NATURE OF INVAGINATING TUBULES IN *FELDERSTRUKTUR* MUSCLE FIBERS OF THE GARTER SNAKE

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It has recently been stated that in the garter snake the muscles which connect the skin with the ribs contain two kinds of muscle fiber, comparable to Krüger's (1) *Felderstruktur* and *Fibrillenstruktur* fibers (2, 3). The former are presumed to be physiologically slow muscle fibers, the latter fast. Major distinguishing features of the former are a severely depleted sarcoplasmic reticulum and the "complete absence of a transverse tubular system" (3). These two features were considered to be the morphological basis for slow contraction. The "transverse" tubular system, which is also known as the intermediary (IT) system (4) and the excitatory tubular (E) system (5), is thought to transmit excitation from the surface membrane into the interior of the fiber. Furthermore, excitation-contraction coupling is thought to result from the release of calcium ions from the cisternal elements of the sarcoplasmic reticulum (6), a reaction which is initiated at the contacts between the E system and the sarcoplasmic reticulum which are either diads, triads or pentads (7).

A muscle fiber which completely lacks E tubules is, therefore, potentially of great interest to physiologists studying excitation-contraction coupling. Accordingly, we set out to identify the garter snake *Felderstruktur* fibers and assess their suitability for experimental study. Since Hess's statement depended upon negative evidence, that is the failure to find E tubules and excitatory/cisternal junctions, we decided to examine first the muscle fibers with the electron microscope.

In this study we found both kinds of fiber referred to by Hess, but we also found a number of fibers which may be regarded as intermediates between the two extremes. However, even in the fibers which contained the least sarcoplasmic reticulum we have found E tubules. These make diadic, triadic or even pentadic associations with the sparse reticulum.

We were thus unable to support the contention of Hess.

MATERIALS AND METHODS

Specimens of the common garter snake (*Thamnophis sirtalis*) were obtained from a pet store. The flat muscles connecting the ribs with the skin were exposed by a simple dissection and held at various lengths whilst being fixed in 5% glutaraldehyde in Millonig's phosphate buffer. They were then divided into small bundles, postfixed in 1% osmium tetroxide in the same buffer, dehydrated, and embedded in Epon 812. Thin sections were made by using the Porter-Blum MT-2 ultramicrotome with a diamond knife. They were stained with uranyl acetate followed by lead citrate and examined on plain copper grids in a Siemens Elmiskop IA.

RESULTS

Muscle fibers identical to the *Fibrillenstruktur* fibers described by Hess were readily visible. We entirely concur with his description of these fibers. In addition, we found other fibers with varying, but much smaller, amounts of sarcoplasmic reticulum and with larger sarcomeres. Attention was focused on the fibers with the least reticulum. In these fibers there are very few invaginating tubules from the surface membrane. Some transverse sections did not include any, but in most sections a few tubules could be seen lying between the filaments (Fig. 1), and a few contacts with the surface membrane were observed (Fig. 2).

It is evident that the transverse tubular system is sparse, but on the present evidence we do not consider that it is ever completely absent in any of these snake muscle fibers. The invaginating tubules do not enter at regularly spaced intervals along the length and around the circumference of "slow" fibers, as they do in the case of "fast" fibers.

The tubules, whilst clearly being homologous with the transverse tubular system, rarely enter radially. Instead, they enter obliquely, as seen in both transverse and longitudinal sections. They run inwards, following a very irregular pathway. In the region of the I bands, they make scallop-edged contacts with the terminal cisternal elements of the local sarcoplasmic reticulum. The majority of these associations between tubules and terminal cisternal elements run obliquely, or even longitudinally, and frequently change direction sharply.

This situation gives rise to the unique appearance of perfect triads in transverse, rather than longitudinal, sections, as is the case in most vertebrate muscle fibers. Although the majority of the contacts are triadic, many diads also occur (Fig. 1), and in one section (Fig. 2 *a*) a perfect pentad had been formed. This was due to the chance invasion of the fiber by two E tubules very close together. Each contacted the same obliquely running sarcoplasmic reticulum element. The triads are located in the I band regions, extending to the zone of overlap of thin filaments with the A band.

The surface membrane of these fibers is not smooth, but has numerous infoldings and pockets.

DISCUSSION

Hess showed a longitudinal section through a "slow" muscle fiber, which did not contain any triads. However, since triads occur along the length of the fibers, rather than transversely as is the case in "fast" fibers, a longitudinal section will tend to cut along the length of the tubules and cisternal reticulum elements. Thus, the normal appearance of triads will not be expected in longitudinal sections, whereas it can be expected in transverse sections. This finding points to the disadvantage of referring to the invaginating elements universally as T (for transverse) tubules. In many muscle fibers the tubules run longitudinally rather than transversely, or both longitudinally and transversely. We think that the symbol E (for externally-connected or excitatory) is preferable to IT (for intermediary) or T (for transverse), especially in view of the present findings. Some longitudinally running tubules were also found by Huxley (8) in frog sartorius fibers.

In transverse sections, triads are quite evident; thus, it is not necessary to postulate any unique excitation-contraction coupling process for the "slow" fibers. Page (9) has recently found triads in frog slow fibers. Slow contraction and relaxation is probably related to a combination of the sparseness of externally connected tubules as well as cisternal elements of the sarcoplasmic reticulum, and to the relatively great distances between these presumed physiologically important contact points and the most distant contractile filaments within the average "radius of activation."

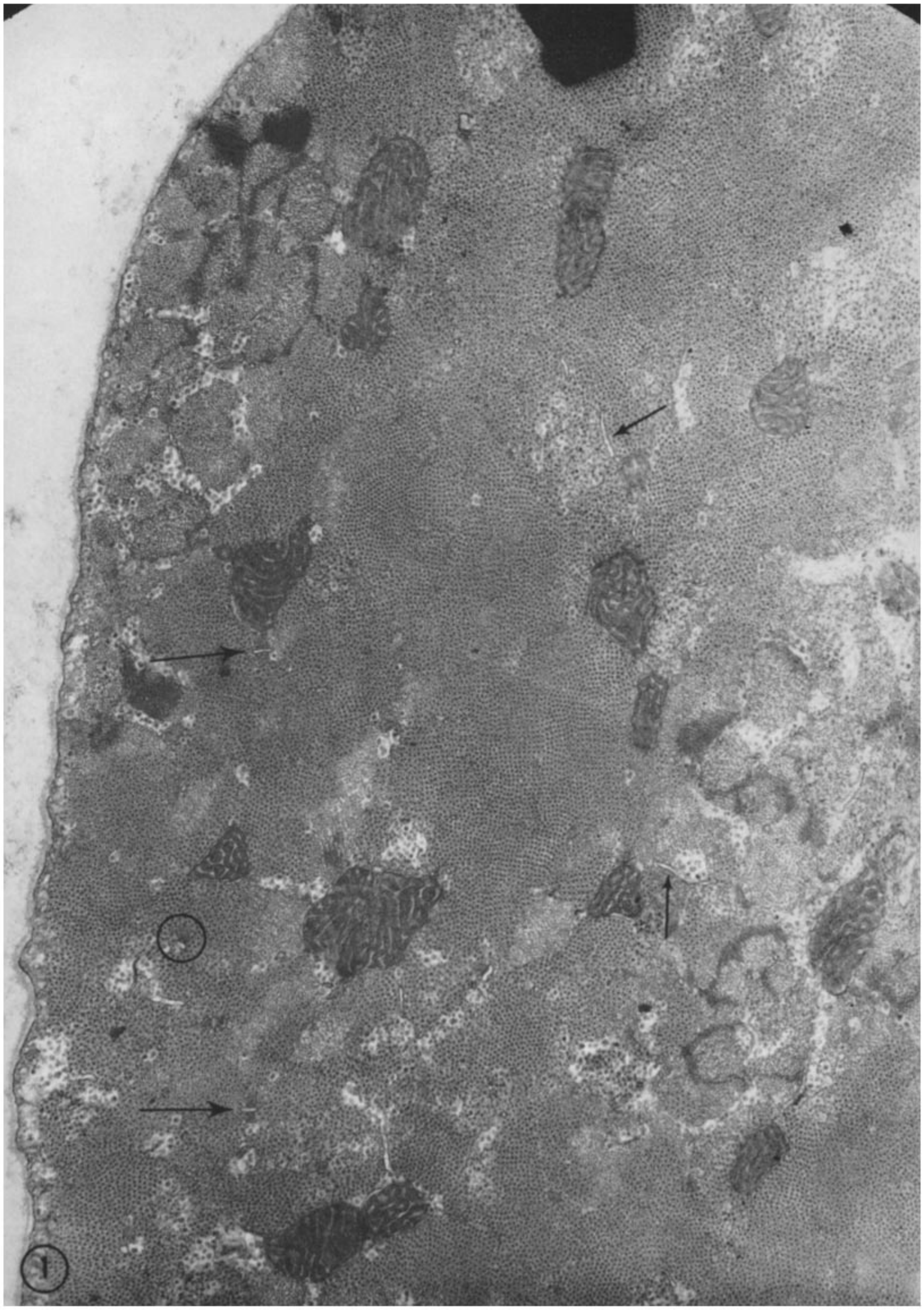

FIGURE 1 Transverse section through the garter snake *Felderstruktur* ("slow") muscle fiber. In this section, two triads (large arrows) and one diad (encircled) may be seen. Branches of the E ("transverse") tubular system are visible throughout the fiber (small arrows). × 28,000.

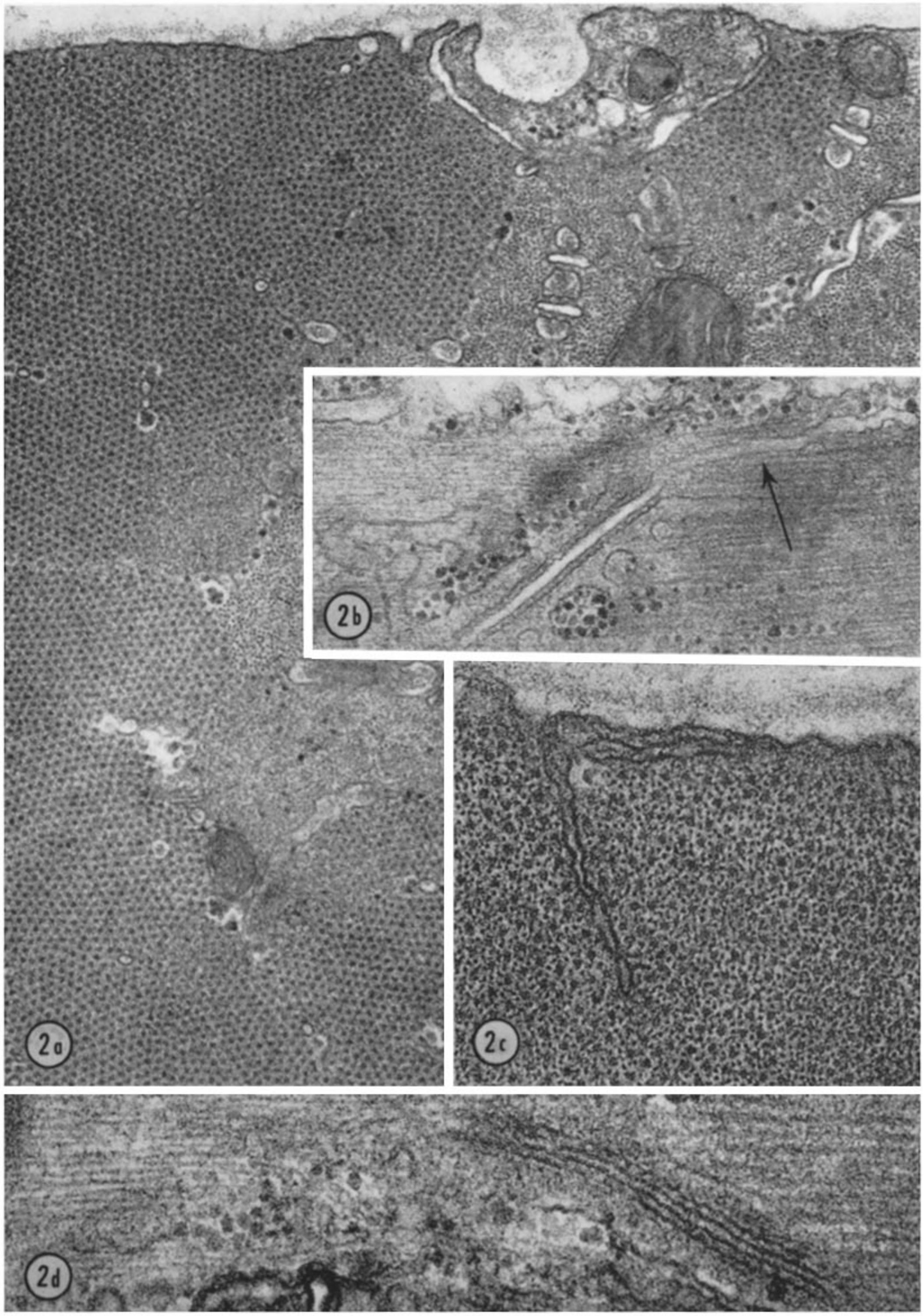

FIGURE 2 Nature of invaginations of E tubular system and of contacts with sarcoplasmic reticulum in *Felderstruktur* ("slow") muscle fibers of garter snake. *a*, A triad and a pentad are present in this section. *b*, Typical appearance of an E tubule (arrow) close to the surface membrane, where it is almost parallel and making oblique contact with SR. *c*, Two E tubules, one oblique and one entering almost radially. *d*, Obliquely running triadic element in body of fiber. Fig. 2 *a*, × 55,000; *b*, × 40,000; *c* and *d*, × 100,000.

SUMMARY

It has been claimed that *Felderstruktur* (slow) muscle fibers of the garter snake lack a transverse tubular system. A close reexamination of the same material suggests that all the muscle fibers have at least a few tubules which arise as invaginations from the surface membrane. They are unusual in that they run obliquely and longitudinally rather than transversely, a situation which is, however, common in striated fibers of invertebrates. Diadic, triadic, and rare pentadic contacts are made with the sarcoplasmic reticulum.

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