Results of the regeneration of abnormal limbs in the adult newt *Cynops pyrrhogaster*

Case No.	Type of abnormality	Regeneration	Day of examination post-amputation
1 rhl	Deformed limb with abnormal tarsal region and 3 digits	Normal	90
2* lhl	6 digits	Identical	90
3 rhl	Limb with 3 digits connected with epidermal lamina	Normal	90
4 rhl	Mirror symmetrical supernumerary structures of II and III digits	Normal	67
5 rhl	Mirror symmetrical supernumerary structure of III digit	Normal	90
6 lhl	Extra digit	Normal	60
7 rhl	Mirror symmetrical supernumerary structure of II digit	Normal	90
8 lfl	Accessory digit-like structure at the metatarsal region	The accessory structure was not regenerated	90
9 rhl	Digits with defects in phalanges	Normal	90
10 lhl	Supernumerary structure of V digit	Normal	48
11 rhl	Mirror symmetrical supernumerary structure of IV digit	Normal	90

*The supernumerary digit was not completely removed. rhl: right hind limb; lhl: left hind limb; lfl left fore limb.

such limbs induced in the flank of *Triturus* larvae by homoplastic grafts of nasal placode[12], by grafting limb bud tissues[13], or by treatment with carcinogens[14]. Yet, Slack and Savage[15] reported that the amputation of mirror symmetrical limbs produced in the axolotls by transplantation of the rudimentary limbs gave rise to mirror symmetrical limbs in 66% of the cases. However, the abnormalities of the limbs used in this study were not induced by transplantation, but their cause is rather enigmatic. Moreover, in this study even unusually abnormal limbs regenerated normally, and amputation of mirror symmetrical parts of the digits (cases 4, 5, 7, 10, 11) gave rise to normal single digits. If the second explanation is true, the present data suggest that the regulatory factor(s) in pattern formation were intact when structural disturbance occurred during development of the embryo or limb.

Whatever the cause of the abnormalities, the present results show very clearly that when amputation is performed proximal to an abnormal structure, normal regeneration results. The regenerates reflect the structural patterns of the limb stump which, in the present study, was normal. This suggests that the abnormalities are a result of local disturbance during blastema formation, or a trauma in the intact limb which may effect the process of pattern formation.

We believe that experiments with abnormal limbs will continue to supplement information on how positional information operates. One question to be addressed is, how does regeneration of abnormal limbs (not supernumerary structures) take place when a part of the abnormal limb is left? Will the same type of abnormality be regenerated? Here, carcinogen-induced abnormal limbs can be used[6]. Such experiments will give information for a deeper understanding of the mechanisms underlying pattern formation.

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The conduction velocity, number and diameter of unmyelinated fibers in Remak's nerve

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Summary. Remak's nerve in the chicken was examined ultrastructurally and electrophysiologically to determine the characteristics of fibers in the nerve trunk. The ratio of unmyelinated fibers to myelinated ones was 111:1. The mean number of unmyelinated fibers was 3555 ± 232 (SEM, n = 5) and they had a mean diameter of 0.502 ± 0.034 (SEM) μm. The compound action potential consisted almost entirely of a large diphasic waveform which had a mean peak conduction velocity of 0.62 ± 0.031 (SEM, n = 5) $m \cdot s^{-1}$ at 37 °C.
Key words. Chicken; conduction velocity; Remak's nerve; unmyelinated fiber diameter.

Remak's nerve (the intestinal nerve) is an autonomic ganglionated nerve trunk which has no mammalian homologue. The available evidence suggests that it contains ascending efferent fibers[1], ascending and descending adrenergic fibers[2,3], and afferent fibers from enteric cholinergic neurones[4]. In this study the fiber composition and the spectrum of fiber diameters in the nerve trunk have been determined using ultrastructural techniques. In addition the peak conduction velocities of unmyelinated fibers in the nerve have been determined from the compound action potential.

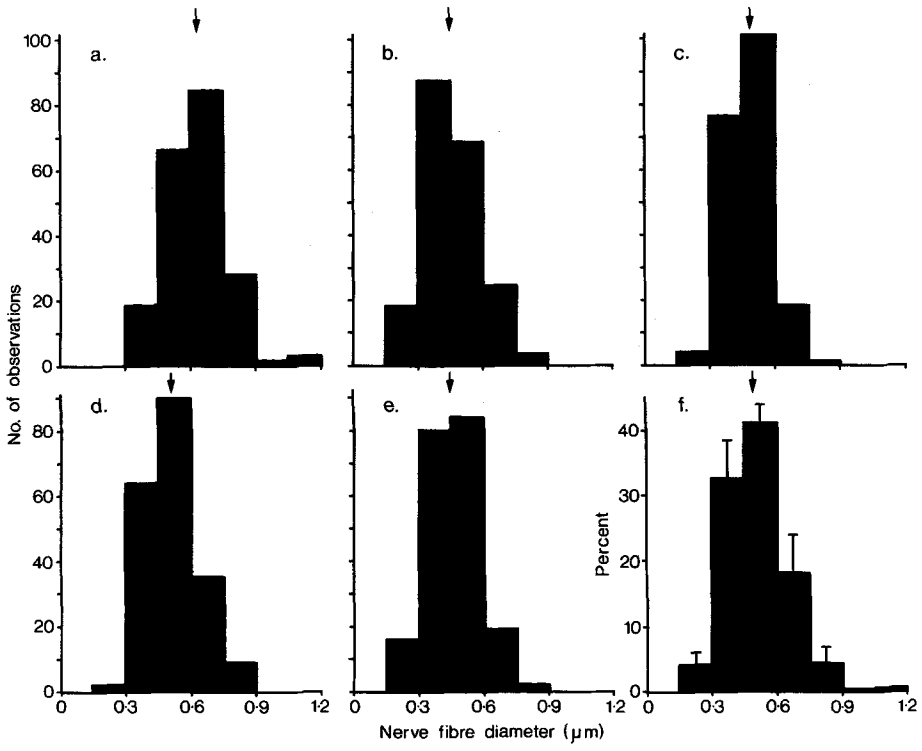

Figure 1. Histograms showing the distribution of diameters of unmyelinated nerve fibers in the five Remak nerve trunks (a–e). The histogram in f was constructed from the data in a–e, the vertical bars represent the SEM. Vertical arrows above each histogram indicate the mean of the distribution in each case.

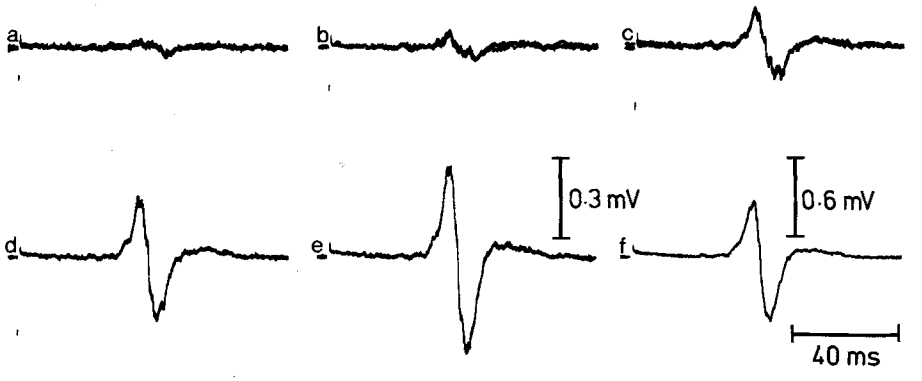

Figure 2. Records of the compound action potential recorded from Remak's nerve in response to increases in stimulus intensity (stimulus duration 500 μs). The threshold voltage was 6.2 V (a), the other stimulus intensities were 7.0 V (b), 8.5 V (c), 9.5 V (d), 11.5 V (e) and 22 V (f). The vertical calibration bar in e applies to a–e. The conduction distance was 22.8 mm, and a peak conduction velocity of 0.502 $m \cdot s^{-1}$ was calculated.

Methods. 10 Brown Leghorn chickens between 6 and 12 weeks of age were used. Five birds were set aside for ultrastructural studies, the others for electrophysiological recording. Remak's nerve at about the level of Meckel's diverticulum was used exclusively. Material for ultrastructural studies was quickly removed, washed with phosphate buffer and fixed in 2.5% glutaraldehyde followed by osmium tetroxide and stained with lead citrate and uranyl acetate. No systematic study was carried out to determine the effect of fixation and the subsequent preparative procedures on the size and shape of the axons. Studies on mammalian myelinated fibers suggest that glutaraldehyde fixation does not appreciably alter the size, shape or position of the myelinated fiber but that subsequent treatment results in an increase in axon diameter in small axons and a decrease in larger ones[5], observations which are at variance with those of others[6]. Photomicrographs of cross sections of the whole nerve were examined at a final magnification of × 6840 and all nerve fibers were counted. Sections of the nerve trunk were then examined at × 12,668 and the diameters of 200 fibers were measured from each nerve.

For electrophysiological recording the nerve was secured to Sylgard (Dow Corning) which lined the base of the organ bath. The bath contained Krebs solution, vigorously gassed with 5% CO_2 in O_2, and kept within 0.5 °C of 37 °C. Tightly-fitting suction electrodes were used for stimulation and recording using conventional electrophysiological techniques.

Results and discussion. a) *Nerve fiber number and diameter.* Counts of the total number of unmyelinated (Um) fibers in transverse sections of the five nerves gave values which ranged from 3006 to 4345 (mean 3555+232 SEM), while the number of myelinated (M) fibers varied from 6 to 61 (mean 32 ± 12, SEM). The two mean values give a Um/M ratio of 111:1, indicating the predominance of unmyelinated over myelinated fibers in Remak's nerve at the level of the small intestine.

The histograms in figure 1, a–e, illustrate the distribution of diameters of unmyelinated fibers in the five nerves examined. All were unimodal and showed no pronounced skew. In all cases the great majority of the fibers had diameters below 1 μm and in four cases (fig. 1, b–e) over 78% of the fibers had diameters below 0.6 μm.

The diameter of the unmyelinated fibers ranged from 0.25 μm to 1.1 μm. The mean unmyelinated fiber diameter varied from 0.44 μm (fig. 1, e) to 0.63 μm (fig. 1, a) to give an overall mean of 0.502 ± 0.034 (SEM) μm for the five nerves (fig. 1, f). The histo-

gram in figure 1, f, was constructed from the data in figure 1, a–e, and shows the proportion of unmyelinated axons in the nerve which fall into each 0.15 μm histogram class. Thus almost three-quarters (73%) of unmyelinated axons have diameters from 0.3 to 0.6 μm. There is a possibility that the preparative procedures used in this study may have changed the size and shape of the axons. Extrapolations from studies on mammalian myelinated fibers suggest that the axons may have either shrunk radially by about 8% or increased in diameter by, on average, 17.3%. However the validity of such extrapolations is somewhat questionable.

b) *Conduction velocity*. As the intensity of the stimulus applied to the nerve trunk was raised, a small deflection was recorded which increased in amplitude to a maximum. In all five preparations a large diphasic wave-form such as that illustrated in figure 2, f, constituted the largest component of the compound action potential. This component also had the lowest threshold (fig. 2, a–e). The peak conduction velocity of this component ranged from 0.5 (fig. 2) to 0.68 $m \cdot s^{-1}$ (mean 0.62 ± 0.031 (SEM) $m \cdot s^{-1}$), which suggests that it is the result of excitation of C-fibers. Since it was also the largest component in the compound action potential, this also suggests, that unmyelinated fibers predominate within the nerve trunk. This agrees with the ultrastructural findings. Other smaller components with conduction velocities either slower or faster than that of the main component were also seen in some preparations.

The relationship between unmyelinated nerve fiber diameter (D) and conduction velocity (CV) had been examined previously in the squid (giant axon $CV = D^{0.57}$ at 20 °C)[7]; the cockroach ($CV = D^{0.78}$ at 20 °C)[8] and the cat ($CV = D$ at 37 °C)[9]. A mean unmyelinated nerve fiber diameter of 0.502 μm and a mean peak conduction velocity of 0.62 $m \cdot s^{-1}$ produces a relationship of $CV = D^{0.69}$ for chicken unmyelinated fibers. This result supports the hypothesis that the relationship between CV and D is a higher power than expected from purely dimensional considerations[10].

Since there have been very few analyses of the composition and size distribution of nerve fibers in avian autonomic nerves[11] the results of the present study should, therefore, be of interest to avian physiologists and anatomists especially those investigating the intestine and its innervation.

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Localization of vitelline-coat lysin purified from testis of a top shell, *Turbo cornutus*

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Summary. The vitelline-coat lysin purified from the testis of *Turbo cornutus* was found, by an immunofluorescence technique, to be located in the acrosome of the sperm, which suggested that the lysin reacts with the vitelline-coat in an early phase of fertilization to allow the sperm to penetrate through the coat.

Key words. Top shell, *Turbo cornutus;* lysin, vitelline-coat; testis; fertilization; sperm acrosome.

Sperm acrosin (EC 3.4.21.10), an acrosomal trypsin-like enzyme, is essential for sperm penetration through the zona pellucida of the ovum of mammals during fertilization[2]. In some marine invertebrates, including sea urchins[3] and acidians[4,5], hydroxylases such as chymotrypsin-like and trypsin-like proteinases have been reported to participate in the penetration of sperm through the egg investments. Also, a protein that reacts with the vitelline-coat in a stoichiometric, nonenzymatic manner has been purified from the sperm of an abalone, *Haliotis rufescens*[6], and from the testis of sea snails, *Tegula pfeifferi*[7] and *Turbo cornutus*[8]; this protein (vitelline-coat lysin) has been thought to play a role in the penetration of sperm through the coat. These vitelline-coat lysins seem to be located in the acrosomes and to be released from the vesicles accompanying acrosome reaction. But direct evidence has been found so far only in *Haliotis rufescens*[6].

We previously reported the purification of a vitelline-coat lysin from the testis of *T. cornutus*, and its properties[8]. In the present study, we prepared the antiserum to the purified lysin and located the lysin in the acrosome of the sperm by an immunofluorescence technique.

Materials and methods. The vitelline-coat lysin was purified from the testis of *T. cornutus* as described previously[8], and the final preparation appeared to be homogeneous on sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis. Dulbecco's phosphate-buffered saline without Ca^{++} or Mg^{++} (PBS) was purchased from Nissui Seiyaku Co. and 1-ethyl-3(3-dimethylaminopropyl)carbodiimide (EDC) was purchased from Wakoi Pure Chemical Industries. Goat antiserum to rabbit γ-globulin (Seikagaku Kogyo Co.) was labeled with fluorescein isothiocyanate (FITC) (Baltimore Biological Laboratory) by the method of Kawamura[9]. A rabbit was bled from the ear vein to obtain normal serum, immunized with purified lysin (1.4 mg each of lysin was injected four times at intervals of 10 days with Freund's complete adjuvant, Difco) and then bled to obtain antiserum. A γ-globulin fraction (antilysin antibody) was purified from the antiserum by the method of Palacios et al.[10]. The equivalence point of the antibody to the lysin was determined by the method described by Garvey et al.[11]. To obtain the antilysin antibody which had been absorbed by the lysin, antilysin antibody (1 mg of γ-globulin/50 μl in PBS) was mixed with an excess of purified lysin (10 μg/15 μl in 0.03 M potassium phosphate-buffer con-