Antigenic Activity of Myosin and Actin

To our knowledge, the immunological behaviour of contractile muscle proteins has not been investigated. We immunized three rabbits and three dogs with rabbit's myosin prepared by the method of Szent-Györgyi¹. We used intraperitoneal injections of 2.5 per cent myosin solution, the myosin being dissolved in 0.4 *M* potassium chloride. The animals received 0.5 gm. of myosin in increasing doses during seventeen days; they lost no weight during the treatment. With these antisera, precipitation tests were performed with the following antigens: rabbit's myosin, *F*-actin, *G*-actin, *F*-actomyosin and normal rabbit serum. The same tests carried out before treatment with normal sera of animals afterwards immunized served as controls. The results are seen in Table 1.

Table 1

	Myosin	Rabbits' <i>F</i> -actin	<i>G</i> -actin	<i>F</i> -actomyosin	Serum protein
Rabbit 1	—	—	—	—	—
" 2	—	—	—	—	—
" 3	—	—	—	—	—
Dog 1	+++ 1:500	—	—	++ 1:125	—
" 2	++ 1:400	—	—	—	—
" 3	++ 1:2000	—	—	—	—
" 1	++ 1:1000	—	—	++ 1:1000	—
" 2	++ 1:500	—	—	—	—
" 3	—	—	—	—	—

Rabbits' myosin injected into rabbits intraperitoneally did not incite antibody formation; this indicates that the presence of myosin in the blood of the same species does not lead to the production of *iso*antibodies. We succeeded in obtaining myosin antisera from two of the immunized dogs. The immune sera did not react with *F*- and *G*-actin; this indicates that the myosin prepared by the method of Szent-Györgyi is free from actin. *F*-actomyosin reacts with the antisera for myosin; this may be explained either by the presence of free polar groups of the myosin molecule in the actomyosin, or by the presence of free unchanged myosin molecules in the antigen solution after the linkage between actin and myosin. The probability of the latter explanation seems to be corroborated by the lower precipitation titre of myosin antisera with actomyosin and by the circumstance that—according to Szent-Györgyi—actomyosin does not represent a stoichiometrically definable compound.

In further experiments, we used *G*-actin from rabbits' muscle prepared by the method of Straub¹. Three rabbits and three dogs were immunized with 0.3–0.6 per cent water solutions of *G*-actin. The animals received intraperitoneally 120 mgm. actin in increasing doses during seventeen days. Three weeks later, precipitation tests were made using the same antigens as in the previous experiment.

Table 2

	Myosin	Rabbits' <i>F</i> -actin	<i>G</i> -actin	<i>F</i> -actomyosin	Serum protein
Rabbit 1	—	—	—	—	—
" 2	—	++ 1:500	++ 1:500	++ 1:500	—
" 3	—	++ 1:250	++ 1:250	++ 1:250	—
" 1	—	++ 1:2000	++ 1:2000	++ 1:1000	—
Dog 1	—	++ 1:2000	++ 1:2000	++ 1:2000	—
" 2	—	++ 1:250	++ 1:250	++ 1:250	—
" 3	—	++ 1:250	++ 1:250	++ 1:250	—

The sera of two immunized rabbits precipitated rabbits' actomyosin with a relatively high titre. It follows that rabbits' actin administered to rabbits parenterally incites antibody formation, that is to

say, it represents an *iso*antigenic, organ-specific protein. The antisera of dogs and rabbits reacted equally with *F*-actin; this indicates that the activation of actin did not result in any changes in the antigenic (chemical) structure of *G*-actin. Antisera for myosin and actin behave similarly towards actomyosin.

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July 26.

¹ Szent-Györgyi, A., "Studies on Muscle" (Szeged, 1944).

Determination of Adrenaline in Blood Plasma by the Fluorescence Method

AMONG chemical methods for determining adrenaline in blood the fluorescence method seems superior¹⁻⁶. It has been used in two basically different versions: either blood plasma^{3,4} or a dialysate of haemolysed blood^{1,2,5,6} has been analysed. It is noteworthy that the first method gives much higher values than the second.

Theoretical objections may be raised against adrenaline determinations on dialysates of haemolysed blood, since the supposed state of equilibrium on both sides of the membrane after four hours of dialysis is assumed, based on the fact that the adrenaline concentration in the outer liquid attains its maximum after four hours. As adrenaline cannot be determined in haemolysed blood, simultaneous control determinations in the impermeate are impossible. Also the eventuality that the adrenaline might be bound to the protein and, therefore, hindered from dialysing through the membrane has been overlooked. Keeping these factors in mind, we have found occasion to compare the two methods more closely.

In so doing we used a modification of Lehmann's and Michaelis' method^{3,7}, which has the essential advantage that, by using a Beckmann spectrophotometer with an accessory fluorescence set, readings are easier to take and less subjective. This method yielded reproducible results; determinations on plasma with known adrenaline contents and on plasma with a known quantity of adrenaline added gave results closely corresponding to calculated ones.

Determinations were made on plasma and blood by the method of dialysis, and directly on plasma. This showed that the dialysis method yields values in the dialysate which closely correspond to those obtained by other authors in tests on haemolysed blood, namely, approximately 0.07 μ gm./ml. A small decrease was noted in the plasma impermeate which, in this case, may be analysed in parallel to the dialysate. The concentration was, however, of the same magnitude as that found in analysing untreated plasma, namely, 1–3 μ gm./ml. Therefore only some few per cent of the adrenaline had passed through the membrane. The same low values in the dialysate were obtained in testing haemolysed blood. Analogous tests on aqueous solutions of adrenaline showed the outer and inner liquids to be in equilibrium after four hours.