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Neuronal Dynamics and Axonal Flow: Axonal Peristalsis*

(time-lapse phase-contrast cinemicrography)

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ABSTRACT **By time-lapse, phase-contrast cinemicrography of sensory nerve fibers of young mice, isolated with their originating spinal ganglia in nutrient solution, the postulated peristaltic surface drive of the cellulifugal propulsion of the semisolid axonal column at a rate of the order of 1 mm/day (1 μm/min) could be verified and quantitatively analyzed. The pulse waves follow each other at about 30-min intervals, regardless of whether the continuity of the axon is preserved or disrupted by degenerative fragmentation. Even when the axonal flow is obstructed, surface peristalsis continues.**

The axon advances as a semisolid column (1) from its origin in the neuronal cell body cellulifugally at an average rate of 1 mm/day, i.e., 1 μm/min ("axonal flow"). Contrary to the much faster "intraaxonal transport" of substances by as yet undisclosed means, its driving mechanism has from the very first (2) been postulated to consist of peristaltic waves in the surface of the nerve fiber "present in each fraction of the peripheral course of a fiber" (3). Experiments and observations with cinemicrographic techniques†, carried on since 1961, have verified the correctness of the postulate and opened the detailed study of the phenomenon to be outlined below.

Although excerpts from the films have been shown frequently (4), this is the first pictorial report. It is basic to a bioengineering study of the microrheology involved, to be summarized in a subsequent article (Biondi, R., Levy, M. J., and P. A. Weiss in *Proc. Nat. Acad. Sci. USA*). Intercostal nerves of young mice, left attached to their ganglia, were dissected and transferred into specially constructed perfusion chambers. The nerve fibers were teased apart locally for better visibility. The preparation, thinly coated by homologous blood plasma and perfused with Earle's balanced nutrient fluid enriched by calf serum at pH 7.4, was kept at a stabilized temperature of 38°. Time-lapse motion pictures of teased fibers were then taken under the phase microscope for up to 10 days, usually by exposure of one frame per min (acceleration in projection: 1440 times).

Myelin sheath, nodes of Ranvier, and Schmidt-Lantermann clefts remained sharply distinct. Endoneurial cells emigrated fast, but the nerve fibers remained in a state of relative paralysis for several hours up to a day or so. Then one after another started to become active. Each nerve contained intact sensory fibers (with their ganglion cells of origin), truncated motor fibers in Wallerian degeneration, and sporadic dead fibers. The former two types showed the expected peristaltic waves and the translatory movement of the axonal column.

Space restrictions limit the following examples to the illustration of four of the most crucial features. In general, the peristaltic wave manifests itself as a travelling constriction or indentation of the surface of the nerve fiber (myelin sheath as well as axolemma), proceeding in intact fibers cellulifugally (i.e., from spinal ganglion cells both proximo-distally, "$p \rightarrow d$", to the receptors and centrally into the dorsal root). The slow surface waves occur in pulses at intervals of about half an hour and advance at an average rate of the order of 1 μm/min (1 mm/day). They proceed regardless of whether the axonal content moves along or is blocked in transit by mechanical obstructions or the fragmentation of fibers undergoing Wallerian degeneration. The more erratic *passive* convection of the axonal content, as evidenced by the shifts of granular inclusions and scraps of myelin, contrasts sharply with the regularity of the *active* surface drive and discounts decisively any conjecture of the flow of the axon being propelled from within‡.

Fig. 1 illustrates this factor; it represents a rare case, in which a localized surface pulse of 1 beat/15 min (at *arrow*) entailed an overt propagated peristaltic wave only once in every nine beats, instead of each time, as ordinarily. The picture shows, from left to right, 17 still frames, seriated at equal intervals of 10 min each (total time represented, 160 min). The $p \rightarrow d$ course of the fiber is from bottom to top; its diameter is 9 μm. The two *pin-pointed black lines* mark two consecutive waves of surface contractions (indentations), following each other at an interval of 130 min and advancing at a rate of 1 μm/2.5 min (0.6 mm/day). The apparent discontinuity of the fiber between its top and middle thirds is actually a loop (at right angles to the optical plane), the kink of which has not prevented the smooth passage of the contractile wave, but has stalled coarse axonal inclusions; note the shift of the granular cluster (upward from and back to the level marked by the *dotted horizontal line*) upon the passage of the surface wave. This example demonstrates in essence *ad oculos* the "damming" of axonal content by a constriction (see also Fig. 4).

Fig. 2 shows an already closed up stretch of a fiber (diam-

* This is article no. VI in a series entitled "Neuronal Dynamics and Axonal Flow." Paper V is ref. 1.

† The instrumentation and the cinemicrography have been largely the masterly achievements of my outstanding collaborator, Dr. A. Cecil Taylor, now Prof. at the Dental School of the University of Texas in Houston, and our technical assistant, Albert Bock. Excerpts from the films for public distribution are being prepared.

‡ The numerical values given in the following are approximations within an uncertainty range of about 15%.

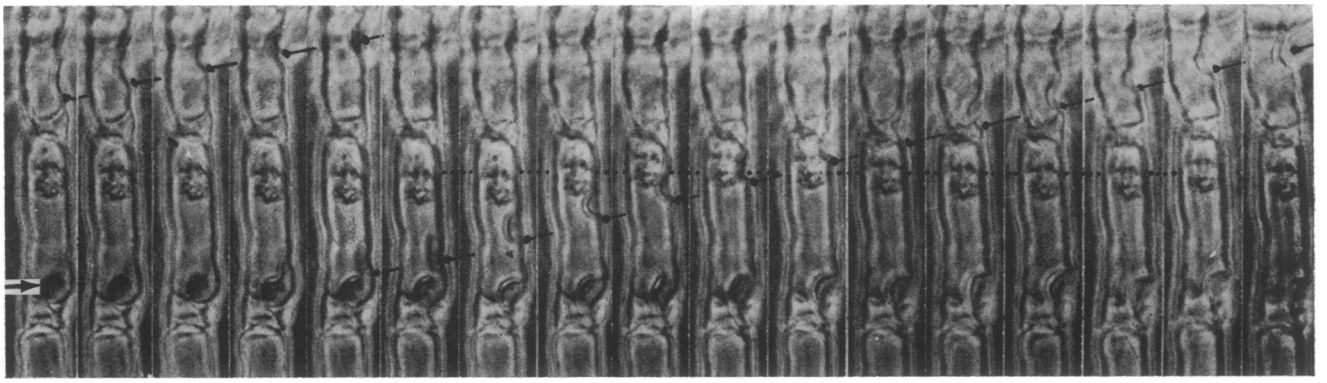

FIG. 1. Single waves after every ninth pulse. ×600.

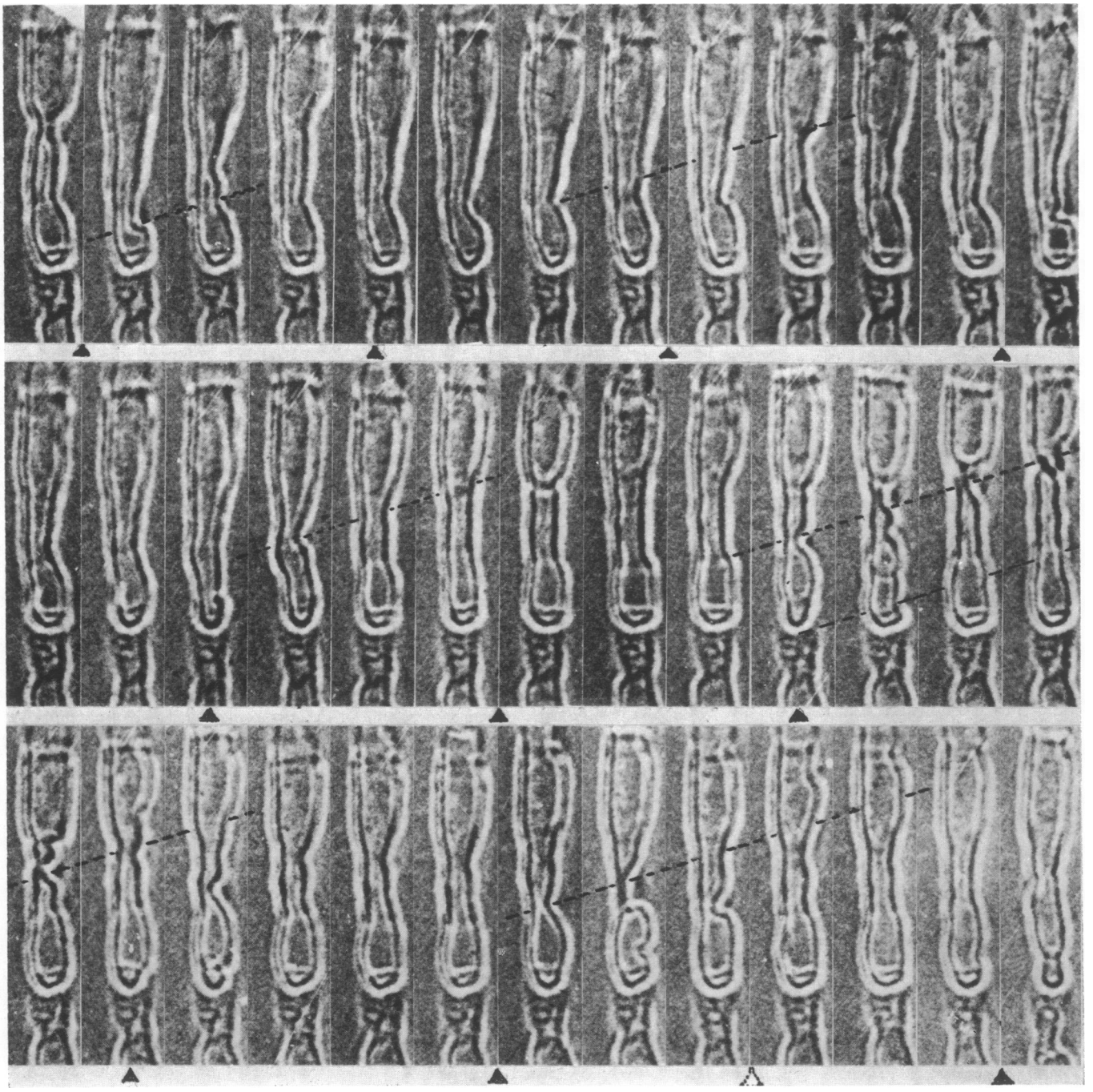

FIG. 2. Continued waves in "ovöids." ×900.

eter nearly 7 μm) in incipient Wallerian degeneration (Cajal's "ovoïd"). The 39 stills, seriated at 8-min intervals (read horizontal rows from left to right, beginning at the top), exemplify the typical succession of peristaltic waves in such a segment of fragmented axons. Nine waves, proceeding from lower to upper edges, are illustrated, the start of each being marked by a *black arrowhead* (the ninth wave might contain an "extrasystole", *open arrowhead*). The average intervals between starts are: for the first six waves, 29 min; for the next four waves, 32 min; for all waves together, 29.6 min. Rates of advance of the consecutive waves are rather constant, as is indicated by the parallelism of their tracings (*broken lines*), amounting to 0.6 μm/min (0.9 mm/day). The peristaltic waves pass unhindered over the tandem chain of internal fragments, while the content of each fragment remains shut in within its borders; each wave produces an intumescence of the blind forward end of the fragment, followed by a slow rebound upon passage of the wave. It remains open whether this rebound is due simply to viscoelastic equilibration of the deformed mass or to an active retrograde surface wave actuated at the blind end. No such rebound was ever observed in undisrupted fibers.

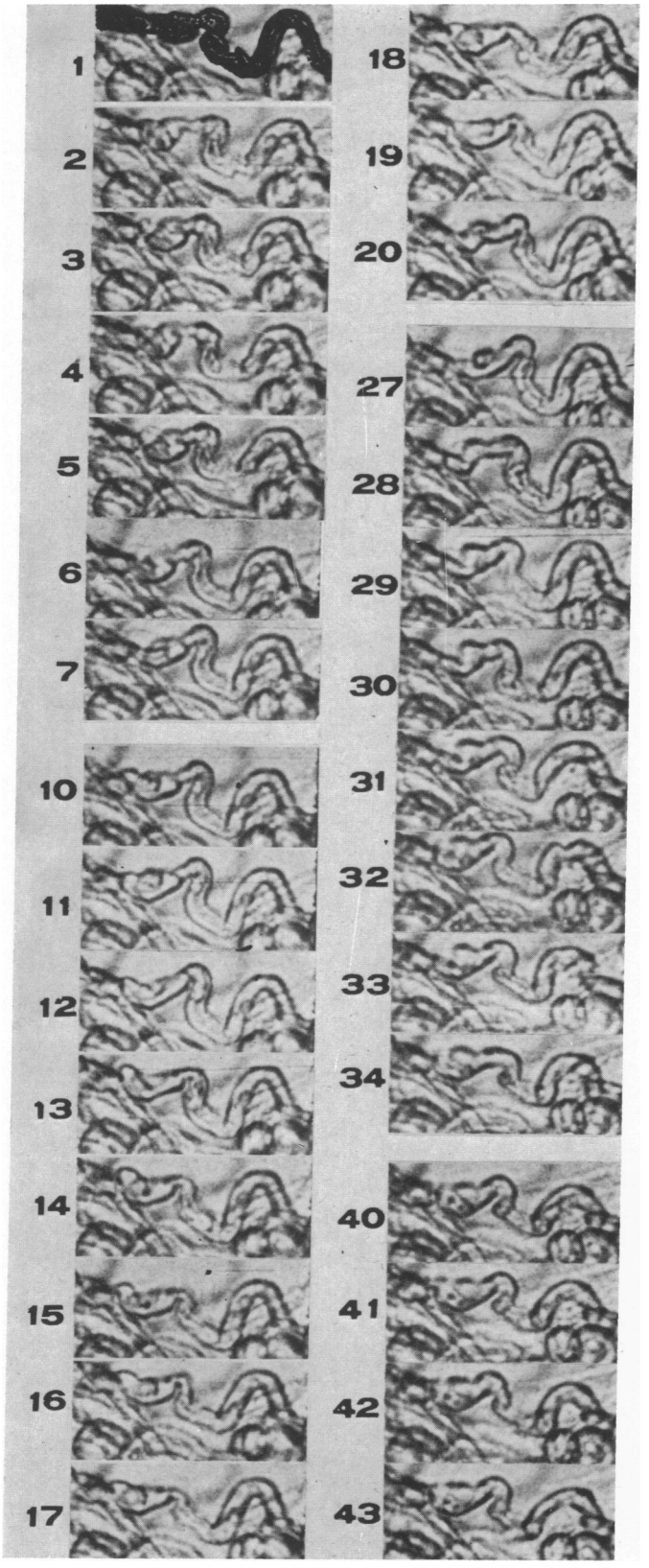

FIG. 3. Meandering fiber. ×610.

Fig. 3 shows an 80-μm stretch of the meandering course of a continuous 5-μm caliber fiber (*ink-traced* in frame 1) in which the advance of the axonal mass could be clearly watched and measured as it moved in spurts, punctuated by transitory stalling at the convolutions, until it overcame the local obstructions at bends (see also Fig. 4). Interframe periods are 4 min; the gaps between 7 and 10 (12 min), 20–27 (28 min), and 34–40 (24 min) correspond to local stalling. The advance of the content thus occurs in rhythmic shoves of about 51 min duration each, with progression and stall alternating at a ratio of 2:1. Velocities during the moving phases averaged 2.5 μm/min, the rate of total advance of the column, clocked for 168 min, being 1.24 μm/min (1.8 mm/day, uncommonly high).

Fig. 4 shows three phases of a fiber containing granular debris and vacuoles for markers, but otherwise continuous, in which passage was temporarily blocked by a kink (at *arrow*). The $p \rightarrow d$ direction of surface peristalsis runs from right to left. The pressure thus exerted on the rear portion of the pictured length of the column, the front end of which was stopped at the kink, resulted in a progressive longitudinal compression, manifested by shortening and dilation of that portion. Two levels (near the moving rear and the stalled front, respectively), marked by the ends of the *two black lines*, were traced from *a* to *c*. The dimensions of the piece between them (*d*, diameter; *l*, length; *v*, volume) and times

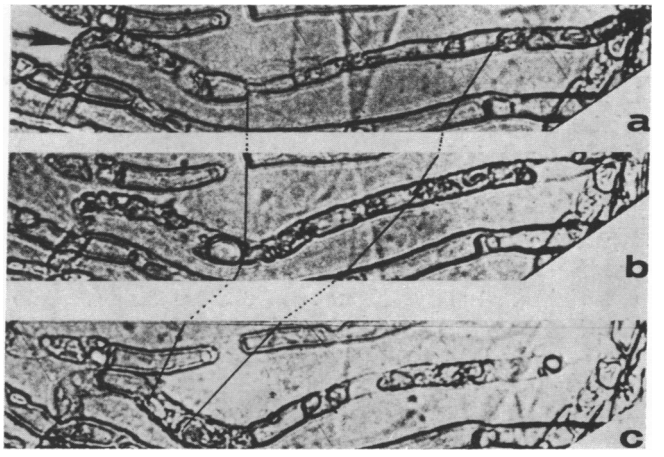

FIG. 4. Kinked and compressed fiber. ×455.

elapsed between the three stages (*i*, interval) were: for *a*, d = 4.4 μm, l = 75 μm, v = 1640 μm^3, *i* (*a* to *b*) = 26.5 min; for *b*, d = 5.4 μm, l = 52 μm, v = 1190 μm^3, *i* (*b* to *c*) = 63.5 min; and for *c*, d = 7.7 μm, l = 14 μm, v = 650 μm^3. Accordingly, besides the geometric deformation, the longitudinal compression has yielded shrinkage of volume (*a* to *b*: − 27%; *b* to *c*: −46%), tentatively ascribable to extrusion of water. In *c*, the pressure head at the front end has just become great enough to widen the stricture of the kink and open the passage for unimpeded downflow. During the block (*a* to *c*), the rates of advance of the two marked levels were, for the rear mark, 1 μm/min, and for the front mark, 0.3 μm/min (for the kink, of course, zero).

COMMENTS

The foregoing examples constitute a small selection of the yield of quantitative information extracted from the analytical study of our films, in combination with the visual observations. The main conclusions are:

(*i*) The cellulifugal drive of axonal flow resides in the axonal surface; even with the added evaluation of our extensive electronmicroscopic studies, I shall still leave the more specific identification of the anatomical substrate of the mechanism (presumably cooperative between Schwann cell and axolemma) open.

(*ii*) The peristaltic surface waves occur in pulses. 254 beats in four fibers, recorded for a total of 120 hr, gave average intervals of 27, 28.1, 28.6, and 29.5 min/pulse, for a total of 28 min/pulse. This rhythm, remarkably constant in view of the variety of neurons and conditions, is about 2000 times slower than our heart beat.

(*iii*) The contractile mechanism is evidently present in every differential fraction of axonal length; it is actuated by the action of the next proximal differential level and, in turn, actuates the next following one, in the manner of a propagated chain reaction.

(*iv*) The rate of the wave propagation has been of significant constancy, essentially of the standard rate of 1 μm/min of normal axonal flow.

(*v*) Axonal content is driven by the peristaltic wave passively; this is evidenced by the correspondence of rates of surface drive and convection of content in unobstructed fibers and the retardation or arrest of flow by mechanical obstructions, which further corroborates the semisolid consistency (1) of the axonal column. A technological (rheological) study of the properties of the driving mechanism itself will follow (Biondi, R., Levy, M. J. & Weiss P. A., in preparation for *Proc. Nat. Acad. Sci. USA*).

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