) have published an alternative method of partial purification of thyrocalcitonin.

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25 August 1964

Chromatography of Ribonuclease-Treated Myosin Extracts from Early Embryonic Chick Muscle

Abstract. *Chromatography, on diethylaminoethyl cellulose, of leg muscle extracts from older chick embryos yields three myosin-containing fractions, whereas material from muscle extracts of 11-day embryos cannot be eluted under the same conditions. A major fraction having the adenosine triphosphotase activity characteristic of myosin can be eluted when 11-day-old embryo muscle extracts are given prior treatment with ribonuclease.*

The preparation of myosin from chick embryos in the form of a single homogeneous fraction has not yet been achieved. The preparation of such material is essential for immunological studies and measurements of tracer incorporation into myosin during embryonic development. Column chromatography, on diethylaminoethyl (DEAE)-cellulose, of muscle extracts from chick embryos 14 days old and older yielded a chromatographically heterogeneous myosin fraction. Under the same conditions of fractionation, extracts from muscle of embryos 11 days old or less gave no protein in the eluate with properties of myosin. Because of the high RNA content of embryonic muscle tissue and the presence of RNA-like material (extractable with hot trichloroacetic acid, and showing a positive orcinol reaction) in myosin fractions from older embryos, ribonuclease treatment of the 11-day embryo muscle extracts was carried out prior to chromatography. Extracts from 11-day chick embryos, from which no myosin fraction could be recovered

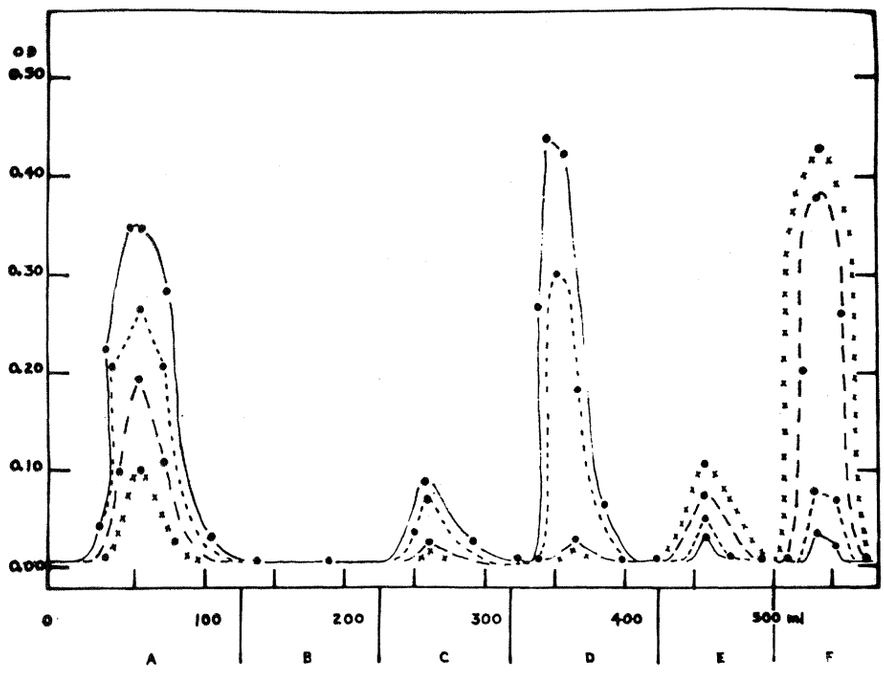

Fig. 1. Chromatography on DEAE-cellulose of ribonuclease-treated and untreated extracts of 11-day embryo muscle. Solid line, optical density at 280 mμ, untreated; short dashes, optical density at 260 mμ, untreated. Long dashes (280 mμ) and crosses (260 mμ), after treatment with 0.005 percent ribonuclease. Fractions eluted as follows: (*A*) 0.02*M* $K_4P_2O_7$; (*B*) 0.125*M* KCl containing 0.003*M* $K_4P_2O_7$; (*C*) 0.18*M* KCl containing 0.005*M* $K_4P_2O_7$; (*D*) 0.36*M* KCl containing 0.01*M* $K_4P_2O_7$; (*E*) 0.9*M* KCl containing 0.025*M* $K_4P_2O_7$; (*F*) 0.1*N* KOH, at a constant *p*H 8.5.

after DEAE-chromatography without ribonuclease treatment, showed a well-defined single peak when chromatographed after ribonuclease treatment.

The main steps in the preparation of the myosin solution for DEAE-chromatography were as follows. (i) Leg muscle (10 to 20 g) from 11-day embryos was excised, minced, and then homogenized in 10 volumes of 0.04*M* KCl containing 0.0067*M* K_2HPO_4, *p*H 7.0. The homogenate was stirred for 90 minutes and centrifuged at 10,000*g* for 20 minutes; the supernatant solution was discarded. (ii) The residue was homogenized in 10 volumes of 0.02*M* $K_4P_2O_7$ containing 0.001*M* $MgCl_2$, *p*H 9.2, and then stirred for 90 minutes. This homogenate was centrifuged at 30,000*g* for 20 minutes, and the residue was discarded. The resulting supernatant solution was then centrifuged at 150,000*g* for 230 minutes. The supernatant solution so obtained was filtered through glass-fiber filter paper (Whatman GFA), concentrated to a volume of 20 to 40 ml with purified carbowax (*1*), and then subjected to gel filtration on Sephadex G-200 (*2*) (3 by 34 cm column), which had been equilibrated with 0.02*M* $K_4P_2O_7$, *p*H 8.5. The major excluded fraction was concentrated and chromatographed on DEAE-cellulose (Schleicher & Schuell). (iii) The DEAE-cellulose chromatography was carried out according to the procedure developed by Love (*3*), in which the exchanged DEAE was equilibrated with 0.02*M* $K_4P_2O_7$, *p*H 8.5. (iv) For the treatment with ribonuclease, 0.001 to 0.005 percent pancreatic ribonuclease (*4*) was added to the concentrated supernatant (150,000*g*) after the *p*H had been adjusted to 8.5 by the addition of 0.1*N* HCl. The solution was stirred at 4°C for 4 to 6 hours and then subjected to gel filtration. All the procedures for extraction and chromatography were conducted at 0° to 4°C. The results of the ribonuclease treatment can be seen directly by comparing the elution patterns for the ribonuclease-treated and untreated muscle extracts from the 11-day embryos (Fig. 1). In embryos 14 days old and older elution from DEAE gave five peaks: a protein fraction (*A*) without adenosine triphosphatase activity; fractions *B–D* with adenosine triphosphatase activity, which is increased by Ca^{++} and inhibited by Mg^{++}; and fraction *E* which contains orcinol-positive material.

No myosin peak was obtained after DEAE chromatography from extracts of 11-day embryonic muscle. Without treatment with ribonuclease the elution pattern for the 11-day embryo extracts shows only very small peaks for fractions *C* and *D*, a larger peak for fraction *A*; the largest peak, fraction *F*, with a high ratio of absorbancy at 260 to 280 mμ, could only be eluted with 0.1*N* KOH. Since material can be extracted from the column with KOH, it may be that much of the protein, and with it the myosin, of this embryonic tissue are held back in the column. After ribonuclease treatment a large peak is found in fraction *D*. This fraction shows Ca^{++}-dependent and Mg^{++}-inhibited adenosine triphosphatase activity. Fraction *A* contains no myosin, and fractions *E* and *F* consist mostly of nucleotides with a small quantity of protein. This fraction is decreased six- to eightfold after ribonuclease treatment.

It seems, thus, that after treatment with ribonuclease the bulk of embryonic myosin can be eluted essentially in the form of one fraction. Therefore, the retention on the column of the 11-day embryonic myosin can be assumed to be due to the presence of RNA which is bound to the myosin molecule. Whether this binding is an indication of some structural association in the cell of myosin with RNA or of a methodological artefact has not been decided. A partial removal of RNA from myosin extracts with ribonuclease has already been observed (*5*). The relation of the RNA in the present preparations to the ribonucleoprotein described by Perry and Zydowo (*6*) and to the RNA found in myosin fractions by Mihalyi *et al.* (*5*) is not clear at the present time.

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6 July 1964