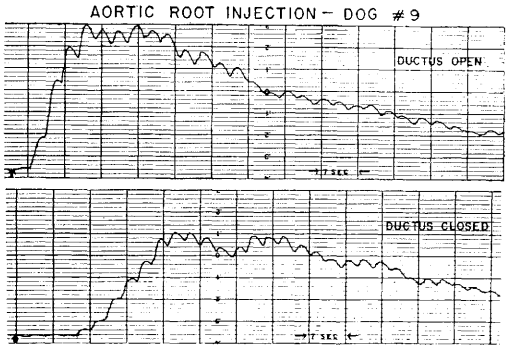

FIG. 3. Direct recordings of radioactivity of expired gas after inj. of Kr^{85} at aortic root.

Discussion. When Kr^{85} in solution is introduced directly into the pulmonary artery, it appears in expired air within 2 seconds. Thus, expired air closely reflects presence of the isotope in pulmonary arterial blood and obviates the need for sampling blood from the pulmonary artery. Detection of a left-to-right shunt by this method depends upon appearance time in expired air. An accurate estimate of this time is best obtained by continuous recording of radioactivity in expired air (Fig. 3).

When Kr^{85} is injected into the left heart or aorta of a normal animal, appearance time is 10 seconds or more; when a left-to-right shunt is present the gas reaches the pulmonary artery almost immediately and appearance time is 5 seconds or less. A basis is thus established for detecting the presence of a shunt. By selective injection at various locations in the left heart and aorta, the site of the shunt may be determined.

Preliminary clinical experience with this method in patients with congenital heart disease indicates that it may offer distinct advantages over present methods of detecting left-to-right shunts.

Summary. Left-to-right shunts were constructed in 8 mongrel dogs by anastomosis of left subclavian artery to left pulmonary artery. When solutions of krypton^{85} were injected into the root of the aorta proximal to a functioning shunt, the krypton gas could be detected in the airway by a Geiger-Müller tube in less than 5 seconds. When similar injections were made in the absence of a shunt, the appearance time was usually 10 seconds or more. Thus, by monitoring expired gas it is possible to detect the presence of a shunt resulting in early appearance of Kr^{85} in pulmonary arterial blood following injection into the left side of heart or the aorta.

1. Broadbent, J. C., Wood, E. H., *Circ.* 1954, v9, 890.

2. Sanders, R. J., Morrow, A. G., *ibid.*, 1958, v18, 856.

3. ———, *Am. J. Med.*, 1959, v26, 508.

Received June 8, 1959. P.S.E.B.M., 1959, v101.

Localization of Myosin in the Conduction Bundle of Beef Heart. (25114)

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Although the characteristic striated fibrillar structure of cells of the conduction bundle of mammalian heart suggests that these fibrils contain protein similar to those of other muscle tissue, the identity of the contractile substance in this area has not been investigated. By means of Coons fluorescent antibody technic Finck, Holtzer and Marshall(1) and Klatzo, Horvath and Emmart(2) have shown that myosin is localized in the A band of striated muscle fibers of both skeletal and heart muscle. Using this immunological technic rabbit antimyosin globulin conjugated to fluorescein has been applied to sections of the conduction bundle of beef heart to determine presence of this contractile protein in the fibrils. The chemical isolation and identity of muscle proteins of the conduction bundle will be reported elsewhere.

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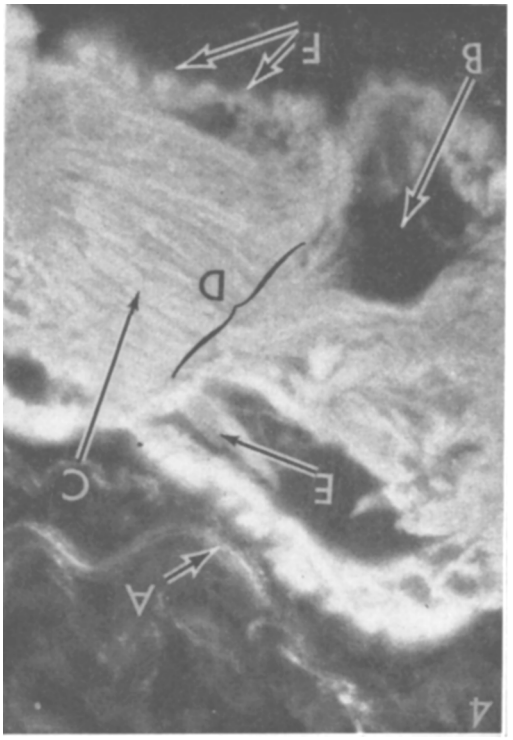
Materials and methods. The antigen. Myosin from calf heart was prepared from ventricles of heart after fat, connective tissue and vessels were removed. In addition, the conduction system was dissected away as far as this could be followed macroscopically. The hearts were obtained while still warm and chilled at once to 0°C and kept cold under dissection. The tissue was ground twice, washed once with water and twice with 0.05 M KCl. It was then extracted twice while stirring with 2 volumes 1.1 M Kl plus 0.1 M phosphate buffer, pH 7.4(3). The supernatant fluids were recovered by centrifugation after each extraction and combined. These latter extracts were diluted with water to concentration of 0.025 M to precipitate the myosin. The precipitate was dissolved by adding M NaCl to final concentration of 0.75 M. This solution was then centrifuged to remove impurities, and further purified by 3 precipitations. The final myosin precipitate was dissolved in 0.67 M NaCl and stored, refrigerated, at +2°C. In this solution nitrogen content was determined by either biuret or Kjeldahl methods and protein concentration held at about 3 mg/ml. Myosin prepared from skeletal muscle was identical with that previously used(2). *Production of antisera.* Myosin prepared from heart muscle was administered intravenously to rabbits 3 times weekly in doses of 8 mg in 2.6 ml of 0.67 M NaCl. After 8 weeks of administration antimyosin sera was obtained. The potency of the antiserum was determined by precipitin reaction *in vitro,* and the globulin fraction reassayed after conjugation to fluorescein isocyanate. *Procedure of staining.* When serum of sufficiently high antimyosin titer was obtained, the γ globulin fractions were conjugated to either fluorescein isocyanate or isothiocyanate and purified as previously described(2,4). The fluorescent antibody solution was applied for 20 minutes directly to the frozen, freshly cut tissue sections without previous fixation. The excess antiglobulin solution was quickly washed away with 4 or more changes of cold .15 M saline adjusted with phosphate buffer to pH 7.4 and the tissue mounted in neutral glycerin diluted 50% in .15 M saline and examined under ultra violet light. Fluorescent normal rabbit globulin was prepared and used for control "staining." In addition, freshly cut sections of all tissues were mounted directly in glycerin without staining and examined for native fluorescence and these fluorescent areas identified by comparison with formalin fixed tissue sections stained by various methods. *Tissue of conduction bundle.* The so-called "bundle of His" or conduction bundle in these studies, was dissected from beneath the endocardium of right ventricle of beef heart within 15-20 minutes after death and frozen immediately in isopentane chilled to −140°C with liquid nitrogen. Frozen tissue was stored in vials packed in dry ice until sectioned. Sections were cut at 7 μ in a cryostat at −18°C.

Results. The histology of the conduction bundle of the mammalian heart has been described in detail by Todd(5), Schiebler(6,7) and others(8,9). Area of the bundle of beef heart in this study includes only the region close to the atrioventricular node and part of bundle of right ventricle. Preparations of this part of the bundle stained with phosphotungstic acid haematoxylin reveal part of the remaining endocardium with dense strands of collagen, reticulin and elastic fibers of the subatrial tissue. Below this compact area these fibers form a dispersed mesh imbedded in ground substance within which blood vessels, nerves and root-like fibers of the Purkinje system are immeshed. Near the node, where branching is extensive, the fibers may be 8 or more cells thick while smaller fibers are only 2 or 3 cells wide (Fig. 1). Sections of this area "stained" with fluorescent antimyosin globulin solution show that only the fibrils of the bundle absorb and retain the fluorescent antibody (Fig. 3). This indicates the specificity of the antibody for the protein of myofibrils of the bundle.

Morphology of the Purkinje cell differs markedly in various areas of the conduction bundle but the basic integrity of the cellular pattern, the characteristic cytoplasmic structures and the structural pattern of the cross striated myofibrils have been clearly defined by means of electron microscopy(10,11,12). These studies suggest that myofibrils composed of fine fibrils are dispersed mostly in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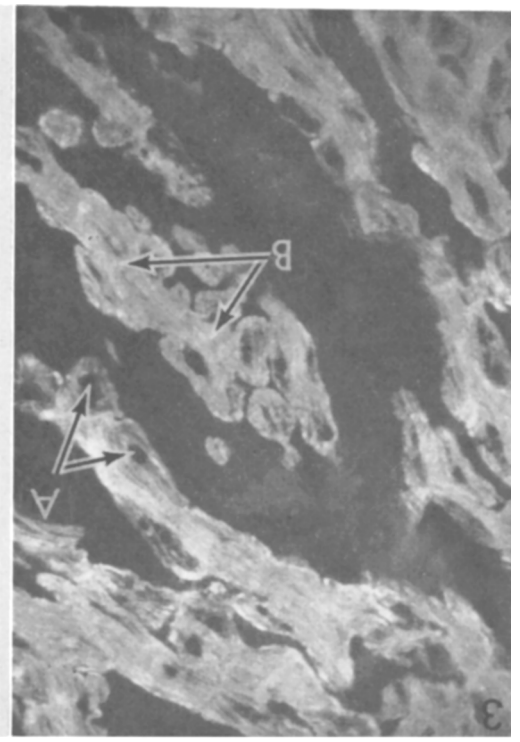
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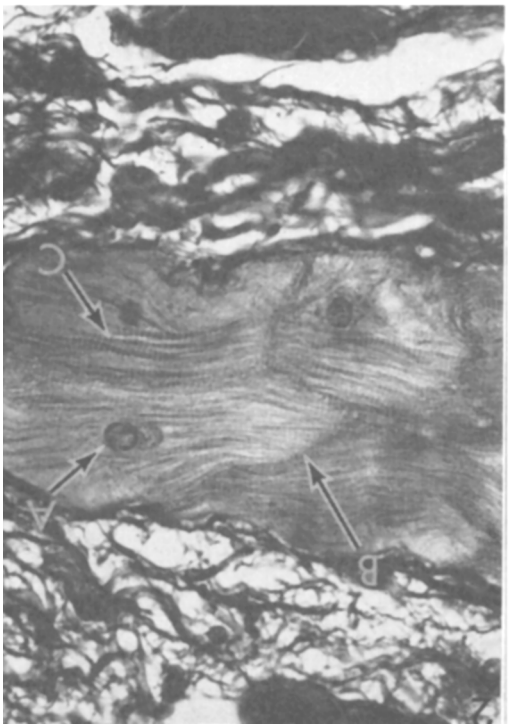
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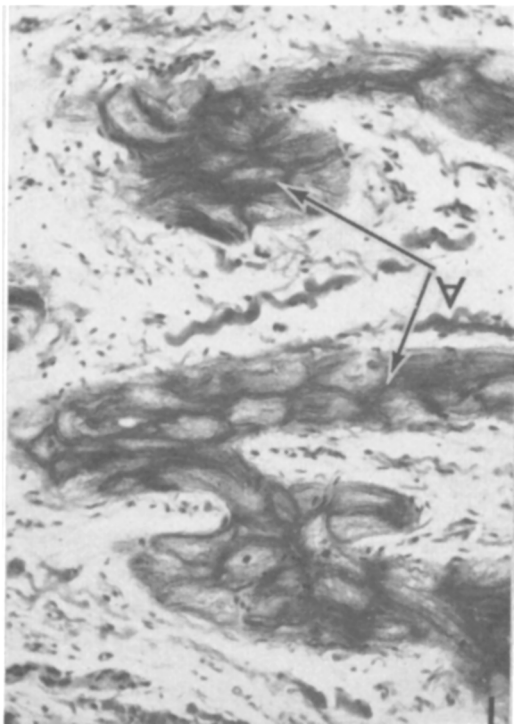
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the peripheral area of the cytoplasm. When sections of the bundle were stained with fluorescent antimyosin solution the center of cell surrounding the nucleus remains essentially unstained (Figs. 3 and 4) with an occasional myofibril penetrating between or near the nuclei in multinucleated cells (Fig. 4-B).

In sections of the conduction bundle of beef heart fixed in 10% formalin and stained with peracetic aldehyde fuchsin, following staining procedure of Fullmer and Lillie(13), the cross striations of myofibrils are clearly discernible (Fig. 2-C). "Staining" of similar sections with fluorescent antibody to myosin demonstrated cross striations of myofibrils (Fig. 4-E), and show clearly the similarity of myofibrils of the conduction bundle and those of the cardiac muscle(2). When fibrils are in a contracted state the bands of myofibrils which extend under the sarcolemma give the cells a scalloped border (Fig. 4). Purkinje cells of the fibrils are intercepted at intervals by intercalated discs with foliated borders which are composed, in part, by transverse myofibrils, and which stain positively with antimyosin solution (Fig. 4-D).

In freshly prepared tissue, strands of collagen, outside the fiber have a native blue fluorescence which, like all fluorescence, appears white in the print (Fig. 4-A). This fluorescence, native to the tissue, fades rapidly and is not seen in frozen tissue kept for several days. In all preparations other fibrillar elements of connective tissue show no evidence of antibody tagging, indicating that such impurities as may be present either in the antigen or fluorescent antibody solution do not interfere with immunological binding or specificity of antibody for myosin.

Discussion. Identification of the contractile protein, in myofibrils of the Purkinje cell as myosin, is dependent upon the antigenicity of myosin and the immunological reaction of the antimyosin globulin solution for specific proteins composing the myosin molecule. Kamikozawa (1923) demonstrated that positive seriological reactions could be obtained with crude extracts prepared from the His bundle of beef heart and rabbit sera immunized against this extract(14). However, His preparations were too crude to justify any conclusions as to identity of proteins present. It was not until 10 years later that the contractile protein of muscle tissue became known as myosin(15,16,17). Within the last 10 years it has been demonstrated that myosin isolated from either skeletal or cardiac muscle is antigenic in animals of different species(18, 19). Since it has been shown that myosin is composed of actin and tropomyosin(20,21, 22) it seems probable that antisera from rabbits injected with myosin possess multiple antibodies to the contractile protein and that in our study these antibodies probably function as a unit in binding to the antigen. Holtzer and Abbott(23) using fluorescent antimyosin globulins on embryonic muscle cells *in vitro* have shown that appearance of cross striations of myofibrils is concurrent with contractility. Changes in contractile proteins which may occur in cell division and maturation need to be followed in greater detail. Variation in histology of different areas of the conduction bundle together with its large cellular unit and dispersed myofibrils affords a unique opportunity for the study of localization of contractile proteins. The results of such experiments will be reported elsewhere.

Summary. 1) Positive binding of fluorescent antimyosin solution to myofibrils of the

FIG. 1. Fibers of conduction bundle (beef heart) stained with phosphotungstic acid haematoxylin (A), × 150.

FIG. 2. Several Purkinje cells of fiber stained with peracetic acid aldehyde fuchsin, Halmi stain (Fullmer and Lillie): (A) nuclei, (B) cell wall, (C) myofibrils showing cross striation, × 300.

FIG. 3. Fluorescence photograph of fibers of conduction bundle—"stained" with fluorescent antimyosin globulin. (A) area surrounding nucleus, negative; (B) positive staining (fluorescence) of myofibrils in Purkinje cells, × 150.

FIG. 4. Fluorescence photograph of several Purkinje cells "stained" with fluorescent antimyosin globulin. (A) collagen fibers in surrounding connective tissue possess a blue fluorescence; (B) area of nucleus; (C) myofibrils in outer cytoplasm; (D) intercalated disc; (E) striated myofibrils; (F) contracted myofibrils along outer sarcoplasm give scalloped appearance, × 500.

Purkinje cell demonstrates presence of myosin in myofibrils of the conduction bundle of beef heart. 2) Antibody to myosin whether prepared from skeletal muscle or heart tissue is capable of binding heart muscle antigen.

We wish to acknowledge our indebtedness to Dr. Koloman Laki for the opportunity for this research and many helpful suggestions.

1. Fink, H., Holtzer, H., Marshall, J. M., *J. Biophys. Biochem. Cytol.*, 1956, Suppl. 2, 175.

2. Klatzo, I., Horvath, B., Emmart, E. W., PROC. SOC. EXP. BIOL. AND MED. 1958, v97, 135.

3. Helander, E., *Acta physiol. scand.*, 1957, v41, Suppl. 141, Gothenburg.

4. Emmart, E. W., Cole, R. M., May, E. L., Longley, J. B., *J. Histochem. Cytochem.*, 1958, v6, 161.

5. Todd, T. W., *Special Cytology II.* E. V. Cowdry, Ed. Publ., P. B. Hoeber, 1932, 1175.

6. Schiebler, T. H., *Z. für Zellforsch.*, 1953, v39, 152.

7. ———, *ibid.*, 1955, v43, 243.

8. Truex, R. C., Curry, J. L., Smythe, M. R., *Anat. Rec.*, 1954, v11, 723.

9. Borg, T., *Acta neerld. morph. norm. et Path.*, 1941, v4, 97.

10. Muir, A. R., *J. Anat.* (London), 1957, v91, 251.

11. Caesar, R. G., Edwards, A., Ruska, H., *Z. für Zellforsch. und mikroskop. anat. Bd.*, 1958, v48, 698.

12. Moore, D. H., Ruska, H., *J. Biophys. Biochem. Cytol.*, 1957, v3, 261.

13. Fullmer, H. M., Lillie, R. D., *J. Histochem. and Cytochem.*, 1958, v6, 391.

14. Kamikozawa, Y., *Acta scholae med. univ. in Kioto*, 1923, v6, 133.

15. Edsall, J. T., *J. Biol. Chem.*, 1930, v89, 289.

16. Von Muralt, A. L., Edsall, J. T., *ibid.*, 1930, v89, 315.

17. Weber, H. H., *Ergebn. Physiol. exp. Pharmakol.*, 1934, v306, 109.

18. Kesztyüs, L., Nikodemusz, S., Szilagyi, T., *Nature*, 1949, v163, 136.

19. Ebert, J. D., *J. Zool.*, 1953, v39, 333.

20. Szent-Gyorgyi, A., *Chemical physiology of contraction in body and heart muscle.* Academic Press, 1953.

21. Weber, H. H., Portzehl, H., *Advanc. Protein Chem.*, 1952, v7, 162.

22. Laki, K., *J. Cell. Comp. Physiol.*, 1957, v49, Suppl. 1, 249.

23. Holtzer, H., Abbott, J., *Anat. Rec.*, 1958, v131, 417.

Received June 10, 1959. P.S.E.B.M., 1959, v101.

The Fetal Lung, A Source of Amniotic Fluid. (25115)

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The physico-chemical features of amniotic fluid in last third of pregnancy are very similar to those found in interstitial fluid(1), particularly in animals in which the allantoic sac is still present during this period, *e.g.*, goats and sheep(2). Amniotic fluid could therefore be produced by an ultrafiltration process. This production requires an organ with large surface supplied by many capillaries, in which hydrostatic pressure is higher than colloid-osmotic pressure of plasma proteins (14-20 mm Hg in goat fetus(3)). Two organs present such properties: kidneys and lungs. Hydrostatic pressure in pulmonary capillaries should be higher than colloidosmotic pressure, as pressure in pulmonary artery is relatively high, due to the nature of fetal circulation. Pressure in the pulmonary artery is higher than that found in the aorta and peripheral arteries, where pressure reaches values of about 70-80 mm Hg(4) at end of pregnancy. The kidney cannot be the only source of amniotic fluid inasmuch as amniotic fluid is present also when the fetal urethra is not patent and the urine must pass through the urachus into the allantoic sac, as in goats and sheep, to 100-120 days pregnancy, *i.e.*, to its last third. This research investigated the possible production of fluid by the lungs of fetus and to measure its rate of flow.

Methods. Experiments were performed on goats and guinea pigs during last third of pregnancy. The goats lightly anesthetized with chloralose (70 mg/g intravenously) were