

Studies with increasing amounts of  $^{59}\text{Fe}$  labelled pork, hog-liver and -haemoglobin both in person with normal and depleted iron stores<sup>3</sup> as well as with haemoglobin and ferritin<sup>2,4</sup>, have revealed that the identity of all the partial retention coefficients  $r_1, r_2, \dots, r_n$  seems to be unlikely, so that in consequence total retention coefficients  $R$  and  $F$  would differ ( $R \neq F$ ). Therefore, an experiment has been designed to find whether the second premises, i.e. the constancy of the specific activity at least of the major iron-containing compounds, holds true.

Various edible portions, obtained from in vivo  $^{59}\text{Fe}$  labelled pigs<sup>5</sup>, were homogenized with phosphate buffer, and after centrifugation, the supernates were fractionated by chromatography on a Sephadex G100 column. The radioactivities of the fractions were determined by an automatic sample changer in a NaI well-type crystal, and the inactive iron contents were measured colorimetrically (reaction with bathophenanthroline disulfonic acid disodium salt) after the fractions within the radioactive peaks had been pooled and concentrated.

The gel chromatography of various muscles resulted in the separation of 5 radioactive fractions I to V (Figure). The absorption spectroscopy revealed that the fractions I, III and IV mainly consist of ferritin, haemoglobin and myoglobin. The nature of fractions II and V, having

molecular weights of about 160,000 and 1,300 respectively, was not established. In speculating, fraction II was assumed to be haptoglobin. The extinction peaks at 410 nm of fractions I and V in the Figure were not typical for haem compounds because no Soret-band could be registered in the absorption spectrum. The extinction curve at 254 nm was similar to those at 280 nm published recently<sup>6</sup>.

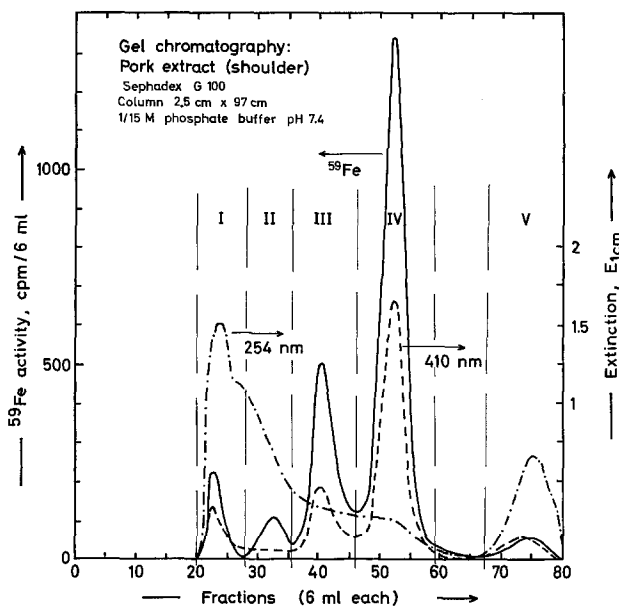
Neglecting fraction V, which did not show  $^{59}\text{Fe}$  radioactivity in all cases (contamination?), and considering the insoluble iron of the muscle homogenates as part of the balance, about 60% of total  $^{59}\text{Fe}$  or Fe was found in the haem fractions (Table). A comparison of the  $^{59}\text{Fe}/\text{Fe}$  ratios indicated that at least the specific activities of the insoluble iron and fractions II and III on the one hand and of fractions I and IV on the other hand should differ (Table). Even if these values need not be typical in other experiments because biological; physical or analytical factors might vary the results, it can be concluded that the second premises will not be generally valid. Therefore, total retention coefficients  $R$  and  $F$  should disagree in such cases ( $R \neq F$ ).

It does not seem unlikely that partial retention coefficients  $r_i$ , partial radioactivity  $a_i$ , partial iron mass  $m_i$  and other interfering factors will link together so that total retention coefficients  $R$  and  $F$  become approximately identical ( $R \approx F$ ). However, as long as these supplementary data are not available, retention studies with  $^{59}\text{Fe}$  or  $^{55}\text{Fe}$  labelled meat should be interpreted with some caution<sup>6</sup> and better be considered in terms of radioiron uptake only.

**Zusammenfassung.** Die Untersuchung von Schweinefleisch, markiert mit  $^{59}\text{Fe}$  in vivo, ergab unterschiedliche  $^{59}\text{Fe}/\text{Fe}$ -Werte in den Fe-haltigen Fraktionen, so dass von der extern messbaren  $^{59}\text{Fe}$ -Retention nicht direkt auf die resorbierbare Fe-Menge des Fleisches geschlossen werden kann.

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$^{59}\text{Fe}$  activity and extinctions at 254 nm and 410 nm of fractions after gel chromatography of the supernates from 6 g muscle homogenates of a 100 kg pig injected i.v. 10 mCi  $^{59}\text{Fe}$  5 weeks prior to slaughter. The radioactivity (cpm) refers to the 6th week post mortem.

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## Composition of the Dorsocutaneous Nerve in *Rana pipiens*

One of the first demonstrations of cutaneous sensory electrophysiology came from the frog nerve-skin preparation<sup>1</sup>. Following that demonstration, recordings from this preparation have contributed toward the understanding of receptor encoding in mechanoreceptors<sup>2-5</sup>, nociceptors<sup>6-8</sup>, and temperature receptors<sup>9</sup>. In addition, this preparation has been used to study effects of drugs<sup>10-13</sup> and thermal acclimation<sup>14</sup> on sensory receptors and the

specificity of neuronal connections<sup>15</sup>. This preparation consists of the dorsal skin of anuran amphibians of the genus *Rana* (species *pipiens*, *esculenta*, *grylio*, *catesbiana*, *clamitans*, *temporaria* have been used) with its accompanying dorsal cutaneous nerves (rami cutanei dorsi mediales). The frog dorsum is innervated by 4-8 pairs of these dorsal cutaneous nerves with receptive fields which overlap with respect to various sensory modalities<sup>2,16</sup>.

Dorsal cutaneous nerves are derived from spinal nerves 2–8 and exit the back among the longissimus and latissimus dorsi muscles.

The electrophysiological characteristics of the dorsal cutaneous nerves are well established. These nerves consist of 4 groups of afferent fibers distinguished on the basis of conduction velocity, response to mechanical stimulation, and extracellularly recorded action potential amplitude<sup>3</sup>. But despite the wide use of this preparation, no previous study has correlated these electrophysiological data with the anatomical spectrum of the dorsal cutaneous nerves.

In the present investigations, electrophysiological recordings were made of both antidromically-evoked compound action potentials and also the compound action potentials elicited in these nerves by electrical stimulation of the dorsal skin surface with concentric bipolar electrodes. All recordings were made with the dorsocutaneous nerve placed on 1 or 2 pairs of platinum wire electrodes with an inter-pair distance of 0.8–1.6 mm. The preparation was bathed in air-equilibrated mineral or silicon oil. A more complete description of electrophysiological techniques has been published elsewhere<sup>9</sup>.

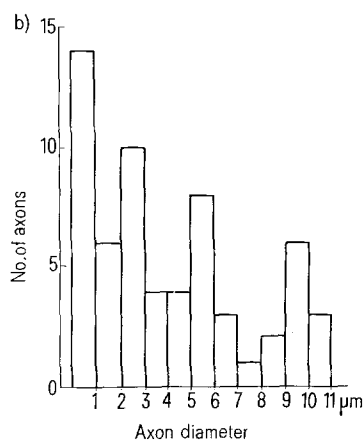
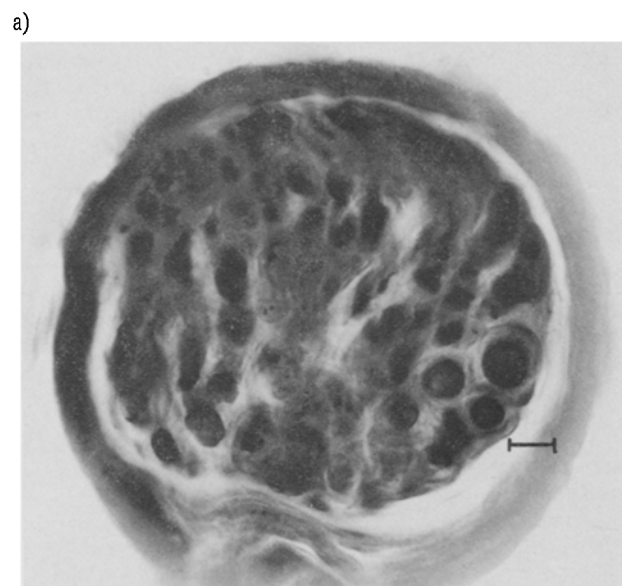


Fig. 1. Cross-section of silver-stained axons (a) showing 63 axons of diameters ranging from less than one to more than 10  $\mu\text{m}$  (b). Calibration mark in (a) represents 15  $\mu\text{m}$ .

For anatomical studies, dorsocutaneous nerves were taken randomly from 5 *R. pipiens* and fixed for 5 days in formalin at 5°C. Nerves were embedded individually in gelatin-albumin, frozen-sectioned at 20  $\mu\text{m}$ , and collected in neutralized formalin. Following additional fixation in the formalin for at least 24 h, the sections were washed in distilled water and the axons stained with a modified original Nauta procedure<sup>17</sup>. The silver-stained sections were rapidly dehydrated in alcohol, cleared in terpineol, mounted on slides, and cover-slipped with a synthetic mounting medium.

Four peaks could usually be distinguished in electrophysiological recordings from the dorsocutaneous nerve in response to antidromic stimulation. These peaks corresponded to fibres with conduction velocities 0.8–1.8 m/sec, 3.5–5 m/sec, 7–9 m/sec, and 14–18 m/sec at 25°C, agreeing quite well with other reports in the literature<sup>3</sup>. 4 peaks also predominate in records from dorsocutaneous nerves following stimulation of the skin, although it is more difficult to calculate conduction velocities directly from these records.

The dorsocutaneous nerve (rami cutanei dorsi mediales) of *Rana pipiens* was  $121 \pm 12 \mu\text{m}$  (SEM) in diameter (12 observations) after fixation and staining (Figure 1a). Although some shrinkage is inevitable as a consequence of fixation, this was minimized by the use of frozen sectioning methods. A representative dorsocutaneous nerve contained  $68 (\pm 4)$  axons, distributed according to the histogram in Figure 1b. Peaks in sizes of dorsocutaneous axons occurred between 0–1  $\mu\text{m}$ , 2–3  $\mu\text{m}$ ,

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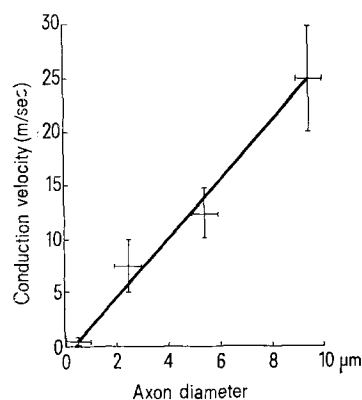


Fig. 2. Correlation between range of conduction velocities determined by CATTON's<sup>3</sup> experiments (solid vertical lines) with ranges of peaks in axon diameters (solid horizontal lines).

5–6  $\mu\text{m}$ , and 9–10  $\mu\text{m}$  (Figures 1a, b). These diameters are smaller than those of cutaneous nerves supplying the calf of frogs<sup>18</sup>.

A graph of the range of conduction velocities found in our experiments and those of CATTON<sup>3</sup> as a function of the range of peaks in the histogram of axon diameters shows that this relationship is linear (Figure 2). The conduction velocity values reported by CATTON are slightly higher than those reported here, but the linear relationship holds equally well for the conduction velocities found in this study. The present study demonstrates that the dorsal cutaneous nerve consists of relatively few axons of several distinct receptor classes. Since unitary action potentials are discernible in records from the whole nerve following physiological stimulation of the skin<sup>9</sup>, it is possible to use this preparation in studies which analyze firing properties of populations of afferents.

**Zusammenfassung.** Der dorsale Hautnerv (rami cutanei dorsi mediales) des Frosches (*Rana pipiens*) enthält nur

eine geringe Axonenzahl. Die Maxima im anatomischen Spektrum stimmen linear mit den entsprechenden Maxima im elektrophysiologischen Spektrum überein.

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### Effect of Potassium and Norepinephrine on the Tone of the Isolated Artery: Changes by Ouabain Pretreatment

The vasodilating effect of an increase in the extracellular potassium concentration by 1–10 mM is well known from investigations on various vascular beds<sup>1, 2</sup> as well as on isolated arteries<sup>3</sup>. The physiological significance of this potassium effect is indicated by the finding that the interstitial  $\text{K}^+$  of skeletal muscle rises during muscular activity, and that the time course of the  $\text{K}^+$  change is similar to that of the functional hyperemia<sup>4, 5</sup>. Furthermore, an increase in  $\text{K}^+$  in the vasodilating range inhibits the vasoconstriction in response to norepinephrine (NE) in the perfused dog forelimb<sup>6</sup> and in the isolated artery<sup>7</sup>. This effect is comparable to the 'functional sympatholysis' in working skeletal muscle<sup>8</sup>. The mechanism of both  $\text{K}^+$  effects is not yet clear. It has been proposed that the dilating effect of

$\text{K}^+$  might be related to changes in the membrane potential inverse to those of the  $\text{K}^+$  diffusion potential and mediated by activation of the sodium pump<sup>9</sup>. Recently that assumption was supported by the finding that ouabain attenuates or prevents the vasodilating action of  $\text{K}^+$  in the perfused dog gracilis muscle<sup>10</sup>. Therefore we investigated the influence of ouabain on the  $\text{K}^+$  induced dilation and on the NE- $\text{K}^+$  interaction in arteries in vitro.

**Methods.** The experiments were performed on helical strips of bovine facial arteries. The arteries were stored overnight at 4°C, cut, mounted in a moist chamber at 37°C and rinsed continuously by physiological salt solution (in mM KCl 2.68, NaCl 136.88,  $\text{MgCl}_2$  0.49,  $\text{CaCl}_2$  1.36,  $\text{NaHCO}_3$  11.88,  $\text{NaH}_2\text{PO}_4$  0.32, glucose 8.0). The strips were prestretched (basic tension 200–300 g/cm<sup>2</sup> or 3–5 g per strip, resp.), and equilibrated for 2 h prior to the experiments. The tension was recorded isometrically.

**Results.** When the potassium concentration of the rinsing solution was increased from 2.7 to 10 mM, the wellknown relaxation of the strips was observed. Reduction in the potassium concentration to 1.3 mM induced a constriction. This response pattern was changed after incubation of the arteries in ouabain containing physiological salt solutions (threshold about  $2 \times 10^{-8}$  g/ml ouabain). Ouabain itself ( $5 \times 10^{-8}$  g/ml, 90 min incubation) increased the tone of the arterial strips by about 100%.

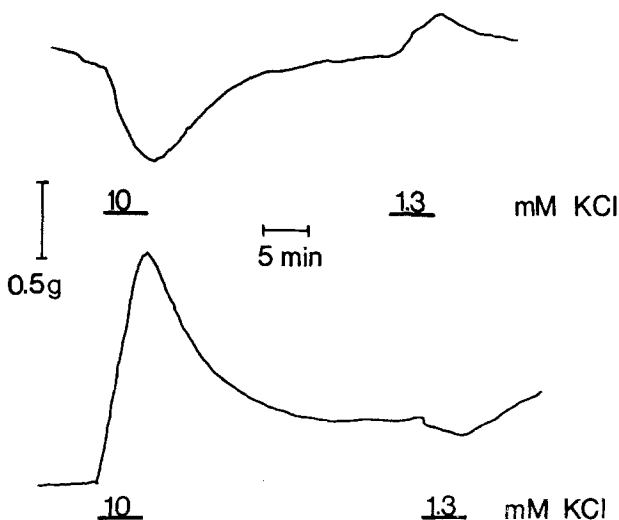


Fig. 1. Effect of changes in the KCl concentration of the rinsing solution (normal value 2.7 mM) on the tone of a helical strip from the bovine facial artery. Upper curve: normal response (basal tension 3 g). Lower curve: response after 120 min incubation with  $5 \times 10^{-8}$  g/ml ouabain (basal tension 4.2 g).

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