Experimental Lipogenesis by Purkinje Fibers.* By Toichiro Kuwabara and David G. Cogan. (*From the Howe Laboratory of Ophthalmology, Harvard University Medical School, Massachusetts Eye and Ear Infirmary, Boston.*)‡

The heart of most animals contains a structure, believed to be a conduction system, extending from the sino-atrial node to beneath the endocardium of the ventricles. This structure is variably differentiated in different species. In ungulates it is conspicuous and consists of specialized muscle cells, called Purkinje fibers, set in fairly well defined bundles of loose connective tissue with an unusual amount of elastic tissue and nerve fibers. The Purkinje fibers differ morphologically from the rest of the cardiac muscle in being larger, having a less dense sarcoplasm, often with double nuclei, and in appearing on cross-section to have an unstained center to the individual fibers. In other species including man, rabbit, and dog the conduction system may be so inconspicuous as to raise the question whether it exists or not (6). For our purposes it is sufficient to state that the conduction system has been studied in a variety of species (although not heretofore in small animals) and has been especially well illustrated in sheep (8).

Aside from its morphologic features, the Purkinje fibers may often be differentiated by a greater content of glycogen (8, 7) and of potassium (7) suggesting a carbohydrate and cationic metabolism distinct from that of the rest of the heart. They are also said to have a high cholinesterase content (2). The purpose of the present article is to describe another feature of the Purkinje system suggesting a distinctive fat metabolism. Specifically, the Purkinje fibers appear to have a capacity to synthesize sudanophilic fat when incubated in serum or serum supplemented by oleic acid, a capacity not shared by other cardiac muscle.

The phenomenon of synthesis of sudanophilic fat by non-adipose tissue has been called aberrant lipogenesis (3). It is a property of many, although not all, cell types and depends on the presence of: (1) an intracellular sulfhydryl-dependent enzyme system; (2) serum; and (3) oleic acid or sodium oleate. Aberrant lipogenesis may be demonstrated *in vivo* by injecting oleic acid into tissues, but it is more effectively studied *in vitro* by incubating suitable tissues for 24 to 48 hours in appropriate media (3 *b*). With tissues that contain little lipid natively, such as fibrous tissue, oleic acid must be added to the media but with lipid-rich tissues, such as liver, there is sufficient endogenously derived oleic acid to provide a substrate for lipogenesis (3 *c*). If palmitic or stearic acid is substituted for oleic acid, a crystalline, non-sudanophilic fat is formed (3 *d*). Separate studies have indicated that the lipids synthesized are predominately triglycerides (4, 5).

The present observations on the heart are based on approximately 25 separate experiments in which blocks of the ventricular and septal walls were incubated at 37°C. for 24 hours in media containing serum. Rabbit serum was used in all experiments but observations on other tissues have indicated that the serum requirement is species non-specific (3 *a*). The size of the blocks and the corresponding amount of serum varied with the size of the animal but ordinarily the serum was 2 to 3 times the volume of the block. This again has been found to be not critical. When oleic acid was used it was added so as to have a concentration of 2 mg./ml. The rabbit heart was most thoroughly studied (14 cases) but comparable observations were also made on the mouse (1 case), rat (2 cases), cat (1 case), sheep (1 case), and beef (2 cases). Following incubation, the tissue was stained en bloc with Sudan IV and then fixed in formalin. The gross specimens were examined with the dissecting microscope and a few were photographed (Figs. 1 *A* and *B*). They were then sectioned in the frozen state, stained by hematoxylin, (sometimes with additional Sudan staining), and finally examined by conventional microscopy.

In the smaller animals only 2 to 3 blocks from each heart were used. The portions excised for incubation were routinely those of the ventricular wall and septum but some observations were also made on the sino-atrial node and bundles.

The controls with which the experimental results were compared included heart tissue that was sectioned and stained without incubation, and heart tissue that was incubated in Tyrode's solution without the addition of serum. Observations on many other tissues had indicated that aberrant

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lipogenesis does not occur in Tyrode's solution (3 *a*).

RESULTS

The Purkinje fibers were found to have a selective capacity to form fat in media of serum or serum fortified by oleic acid. This applied to the entire conductive system, including the sino-atrial node, and was demonstrable in all species. While the addition of sodium oleate enhanced the lipogenesis it was not essential for it. In this respect the heart is similar to certain parenchymatous tissues in which the endogenous oleate has been inferred to provide sufficient substrate for lipogenesis. In contrast to the Purkinje system, the rest of the cardiac muscle showed no lipogenesis on incubation with or without oleate. Indeed, the fat formed in the Purkinje system on incubation may be an efficient means of outlining this system in small animals where the usual morphologic criteria are equivocal. On the other hand, no lipogenesis occurred in control specimens incubated in Tyrode solutions instead of serum.

Whole mounts stained with Sudan showed the lipogenic area outlining the Purkinje system (Fig. 1) similar to that which others have observed when India ink was used to outline the system (1). No such pattern was seen on the pericardial surface or on the cut edges of the myocardium which thus provided an internal control.

Cross-sections through appropriate areas of the heart wall showed selective lipogenesis by the Purkinje fibers (Fig. 2). The fine fat droplets were scattered throughout the cytoplasm, possibly corresponding to the myofibrils, with some tendency to aggregate in the immediate perinuclear and peripheral cytoplasm.

In the present experiments, as in the reports of others, the Purkinje system was found to be much richer in glycogen than was the rest of the heart. On incubation, this glycogen disappeared prior to the formation of fat. Glycogen similarly disappears from liver on incubation but we have not studied this phenomenon further.

Fat natively present in the hearts of certain stock animals showed a distribution opposite to that of experimental lipogenesis. Thus beef and sheep hearts often showed a diffuse sudanophilia of cardiac muscle with selective sparing of the conduction system (Fig. 2 *A*). Similarly, rabbits which had been poisoned with systemic iodoacetate showed a sudanophilia of the heart muscle with sparing of the Purkinje fibers. Nor did the present experimental lipogenesis correspond to the "thrush-breast" distribution of fat in the hearts of some patients dying with anemia.

DISCUSSION

The significance of the foregoing observations is obscure. Indeed, the fact that spontaneous fat formation in the heart during life has the opposite distribution from that induced *in vitro* may cast doubt on any practical significance. On the other hand, the present experiments do add to the evidence that the conduction system has metabolic peculiarities which differentiate it from the rest of the heart muscle. Heretofore, this evidence has rested solely on its greater glycogen, cholinesterase, and potassium content.

The fat formed appears to result from an active process of synthesis such as has been shown with aberrant lipogenesis in certain other types of tissue (3 *c*). In the case of the heart, this lipogenesis is limited to the Purkinje system. Chemical analyses of other tissue from which considerable quantities of the fat may be obtained have indicated that this is predominately triglyceride in which the fatty acids of the medium are incorporated. It is neither liberation of preformed fat nor phagocytosis (such as has been shown in digoxin-injured myocardium (9)). A somewhat similar selectivity of lipogenesis in certain muscle fibers has been found in diaphragm and tongue muscles.

Another noteworthy feature of the present experiments is that they show, apparently for the first time, that small animals contain a ramification of specialized cells analogous to the Purkinje fibers in larger animals.

CONCLUSION

The Purkinje fibers differ from other cardiac muscle in having a capacity of synthesizing sudanophilic fat when incubated in media consisting of serum or serum fortified with oleic acid. This capacity of the Purkinje fibers is similar to that which has been described under the heading of aberrant lipogenesis for certain other types of tissues.

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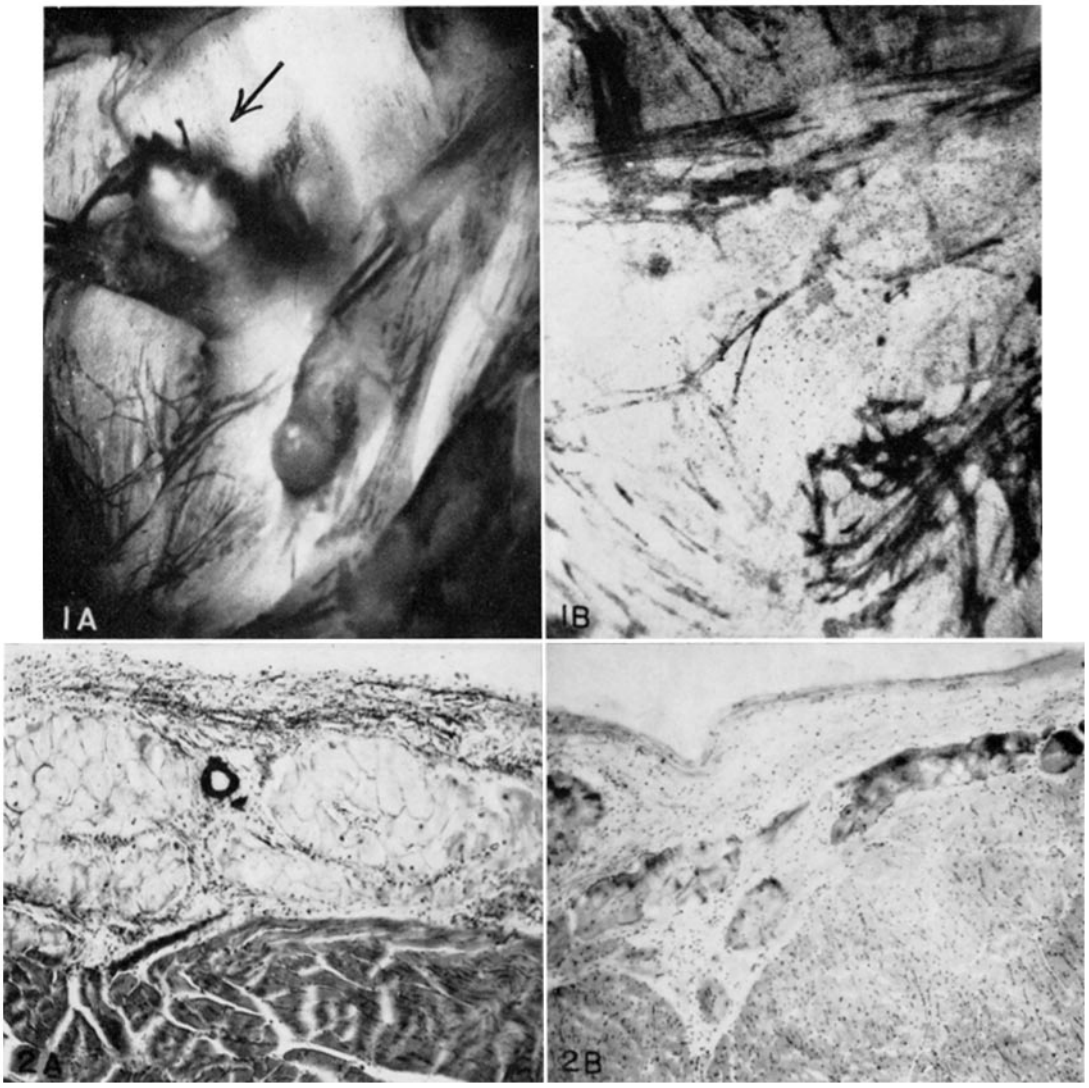
EXPLANATION OF PLATE 252

FIG. 1. Endocardial surface of ventricular wall of rabbit heart stained *en bloc* with Sudan after incubation for 24 hours in a medium of serum fortified by oleic acid (see text).

A. Portion of right ventricle enlarged approximately 20 times. Noteworthy are the ramifications of the sudanophilic fibers below and around the papillary muscles (which are indicated by the arrow). The tricuspid valve is just visible in the left upper corner, while the apical portion of the heart is in the left lower corner.

B. Portion of septal wall of left ventricle just below the aortic valve. Magnification approximately 50 times. The interlacing fibers stand out by reason of their sudanophilia.

FIG. 2. Cross-sections of endocardial portion of beef heart stained with Sudan-haematoxylin to show Purkinje fibers: *A*, before incubation; and *B*, after incubation. Before incubation, the Purkinje fibers stand out beneath the endocardium as bundles of pale staining cells from which the glycogen has dissolved out. The staining of the background fibers is the diffuse sudanophilia which one sees in all muscle fibers and which is unlike the dense red globular stain of aberrant lipogenesis. After incubation the Purkinje fibers stand out strikingly by reason of their intense sudanophilia. Much of the native lipid in the muscle fibers disappeared during the incubation.



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