

Antibodies against the Various Types of Skeletal Muscle Fibres

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Summary. Antibodies against skeletal muscle tissue may be directed against all muscle fibres ('overall' type) or against the distinct types of muscle fibres ('zebra' type). The antibodies of the 'overall' type are frequently demonstrated in sera of patients with myasthenia gravis, rheumatoid arthritis, pernicious anaemia, Hashimoto's disease and idiopathic adrenocortical atrophy. The antibodies of the 'zebra' type are probably without a clinical significance. They can be divided into antibodies against the three distinct histochemical types of skeletal muscle fibres, namely into anti-red fibre, anti-white fibre and anti-medium fibre antibodies.

INTRODUCTION

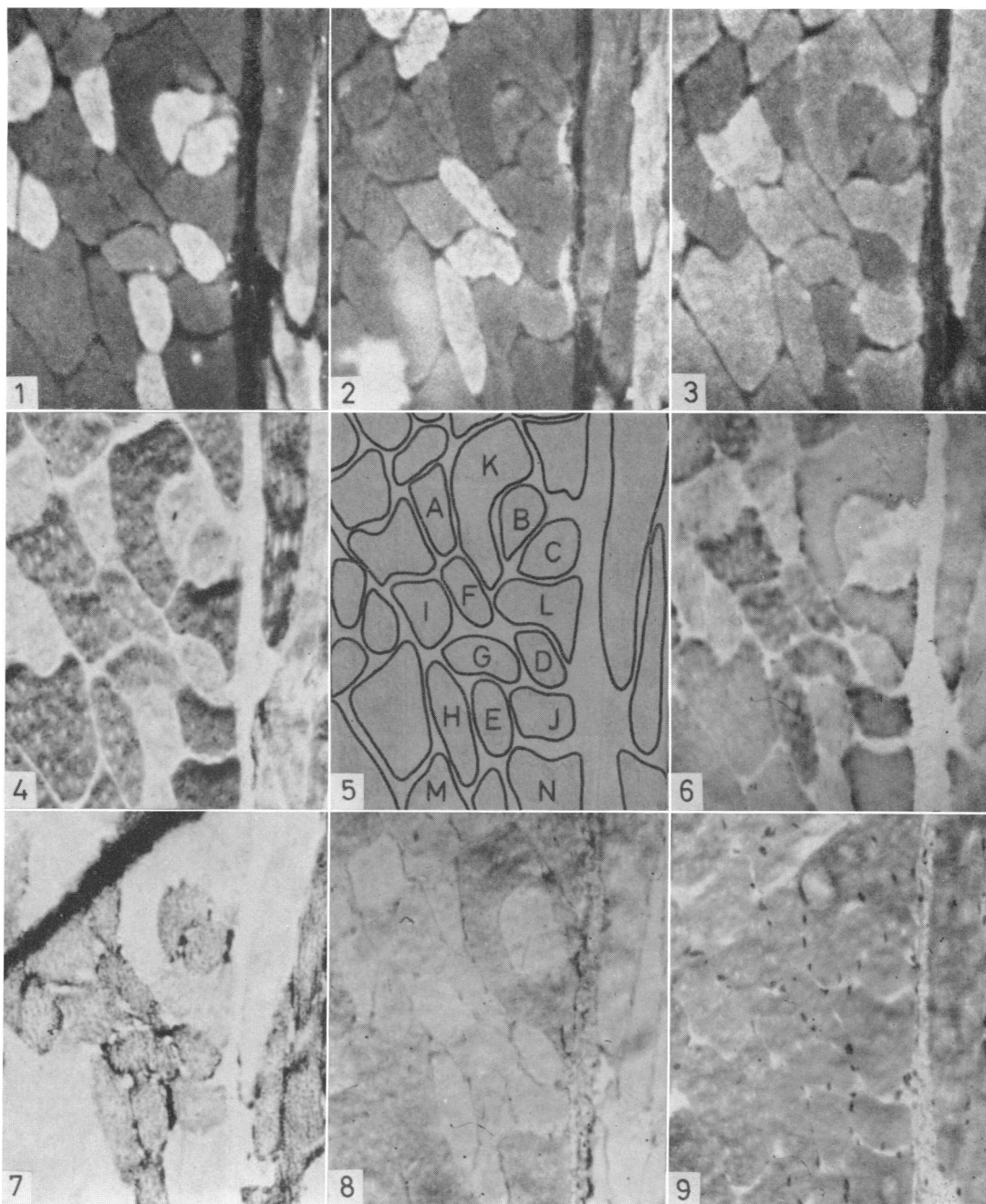
For nearly a century it has been known that skeletal muscle fibres can be divided into two types (Denny-Brown, 1929; Knoll, 1891; Ranvier, 1873, 1874, 1880, 1887, 1889; Wohlfart, 1937). In the last decade, especially since the development of histochemical techniques, it has become clear that an intermediate type exists as well (Dubowitz and Pearse, 1960; Engel, 1962; Ogato and Mori, 1964; Stein and Padykula, 1962). The following types are now distinguished: the red or so-called type I fibres, the medium fibres and the white or so-called type II fibres (Ogato and Mori, 1964).

TABLE 1
ENZYME ACTIVITIES IN THE DIFFERENT SKELETAL MUSCLE FIBRES

	Red I	Medium	White II
ATPase	—	+	+
Phosphorylase	—	(+)	+
SDH	+	(+)	—
LDH	+	(+)	—
PAS	—	(+)	+

The red fibres (Table 1) show a high activity of succinic dehydrogenase (SDH) and of diphosphopyridine-nucleotide dependent lactate dehydrogenase (LDH) while there is low activity of phosphorylase and adenosine triphosphatase (ATPase). In general the periodic acid Schiff (PAS) reaction is very weak in type I fibres. The medium fibres show less activity of SDH and LDH, moderate activity of phosphorylase and generally an intermediate PAS reaction. The ATPase activity of these fibres is high.

Antibodies to Muscle Fibres



Phosphorylase activity was demonstrated with the method of Takeuchi and Kuriaki, as described by Barka and Anderson (1963, p. 291).

Succinic dehydrogenase (SDH) activity was demonstrated with the method of Nachlas, Tsou, Desouza, Chang and Seligman, modified by Barka and Anderson (1963, p. 315).

Indirect immunofluorescent technique

This technique was the same as described by Feltkamp *et al.* (1963).

RESULTS

To compare results of the histochemical procedures with those obtained by the indirect immunofluorescent technique, serial sections of rat diaphragm were made; some were used for histochemical study, and some for examining three different sera by the immunofluorescent technique. These sera were chosen because of the different picture of the 'zebra' type fluorescence. Serum 1 was from a patient with rheumatoid arthritis, sera 2 and 3 from patients with myasthenia gravis. A clear correlation could be made (Figs. 1-9). Serum 1 contained antibodies against only the red fibres, serum 2 against the medium fibres, and serum 3 against the medium and white fibres.

Of 111 patients with myasthenia gravis only three (3 per cent) produced antibody giving alternating muscle fibre fluorescence of the 'zebra' type on rat diaphragm (Feltkamp *et al.*, 1963). Also, this type of fluorescence was found in the sera of only 7 per cent of 123 patients with definite rheumatoid arthritis examined by Feltkamp, van der Geld, Oosterhuis, den Oudsten and Hijmans (1964). These 123 patients represented 95 per cent of all patients with definite rheumatoid arthritis living in an urban district of 26,000 inhabitants. In the present work we investigated the frequency of this type of muscle fluorescence in a greater number of patients with these two diseases and in patients with other idiopathic auto-immune diseases, namely Hashimoto's disease, pernicious anaemia and idiopathic adrenocortical atrophy. An equal number of sera from normal control subjects, matched for sex and age with each group of patients, was also examined. In the group with idiopathic adrenocortical atrophy two controls were taken for each patient. The same conjugate was used for testing throughout. The results, compared with the frequency of the 'overall' type of skeletal muscle fluorescence, in which all fibres show a nearly uniform fluorescence, are presented in Table 2.

TABLE 2
FREQUENCY OF THE TYPES OF MUSCLE FIBRE FLUORESCENCE

	No.	'Overall' type		'Zebra' type	
		Patients (%)	Controls* (%)	Patients (%)	Controls* (%)
Myasthenia gravis	125†	<u>38</u>	2	3	6
Rheumatoid arthritis	132†	<u>19</u>	1	4	9
Pernicious anaemia	94	<u>13</u>	1	12	6
Hashimoto's disease	76	<u>11</u>	1	3	9
Idiopathic adrenocortical atrophy	21	<u>19</u>	2‡	14	7‡

*Matched for sex and age with each group of patients.

†Includes sera previously reported on (Feltkamp *et al.*, 1963, 1964).

‡Two controls for each patient.

Underlined values = significant difference from controls ($P < 0.05$).

It should be noted that the border between the 'overall' type and the 'zebra' type is not sharp. Reading the slides one sometimes has difficulty in choosing between the two types.

In each disease the frequency of antibodies of the 'overall' type is significantly higher than in the respective control groups, but the frequency of the 'zebra' type is not significantly above the control figure in any group of patients. The fact that there is a tendency for a lower frequency of 'zebra' type antibodies in the patients' sera than in their controls could be the result of difficulty in demonstrating the 'zebra' type antibodies when the 'overall' type are also present. If cases showing 'overall' staining are subtracted, this tendency is indeed somewhat less (Table 3). All sera, from both patients and control

TABLE 3
FREQUENCY OF 'ZEBRA' TYPE FLUORESCENCE IN SERA THAT DO NOT
PRODUCE 'OVERALL' FLUORESCENCE

	No.	'Zebra' type	
		Patients (%)	Controls* (%)
Myasthenia gravis	77	5	8
Rheumatoid arthritis	107	5	9
Pernicious anaemia	82	13	5
Hashimoto's disease	68	3	9
Idiopathic adrenocortical atrophy	17	18	9†

*Matched for sex and age with each group of patients.

†Two controls for each patient.

subjects, that produced a fluorescence of the 'zebra' type were differentiated with respect to antibodies against red and white fibres by testing on sections of rat diaphragm, rabbit m. soleus and rabbit m. vastus lateralis. Results were not compared in all cases with those obtained on serial sections by histochemical staining methods. Differentiation of specific fluorescence of the medium fibres was therefore not possible. It appeared that the ratio of antibodies against red fibres to antibodies against white fibres was 1:6. This ratio was about the same in the auto-immune diseases studied and in the controls.

DISCUSSION

It appears that some human sera contain antibodies that react only with one or more of the distinct types of skeletal muscle fibres which can be demonstrated with histochemical procedures. Although these sera were not tested on human skeletal muscle tissue, it may be expected that they would react with this tissue in the same way, for it is probable that these antibodies are autoantibodies.

Only a small number of idiopathic auto-immune diseases have been investigated here, so it is possible that some other such conditions may show a higher incidence of the 'zebra' type fluorescence. It seems improbable that such a correlation with an auto-immune disease will be found, however, since most of these diseases show an overlap of serological aberration. It is likely that these antibodies are without clinical significance and occur frequently in normal subjects. They are directed against antigens in the different types of skeletal muscle fibres which could be the enzymes whose distinct

activity is found in the various muscle fibre types, or unknown antigens which co-exist with these enzymes.

Absorption experiments or precipitation tests with these enzymes or muscle antigens might throw further light on this problem.

Sera with antibodies of the 'zebra' type may perhaps find their greatest use in the field of what might be called seromorphology, and so become a tool in the hands of anatomists, embryologists and neuropathologists (Coombs, 1963).

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