the method described by Nossal *et al.* (*3*), apparently because of a "wobble" component to the motion in the bacteria.

Three theoretical curves are displayed in Fig. 1. The upper curve is the calculated occupation number correlation function for a Brownian particle with a value of D appropriate for a particle 1 μm in diameter. No decay is visible over the time range covered by Fig. 1. The solid curve is that of a free particle. A comparison of the data with these curves indicates that while nonswimming bacteria behave as Brownian particles, the motile bacteria behave as neither diffusing nor free particles. The broken curve in Fig. 1 is that obtained from the interpolation procedure described above, assuming $\langle v^2 \rangle^{1/2} = 39$ μm/sec and $\langle L \rangle = 17$ μm. Although this curve falls outside the experimental scatter at some points, it must be realized that the assumed δ-function distributions for speed and step length and random angle distribution are a compromise with reality. Nossal *et al.* (*3*), for example, found a strongly skewed velocity distribution, and the data of Berg and Brown (*9*) suggest an exponential step distribution. In addition, Berg and Brown indicate that the angle between steps is not random, but steps are skewed toward small angles. The inclusion of more realistic velocity, step, and angle distributions would complicate the interpretation of the data considerably. Fortunately, however, inclusion of the skewed velocity distribution would result in a longer measured mean free path, while more realistic step-length and angle distributions would imply shorter $\langle L \rangle$. These effects thus tend to cancel, lending validity to the mean free path of 17 μm reported here.

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Axonal Transport of Dopamine-β-Hydroxylase by Human Sural Nerves in vitro

Abstract. *Dopamine-β-hydroxylase activity accumulated above a ligature on biopsy samples of normal human sural nerves incubated in vitro. The rate of accumulation indicated that this enzyme was transported distally at a velocity of 2 millimeters per hour. Axoplasmic transport of dopamine-β-hydroxylase was greatly reduced in sural nerves from a few patients with peripheral neuropathies.*

For many years it has been known that peripheral nerves can transport proteins down their axons at rates of a few millimeters per day (*1*). Recently, it has been discovered that certain proteins flow distally at much higher rates of several millimeters per hour (*2*). The question of how nerves are able to move substances from cell body to terminal regions has attracted much attention. Less well explored is the role of axonal transport in maintaining neuronal structure or function. In this study of human sural nerves in vitro, we have examined transport of dopamine-β-hydroxylase (DBH), the enzyme that catalyzes the final stage in the biosynthesis of norepinephrine (*3*). We undertook these experiments with the aim of characterizing axonal transport of DBH well enough in normal nerves to permit meaningful analysis of the role of transport in disease of peripheral neurons. Results obtained with normal nerves are reported here along with initial observations that point to abnormalities of transport in certain kinds of neuropathy.

As part of an ongoing study of the histology and biochemistry of peripheral nerves, a fascicular biopsy of sural nerve, 3 to 5 cm in length, was obtained at ankle level with informed consent from ten healthy human volunteers, ages 21 to 28 (*4*). The biopsy specimen was about one-third of the thickness of the nerve and had no fascicles entering or leaving. One milliliter of lidocaine (0.75 percent) was instilled directly into the nerve about 2 cm above the site of transection. An oblique cut marked the distal end of the specimen. The nerve was moistened with isotonic saline solution while the epineurium was trimmed off under a dissection microscope. The nerve was then blotted, weighed, and transferred to a beaker containing 200 ml of a bicarbonate-buffered physiological salt solution (*5*). This solution was maintained at 37°C and continuously gassed with 95 percent O_2, 5 percent CO_2; its *p*H was constant at 7.4. After 15 minutes of incubation in this solution, the nerve was ligated with silk thread at the proximal end (ligature 1), at a point about 9 mm from the distal end (ligature 2), and at the distal end (ligature 3). After a further incubation for a variable period of time, the nerve was removed and cut into 3-mm segments which were individually homogenized in glass homogenizers containing 0.6 ml of ice-cold buffer (0.005*M* tris, *p*H 7.4; bovine serum albumin, 0.2 percent; and Triton X-100, 0.1 percent). Homogenates were centrifuged at 15,000*g* for 10 minutes. The supernatant fractions were assayed for DBH activity in 200-μl aliquots with tyramine as a substrate, according to a previously described method (*6*). Assays were run in pairs at an optimum copper concentration (13 μM). Ten microliters of partially purified bovine adrenal DBH was added to duplicate samples as an internal standard to correct for possible variations of activators or inhibitors of DBH (*7*).

Figure 1 shows how DBH activity was distributed along the sural nerve at varying times after ligation. Most striking is the time-dependent increase in DBH activity of the segment immediately proximal to ligature 2. This increase was apparent at 1.5 hours and was dramatic by 5 hours after ligation. Elsewhere along the nerve, changes in DBH activity were minor. After the three longest incubations, there was some increase in DBH activity in the most proximal segment; this could correspond to a small amount of retrograde transport. Inconsistent increases occurred in the DBH activity of the most distal nerve segment.

Total DBH activity per milligram (wet weight) of nerve was unrelated to the time of incubation ($r = -.05$). Since DBH was apparently neither formed nor lost during incubation, the

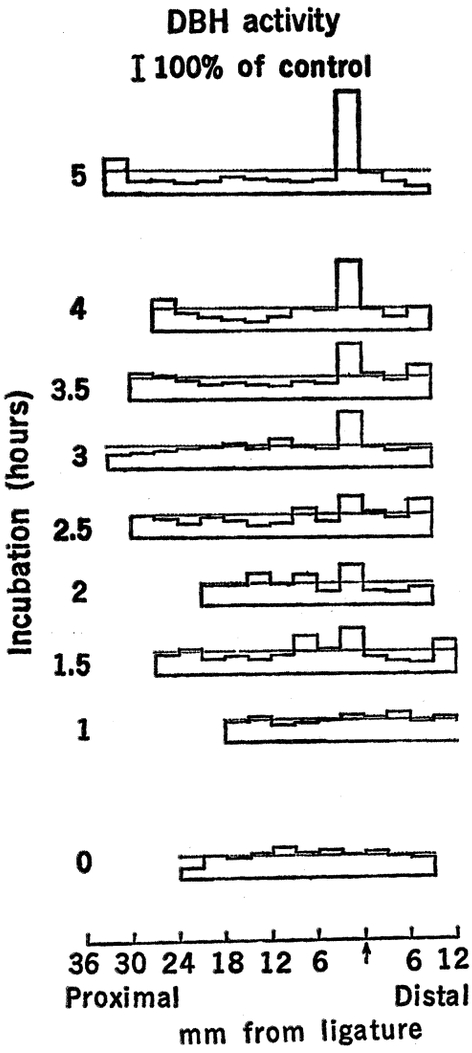
mean activity of the segments between ligatures 1 and 2 was used as a baseline against which to measure accumulation. DBH accumulation was expressed as $(L - C)/C$, where L is the activity in the segment just above ligature 2, and C is the mean activity of all segments between ligatures 1 and 2. This relative measure equals the number of control segments that would have to be emptied of DBH in order to account for the activity in the segment above the ligature.

Figure 2 represents this measure of accumulation in the segment just above ligature 2 as a function of time after ligation. Accumulation clearly was related linearly to time for at least 5 hours. The slope of the regression line can be used to estimate velocity of transport of DBH. This slope indicates that 62.5 percent of the baseline DBH activity per 3-mm segment accumulated each hour just above ligature 2. Therefore, the velocity of proximo-distal transport must have been $0.625 \times 3 = 2$ mm/hour, or greater, if some of the DBH in the nerve was not subject to transport. Transport in vitro may be slower than it is in vivo. Nevertheless, 2 mm/hour is a high velocity that is comparable to those reported for transport of DBH down rat sciatic nerves in vivo (*8*).

Rate of accumulation of DBH activity was correlated with length of nerve proximal to the middle ligature ($r = .812$, $P < .05$). Thus, unequal lengths of this portion of the nerves (Fig. 1) may have contributed to the variability about the regression line in Fig. 2. Effects of length may also explain why little DBH activity accumulated above the most distal of the ligatures, since the proximal ligatures were three times farther apart than the distal ligatures.

DBH transport was examined after a 3-hour incubation of sural nerves from a few patients with peripheral neuropathy. In one patient (age 17) with Charcot-Marie-Tooth disease of the neuronal type, accumulation was only 10 percent of the control value estimated from the regression line in Fig. 2; in this same patient DBH activity per milligram of nerve was 100 percent of control. In a second patient (age 45) with Charcot-Marie-Tooth disease of the hypertrophic type, DBH accumulation was 16 percent of control, whereas activity per milligram was 42 percent of control. In a third patient (age 14) with hypertrophic neuropathy of the Dejerine-Sottas type, there was no measurable DBH accumulation, whereas activity per milligram was 125 percent of control (*9*). In all three cases, DBH accumulation fell below the limits of 98 percent confidence for the distribution of normal points about the regression line in Fig. 2.

These results indicate that, in certain types of peripheral neuropathies which may involve the autonomic nervous system (*10*), much less than the normal amount of DBH moves down the axons of adrenergic nerves. Since the abnormal nerves that were studied had baseline levels of DBH activity that ranged from above normal to moderately subnormal, it is hard to argue that they contained fewer adrenergic fibers than



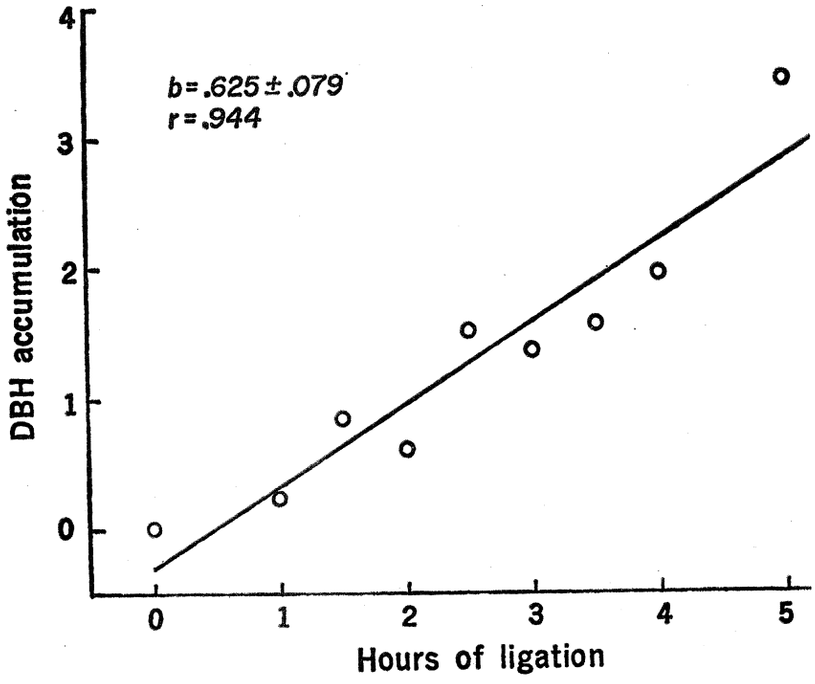


Fig. 1 (left). Distribution of DBH activity along sural nerves in vitro. Each profile represents the spatial variation in activity in an individual nerve incubated for a particular length of time after application of ligatures. The DBH activity is expressed for each nerve as a percent of the mean activity for that nerve; 100 percent of the mean is indicated in each profile by a fine horizontal line. Overall, the mean activity for these normal nerves was 96 pmole of octopamine produced per hour of incubation per milligram (wet weight) of nerve. The arrow on the x-axis marks the position of ligature 2. Fig. 2 (right). Rate of accumulation of DBH activity. Accumulation is expressed as described in the text. The indicated regression line was fitted by the method of least squares. The coefficient of regression of accumulation on time, b, and the correlation coefficient, r, are shown.

did the controls, or that synthesis of DBH was reduced. In any case, a loss of fibers or a reduction in synthesis should not have affected our measure of transport, which depended on levels of DBH actually present in the nerves. Reduced accumulation points, therefore, to a defect in the system for transport of DBH in these abnormal nerves. Because DBH is ordinarily associated with catecholamine storage vesicles in adrenergic axons (*11*), reduced transport of this enzyme could reflect abnormalities in the vesicles or in the packaging of DBH into vesicles. On the other hand, reduced transport of DBH might well reflect an abnormality in the machinery for rapid axonal transport per se (*12*). These alternatives require further study. It does appear, however, that examination of the axonal transport of DBH and other marker substances may yield new insights into the mechanism underlying some peripheral neuropathies.

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Heart Muscle Viability following Hypoxia: Protective Effect of Acidosis

Abstract. *The mechanical performance of hypoxic heart muscle is further depressed by an acid* p*H. In contrast to preparations at normal or alkaline* p*H, however, hypoxic preparations at acid* p*H do not develop contracture and exhibit full recovery of mechanical activity upon reoxygenation.*

It is generally agreed that an acid *p*H, particularly in the presence of myocardial hypoxia, is detrimental to cardiac contractile function and to the organism as a whole. An alkaline *p*H, on the other hand, improves the contractile function of hypoxic heart muscle.

Agents that enhance the performance of ischemic or hypoxic heart muscle, however, may accelerate deterioration (*1*), whereas myocardial depressants decrease cardiac energy requirements and preserve viability. In view of these observations and the results of the present experiment, it may be necessary to reevaluate our concepts of the significance of *p*H on hypoxic myocardium. Experiments were carried out to determine the effects of *p*H on heart muscle function during hypoxia and on recovery of mechanical activity after reoxygenation.

Papillary and trabecular carneae muscles were dissected from the left ventricles of rats killed by decapitation and were mounted in a muscle chamber containing Krebs-Henseleit solution (*2*) gassed with 95 percent O_2 and 5 percent CO_2. Glucose (5 m*M*) was present in the bath solution, kept at a constant temperature of 28°C. Muscles were stimulated 12 times per minute by parallel platinum electrodes delivering 5-msec square-wave pulses at voltages 10 percent greater than the minimum required to produce a maximum mechanical response. Stimulation thresholds did not change significantly throughout the study. Experiments were carried out with preparations contracting isometrically at the apexes of their length tension curves, and unstable or poor preparations were discarded. Developed tension at *p*H 7.4 under oxygenated conditions was 5.72 ± 0.42 g/mm². Resting tension was less than 20 percent of developed tension and did not vary significantly prior to hypoxia.

The *p*H of the solution was changed by addition of 1*N* HCl or NaOH. With a change to *p*H 6.8 or 7.8 alone, tension declined to 93.7 or 90 percent of control values, respectively. After 15 minutes of stable contraction at the new *p*H, hypoxia was initiated by changing the gas mixture to 95 percent N_2 and 5 percent CO_2. After 60 minutes of hypoxia, reoxygenation with

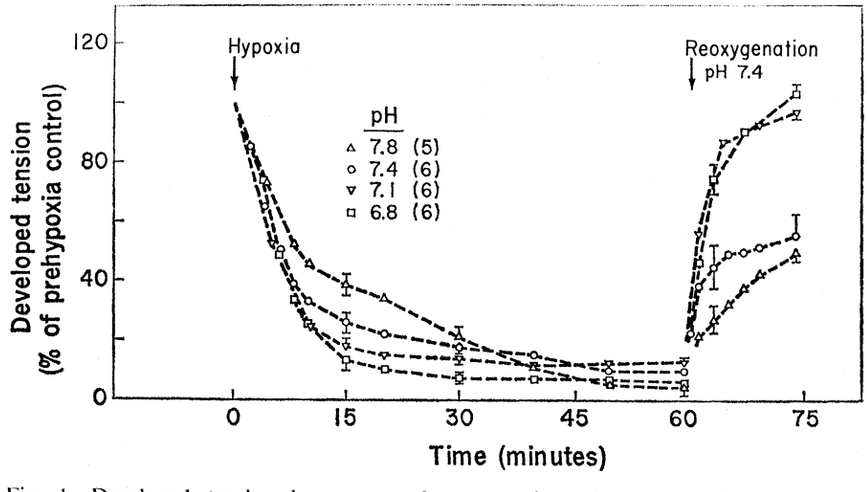

Fig. 1. Developed tension by rat muscle preparations during 60 minutes of hypoxia at varying *p*H values and 15 minutes of reoxygenation at *p*H 7.4. Glucose (5 m*M*) was in the medium. Prehypoxia developed tensions at each *p*H were as follows: *p*H 6.8, 5.25 ± 0.47 g/mm²; *p*H 7.1, 6.73 ± 0.63 g/mm²; *p*H 7.4, 5.70 ± 1.30 g/mm²; and *p*H 7.8, 4.96 ± 0.37 g/mm². The numbers in parentheses following the *p*H values indicate the number of muscle preparations in each group. Brackets represent ± 1 standard error of mean. Although tension is most depressed at an acid *p*H during hypoxia, full mechanical activity is restored upon reoxygenation.