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Autoantibodies to cardiac conducting tissue and their characterization by immunofluorescence

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SUMMARY

(1) A new antibody has been found by immunofluorescence which reacts with cardiac conducting tissue using ox heart false tendons. It was detected in eight out of ninety-three cases of idiopathic heart block (8·6%), in one out of twenty-two cases of secondary heart block (4·5%) and in seven of 165 normal controls (4·2%), in titres varying from 1:10 to 1:40. Previous authors had indicated that this tissue might contain unique antigens.

(2) Sera reacting with type I fibres in skeletal muscle (red zebra) were found to be of two varieties, one of which stained conducting fibres diffusely while it gave minimal staining of cardiac muscle; the other reacting with myofibrils in Purkinje cells and heart.

(3) Some sera with high titre smooth muscle antibodies (SMA) reacted with conducting tissue together with skeletal and cardiac muscle, suggesting that the four tissues have at least one antigen in common.

(4) Known non-organ specific antibodies behaved as expected on beef conducting fibres: striational fluorescence of myasthenia gravis sera reacted with the same patterns on Purkinje myofibrils; AMA and ANA produced IFL in expected locations; ribosomal antibodies reacted strongly, while LKM and reticulin antibodies showed no reactivity.

(5) Although the incidence of specific Purkinje fibre antibodies was not significantly raised in idiopathic heart block, the clinical associations suggest that some cases might be related to autoimmunity possibly involving cell-mediated mechanisms as in polymyositis.

INTRODUCTION

The rhythmicity of the heart is dependent on the specialized excitable tissue forming the sinus and atrioventricular nodes, the bundle of His, the right and left bundle branches and a subendocardial network of Purkinje fibres which also cross the ventricular cavities in the 'false tendons'. Impairment of function of the bundle branches occurs in about 5% of asymptomatic old people (Campbell, Caird & Jackson, 1974). Complete heart block is uncommon, the incidence being about sixty-three per million (Eraut, Evans & Shaw, 1973). It is usually of unknown aetiology: at post mortem, the Purkinje system is replaced by fibrous tissue with some round cell infiltration described by Lenègre (1964) and called idiopathic bundle branch fibrosis.

The selectivity of damage to the conducting system in these disorders stimulated the search for evidence of humoral immunity to the Purkinje system in patients with heart

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block and in normal subjects. Using known antibodies from patients with a variety of disorders an attempt was made to categorize the antigens present in conducting tissue and to identify any differences between Purkinje and myocardial cells and also smooth and striated muscle.

MATERIALS AND METHODS

Patients' sera. Sera were collected from a hundred patients aged 33–96 (mean 72·4) yr with complete heart block present for between 3 months and 23 yr (mean 6 yr) treated by pacemaker implantation. Details of these patients and their serology are described elsewhere (Fairfax & Leatham, 1975). Controls consisted of a hundred sera from blood donors aged between 50 and 65 (mean 56·5 years) and from a selected group of sixty-five geriatric patients aged 95–102 (mean 99·4) yr. In addition, fifteen sera obtained before death were tested in patients with proven causes of heart block at post mortem. All sera were tested fresh or stored at −20°C for up to 2 yr. Sera containing a variety of non-organ-specific autoantibodies were tested on conducting tissue for comparison with the specific Purkinje fibre fluorescence pattern.

Serological methods. Unfixed 5-μm cryostat sections of ox false tendon, skeletal muscle and myocardium were used. Ox and sheep tissues were chosen because in ungulates, the Purkinje fibres are larger than cardiac fibres and therefore readily distinguishable under the ultraviolet microscope. The presence of Purkinje fibres in the composite blocks was also checked by histochemical reactions including myosin ATPase, and phosphorylase. The tissues were obtained fresh from the slaughter house, snap-frozen in isopentane within 4 hr of death and kept at −70°C. The standard sandwich technique was employed with FITC-conjugated anti-human-Fab and monospecific conjugates of antisera to the three main immunoglobulin classes and β1C. All sera were initially tested at 1/10 dilution, and titrated to end point.

Fixation experiments. The cryostat sections were fixed for 3, 10 and 30 min in acetone, methanol, 1% formaldehyde and 0·2% glutaraldehyde.

RESULTS

Specific Purkinje fibre immunofluorescence

In eight patients with idiopathic heart block, including three cases proven at post mortem, a diffuse positive fluorescence was observed on Purkinje fibres with anti-human Fab conjugate (Fig. 1). The antibodies gave negative results on cardiac and skeletal muscle, rat kidney, liver, human thyroid, stomach and thymus. Further testing showed the antibodies to be of the IgG class and to fix complement. The sera were positive in low titres, up to 1:30 dilution. A similar pattern was seen in three blood donors and four old people, the highest titre being 1:40 (Table 1). The difference between the idiopathic heart block patients and the other groups did not reach statistical significance ($P<0·3$).

Of the twenty-two cases with heart block of other aetiology, including calcified heart valves and ischaemic heart disease, one had this antibody. One other patient with acute infarction heart block showed a subsarcolemmal pattern on cardiac muscle and the antibody stained Purkinje tissue weakly around the membrane edge.

Non-organ-specific antibodies

Six heart block patients had striational antibody to cardiac muscle, to all types of skeletal muscle fibres and to Purkinje myofibrils in titres of 1/20–1/40. One of these had multiple autoimmune disease, namely hypothyroidism, vitiligo and pernicious anaemia. Two patients had mitochondrial antibodies to 1/160 and 1/640 (Fig. 2) without clinical evidence of cirrhosis or other disease. Low titre antibodies to muscle striations were also seen in nine of the controls. No 'Zebra' patterns were observed in any of the groups.

Immunofluorescent patterns on Purkinje fibres with other sera

(1) *Muscle antibodies.* (a) *Striational immunofluorescence.* Sera from patients with myasthenia gravis were tested on Purkinje tissue. They contained antibodies to either I or A

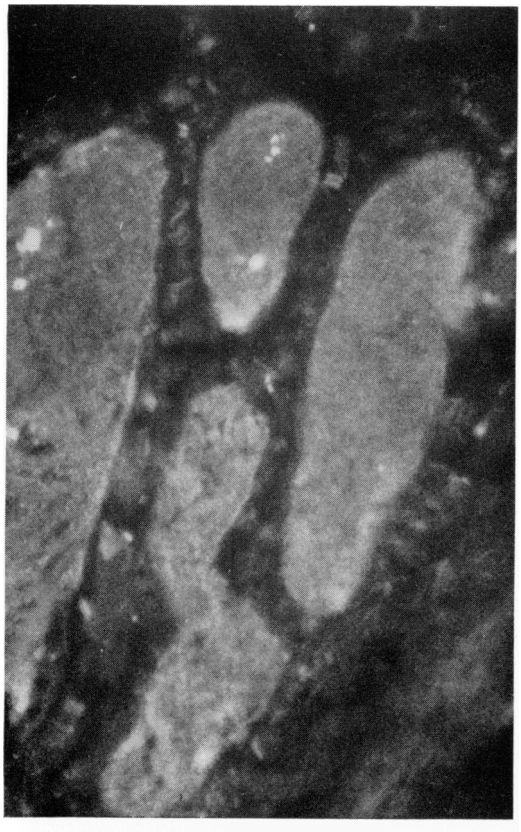


FIG. 1.

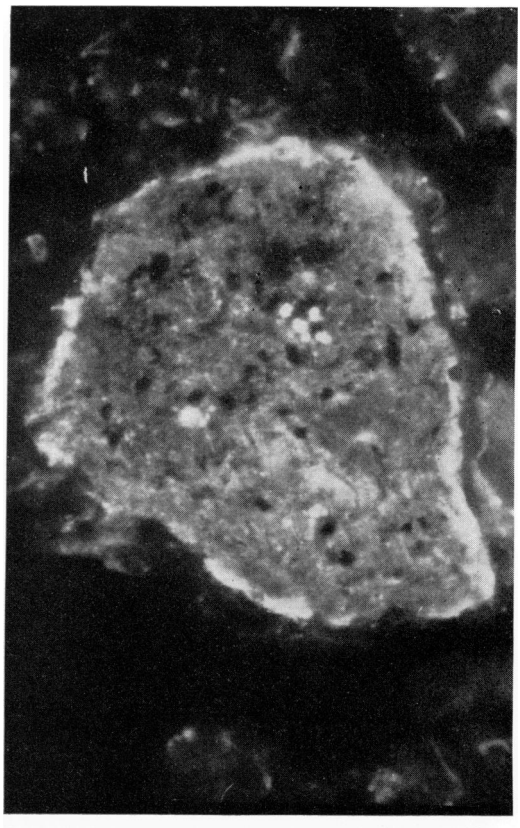


FIG. 2.

FIG. 1. Unfixed cryostat section of beef heart conducting fibres stained by indirect immunofluorescence with FITC conjugate. The Purkinje fibres are cut transversely and show a tissue-specific diffuse fluorescence pattern. (Magnification ×315.)

FIG. 2. Conducting tissue stained with serum containing mitochondrial antibodies (AMA). The brightest IFL was seen at the periphery of each fibre corresponding to the location of mitochondria around the myofibrils.

TABLE 1. Prevalence of autoantibodies to Purkinje tissue, heart, skeletal and smooth muscle in heart block cases and healthy controls

Patients and controls	Number tested	Number positive immunofluorescence at 1:10 on ox tissues: Specific Purkinje (diffuse)	Cardiac and skeletal striational	Cardiac subsarcolemmal	Smooth muscle (vessels)
(1) Idiopathic bundle branch fibrosis					
Presumed (clinical)	85	5 } 8·6%*	4	0	3
Proven (post-mortem)	8	3 }	2	0	0
(2) Heart block known aetiology					
Clinical	15	1 } 4·5%	0	0	1
Post-mortem	7	0 }	0	1	0
(3) Control groups					
Blood donors (aged 50–65 years)	100	3 } 4·2%	6	0	3
Geriatrics (aged 95–102 years)	65	4 }	3	0	4

* $P < 0{\cdot}3$, i.e. n.s. between group 1 and groups 2 and 3.

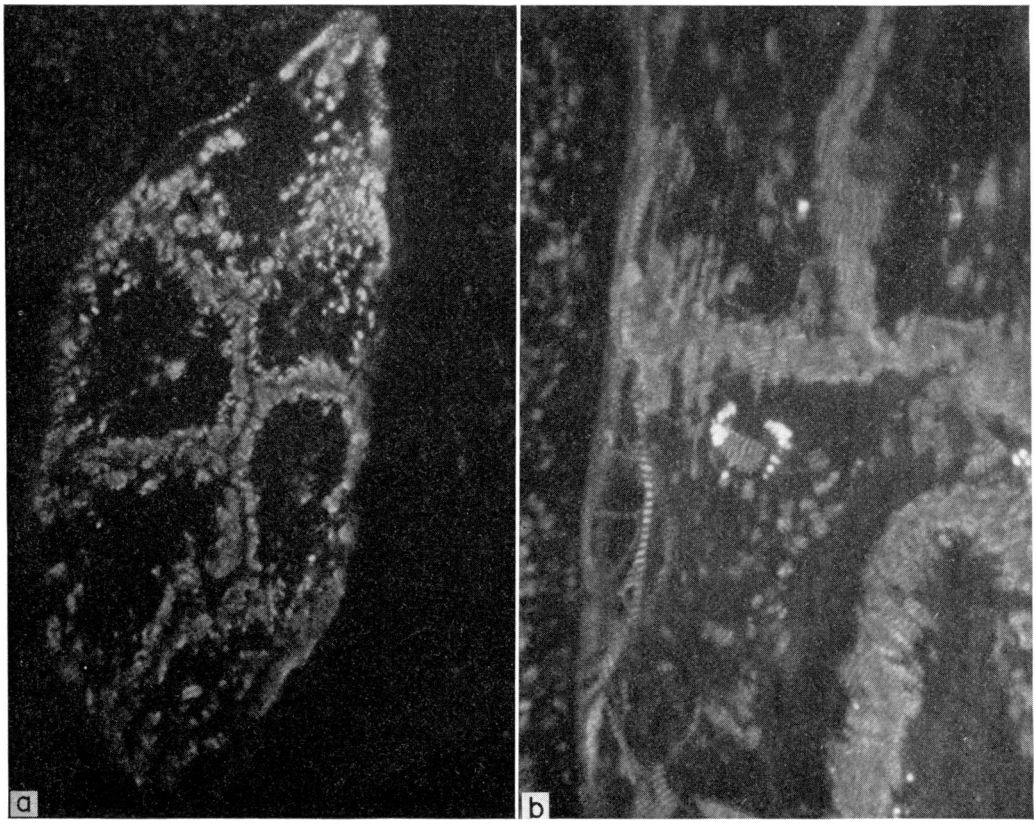


FIG. 3. (a) Bundle of seven Purkinje fibres stained with a myasthenia gravis serum containing striational muscle antibodies. The myofibrils are stained as in skeletal and cardiac muscle. (b) Same preparation cut longitudinally, showing the irregular orientation of myofibrils. The bright dots are lipofuscin granules which give an orange autofluorescence.

bands on skeletal and cardiac muscle. In all cases the myofibrils, which are situated peripherally in ox Purkinje cells, stained positively (Fig. 3). These myofibrils lie predominantly along the longitudinal axis of the cells, but are much less regularly arranged than in skeletal or cardiac muscle, and the bundles, which avoid the nuclear and central homogeneous cytoplasm of the cells, appear to be often in discontinuous bundles, as is also seen in electron micrographs of this tissue (Hayashi, 1962). One myasthenic patient had antibody which stained the Z band of cardiac muscle only, and this reacted similarly with the myofibrils of Purkinje tissue.

(b) *Fibre type-specific antibodies* ('Zebra sera'). Antisera were tested which contained antibodies to either type I or type II skeletal muscle fibres as defined by the myosin ATPase reaction (Padykula & Herman, 1955). No staining of cardiac or Purkinje fibres occurred with type II antisera. Two sera reacting with type I fibres (Feltkamp & Feltkamp-Vroom, 1965) gave different reactions on heart and Purkinje tissue despite similar staining of the type I skeletal muscle fibres (Fig. 4) and equal titre (1/80). The first serum stained heart tissue weakly to a dilution of 1/10, but gave a diffuse pattern on Purkinje tissue to 1/80 (Fig. 5). The second serum reacted with cardiac muscle to 1/80 and stained the myofibrils of Purkinje tissue to the same titre (Fig. 6). Weak staining of Purkinje myofibrils was occasion-

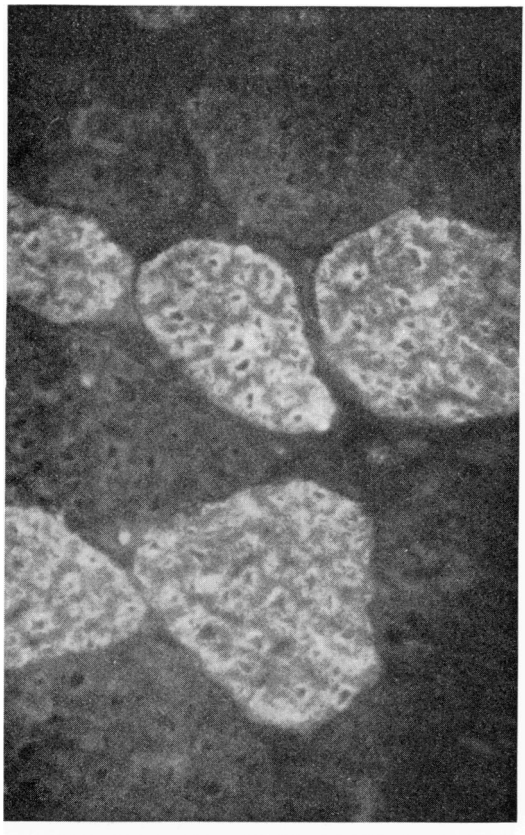


FIG. 4.

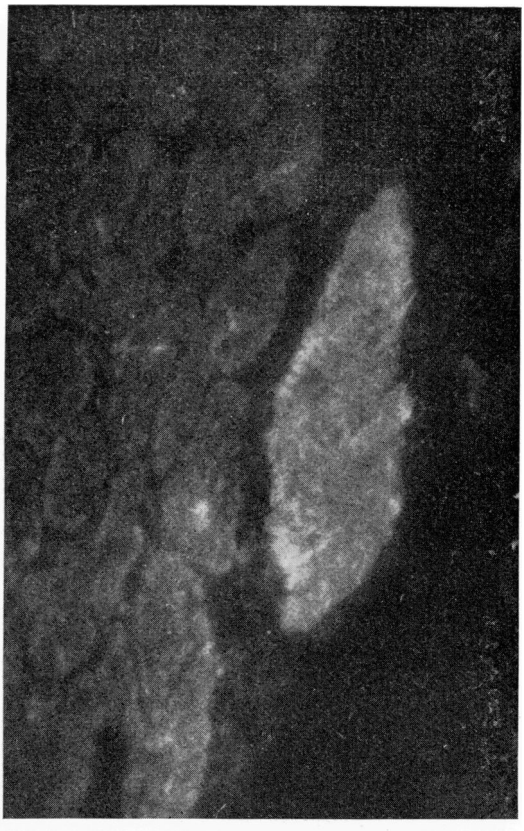


FIG. 5.

FIG. 4. Ox striated muscle cut transversely, showing identical pattern with two different sera to Type 1 muscle fibres (red zebra).

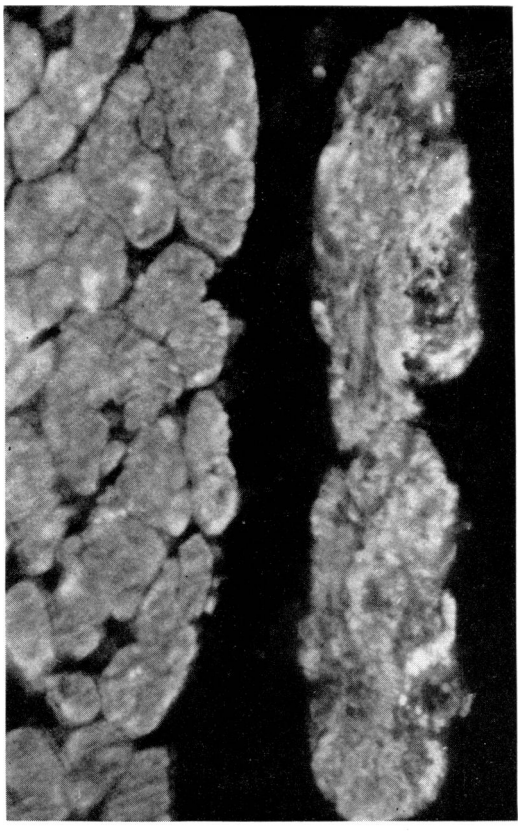
FIG. 5. Section containing cardiac muscle (left) and Purkinje fibres (right). Conducting tissue was diffusely stained with one variety of 'red zebra' serum, which did not react with cardiac muscle.

ally seen with normal sera tested at less than 1 : 10 dilutions. This suggests that 'red zebra' sera may react with at least two distinct antigens in type I muscle fibres.

(c) *Polyclonal smooth muscle antibodies* (*SMA*). Thirty-two sera positive for smooth muscle in titres of 1/80 or less on rat kidney vessels gave negative results on ox skeletal, cardiac and Purkinje tissue. Five out of nineteen sera with titres of 1/160 or above mostly from patients with chronic liver diseases were positive on Purkinje tissue, cardiac and skeletal muscle, giving a diffuse, non-striational pattern, and staining equally the different fibre types in skeletal muscle. Sera with titres of less than 1/80 failed to react on concentration by dialysis: therefore, only a small proportion of SMA are directed against an antigen common to smooth and skeletal muscle, heart, and Purkinje tissue.

(2) *Nerve antibodies*. Three sera containing antibodies to non-myelinated nerve fibres in titres of 1/40 to 1/80 in ox tissue were tested on Purkinje fibres: these were negative at 1/10 dilution. The sections of false tendons, however, showed numerous bundles of nerve fibres running adjacent to the strands of Purkinje fibres (Fig. 7).

(3) *Other known autoantibodies*. Sera with antibodies to nuclei (ANA), mitochondria (AMA) (Doniach & Walker, 1974), ribosomes (Bianchi *et al.*, 1974), cardiolipin (Wright & Doniach, 1971), liver and kidney microsomes (LKM) (Rizzetto, Bianchi & Doniach, 1974)



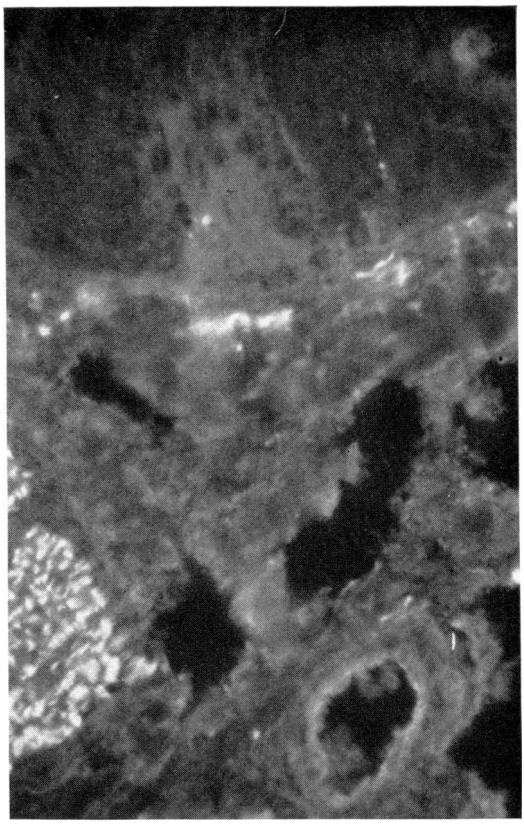


FIG. 6. FIG. 7.

FIG. 6. Similar section as in Fig. 5 stained with another type of 'red zebra' serum which reacted equally with myofibrils of cardiac and conducting tissues.

FIG. 7. Ox false tendon stained with serum of a patient having antibodies to nerve fibres. Negative appearance of conducting fibres (top). The positively stained nerve fibres were in close relationship to the conducting tissue. An unstained vessel is seen bottom right.

and various reticulin fibres (Rizzetto & Doniach, 1973) were applied to Purkinje tissue to compare the patterns with the specific antibody to conducting tissue.

Nuclear antibodies stained the large, central, single, or double nuclei in the cells. Mitochondrial antibodies gave a fine granular appearance with the brightest fluorescence at the edges where the mitochondria cluster around the myofibrils (Fig. 2); cardiolipin fluorescence (CLF) followed the distribution of the mitochondria with a less granular appearance than AMA; and ribosomal antibody gave a diffuse granular pattern as it does in many other tissues. Immunofluorescence is thus a possible way of identifying Purkinje ribosomes which are difficult to distinguish from glycogen granules by electron microscopy (Thornell, 1974) LKM antibodies did not react with Purkinje fibres, and the antigen is known to have a restricted organ specificity. Reticulin antibodies also gave negative results on conducting tissue.

Fixation experiments

Methanol, 1% formalin and 0·2% glutaraldehyde rapidly destroyed the specific antigenic characteristics of Purkinje fibres. Acetone fixation for between 3 and 30 min significantly diminished the immunofluorescent patterns of the composite muscle block.

DISCUSSION

It is now generally agreed that the spread of the electrical impulse in the heart is by the Purkinje system (Davies, 1971) and is myogenic, i.e. directly from cell to cell and not involving a neurotransmitter substance. The literature is sparse on the immunological properties of the conducting system. Using Coons fluorescent antibody technique, Helander and Emmart (1959) demonstrated the presence of myosin in the myofibrils of ox-conducting tissue, and showed that antisera raised to 'myogen' of cardiac cells cross-reacted with Purkinje cells (Emmart & Helander, 1960). Helander then showed that there appear to be qualitative differences between the proteins of Purkinje tissue and myocardium (Helander, 1965). St. Szabó *et al.* (1966) immunized rabbits with ox Purkinje tissue and detected antibody activity to Purkinje cells by complement fixation and tanned cell haemagglutination which were not absorbed by ox myocardium.

In a detailed study of canine, porcine and bovine hearts Snijder & Meijer (1970) considered Purkinje tissue to be similar to type II skeletal muscle fibres since both are rich in glycogen and myosin ATPase but contain smaller quantities of oxidative enzymes, e.g. dehydrogenases and cytochrome oxidase compared with type I fibres and myocardium. However, Purkinje fibres contain monoamine oxidase and cholinesterase (Carbonell, 1956) unlike myocardium and skeletal muscle. It is therefore of particular interest that the seromorphology of Purkinje tissue does not fit into this scheme in that antisera to type II fibres (White fibres) did not react with conducting tissue.

The Feltkamps (1965) described only one antibody reacting with type I skeletal muscle fibres and at that time cardiac muscle was not included in the tests. In the present study two distinct 'red zebra' antibodies have been distinguished, one staining both type I fibres and heart equally, and one staining mainly type I fibres in striated muscle. On conducting tissue, these two sera behaved differently. The first gave a diffuse staining while the second reacted only with the myofibrils. It is emphasized that the specific conducting tissue antibody which also gave a diffuse pattern, did not react with type I fibres in skeletal muscle and thus the reacting antigens are different.

The antigenicity of Purkinje tissue indicates that it behaves as a specialized form of muscle and does not react with antibodies to nerve tissue. Ox Purkinje fibres have been shown to contain at least one antigen common to heart, skeletal and smooth muscle, possibly actin; antigens common to heart and skeletal muscle; and finally antigens unique to the tissue alone.

The finding of low titre antibodies in patients with heart block and in a similar percentage of normals does not support a hypothesis of humoral immunity involved in the pathogenesis of bundle branch fibrosis. In this context, however, it is of interest that in experimental autoallergic myositis, tissue injury does not correlate with the present of antibodies to muscle (Dawkins, Eghtedari & Holborow, 1971) and whereas antibodies to heart muscle have not been proven to be pathogenic *in vivo* (Kaplan & Frengley, 1969) passive transfer of lymphocytes sensitized to heart tissue leads to an experimental myocarditis in rats (Friedman *et al.*, 1971). Bieber, Stinson & Shumway (1969) studied the conducting system of dog heart homografts and found that mononuclear cell infiltration during graft rejection predominantly damaged the conducting tissue compared with the myocardium. Moreover, one of the three dogs with chronic graft rejection had fibrosis of the bundle of His and the left bundle branch as an end result of this cell-mediated immune response—a pathology similar to that in patients with idiopathic bundle branch fibrosis. The discovery therefore of recognizable antigenic differences between Purkinje cells and myocardium in this study suggests that a possible cell-mediated immune response could be involved in the genesis of heart block in man.

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