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PROXIMO-DISTAL FLUID CONVECTION IN THE ENDONEURIAL SPACES OF PERIPHERAL NERVES, DEMONSTRATED BY COLORED AND RADIOACTIVE (ISOTOPE) TRACERS[1]

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Peripheral nerves contain four different kinds of channels in which substances may be transported: the blood vessels, the lymphatics of the sheath, the axons, and the spaces between the axons which we shall call the endoneurial spaces. The vascular supply of nerves has been dealt with in excellent review papers of recent date (Adams, 1942, 1943; Bentley and Schlapp, 1943), and the more intricate problem of intra-axonal and inter-axonal traffic has been very competently discussed in the monograph of Howe and Bodian (1942), according to which toxins and neurotropic viruses seem to spread preferentially inside the axis cylinders, taking the ascending direction (see also Speransky, 1935).

Observations of edema formation in constricted peripheral nerves, on the other hand, intimated that fluid outside and between the nerve fibers moves in the descending direction (Weiss, 1943a; Weiss and Davis, 1943).

The centrifugal direction of this postulated endoneurial seepage was, however, at variance with earlier reports according to which the direction of fluid convection in peripheral nerves is mostly towards the spinal cord (e.g., Teale and Embleton, 1914, 1919; Yuien, 1928). Those earlier claims were based on observations on the spread of various substances, including bacterial suspensions injected into large nerves. However, the excessive amounts of fluid used in the injections (e.g., 0.2–0.5 cc. of fluid into a rabbit sciatic) must have created such unnatural hydrostatic pressure conditions in the nerves that the natural physiological flow might have been fully obscured by this artifact.

In view of the conflicting and inconclusive state of the problem, the series of experiments here described was undertaken. Marker substances used were India ink, Chinese ink, potassium ferrocyanide (Weed, 1917) with subsequent Prussian Blue precipitation, and radioactive tracer substances.

The results obtained on a total of 420 nerves prove that in the limb nerves of rats and guinea pigs, endoneurial transport is directed toward the periphery, and not toward the cord.

All experiments were done with the sciatic nerve or its two major divisions or with brachial nerves of albino rats and guinea pigs. As rat nerves are poorly

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fasciculated, any injection into the trunks leads directly into the endoneurial spaces. All injections in situ were performed under nembutal anesthesia. The nerves were exposed, and all adhering liquid was blotted off.

I. *India ink tests.* Injection of cca. 0.1 cu. mm. of India or Chinese ink suspension into 46 nerves in situ showed in the majority of cases greater spread of ink particles in the distal than in the proximal direction during the first several hours after the operation. Clumping of the granules and their adhesiveness to the tissue, however, led to early clogging of the endoneurial channels, and the method was abandoned as inadequate.

II. *Prussian blue tests. Method.* Convection of colloidal and particulate dyes in nerves has been studied previously (Teale and Embleton, 1919; Yuien, 1928; Uljanov, 1929; Yuien and Sato, 1929; Perdrau, 1937), but since our experiences with ink clumping contraindicated the use of particulate matter, we turned to the methods of Weed (1917) and Wishnewsky (1928) using potassium ferrocyanide in solution followed by Prussian Blue precipitation. $K_4Fe(CN)_6$ is introduced into the nerve, allowed to diffuse for varying periods of time, and then the nerve is placed into a 10 per cent solution of ferric chloride, which precipitates Prussian Blue. Bathing the nerve for two minutes in H_2O_2 before immersing in $FeCl_3$ intensifies the color of the Prussian Blue reaction.

In the first group of experiments the ferrocyanide was injected in solution. Pipettes tapering to 0.2 mm. width at the mouth or minute cotton wicks inserted through a lateral puncture were used to introduce the solution. Approximately 0.1 cu. mm. of the substance was injected by slight pressure. A drop of paraffin oil was used to seal the wound and prevent escape of the injected mass. The injected amount was so small that in no case was there any perceptible disruption of the nerve, except a slight herniation of fibers through the hole in the sheath after withdrawing the pipette.

In order to eliminate all injection pressure, we finally turned to introducing $K_4Fe(CN)_6$ in crystalline form. The sheath was punctured with a glass needle, and a minute flake of the salt was placed lengthwise between the nerve fibers without injury to the latter. The hole was sealed with paraffin oil. The crystal became completely dissolved within 5 to 10 minutes.

All experiments were done with rat hind-limb nerves in situ, either intact or severed, some in dead animals for controls. After from one half to nine hours, the nerves were excised with special care to avoid displacement of the endoneurial liquid.

The injection point was always clearly discernible. After some practice, it was easy to tell where the stained zone ended, and checks by different observers on the same preparations never disagreed by more than one millimeter. Microscopic examination proved the location of the stain to be exclusively endoneurial, i.e., between the nerve fibers; the substance had not penetrated into the axons themselves.

The anatomical uniformity of the nerve stretches used for these tests has been confirmed by diffusion tests in nerves injected several hours after the death of an animal. Table 1 shows the progress of longitudinal diffusion in such nerves:

there is no significant asymmetry between the ascending and descending directions.

A. Fluid injections. In contrast to the results with dead nerves, fluid injected into live nerves in situ assumed in most cases an asymmetrical distribution, extending farther in the distal than in the proximal direction. Cases with fluid injection, no matter how performed, are lumped in the following compilation.

Out of a total of 77 cases, fourteen (i.e., 18 per cent) showed symmetrical spread in both directions; fifty-five (i.e., 72 per cent) greater spread in the distal direction, the excess averaging 4.6 mm.; while only eight (i.e., 10 per cent) showed greater spread in the proximal direction, with an average difference of 2.2 mm. Or, if cases with no more than 1 mm. asymmetry are counted as symmetrical, the figures are: symmetrical, 23 (30 per cent); greater distal spread,

TABLE 1

Diffusion of $K_4Fe(CN)_6$ in nerves of dead animals

NERVE	DURATION OF EXPERIMENT	EXTENT OF DIFFUSION			ASYMMETRY (D − P)
		proximad (P)	distad (D)	total (P + D)	
	hrs.	*mm.*	*mm.*	*mm.*	*mm.*
Tib.	1	3	3	6	0
Per.	1	3	3	6	0
Tib.	1½	3.5	3.5	7	0
Per.	1½	3.5	3.5	7	0
Tib.	5	6.5	6.5	13	0
Per.	5	6.5	6.5	13	0
Tib.	7	8	9	17	1
Per.	7	8	9	17	1
Tib.*	1	9	9	18	0
Tib.*	5	13	13	26	0

* Nerves with implants of crystals instead of injections.

78 (62 per cent), with an average difference of 5.1 mm.; greater proximal spread, 6 (8 per cent) with an average difference of 2.7 mm.

There is thus a decidedly greater spread of substance in the descending than in the ascending direction. This effect appears no matter whether the injection pipette is pointed proximal or distad. It is less marked after injections in perpendicular direction, presumably because this operation involves practically the whole width of the nerve.

Figure 1 gives a graphic summary of the results arranged according to time. For the reason just mentioned, the cases with transversal injection have been excluded. Of the 29 cases with ascending or descending injection, all but two showed a positive distal differential. While the stained area has reached its full extent as early as one half hour after injection, the stain has shifted progressively in the distal direction, as can be seen from the increasing distance between the center of the stained area (white circle), and the injection point (dark circle). This shift is most marked during the first half hour, then declines rapidly, and

seems to have ceased during the second hour. This indicates that the injected substance produces changes in the tissue which gradually obliterate the channels, or otherwise interfere with the mechanism, of convection.

Separate computations for the 23 peroneal and 32 tibial nerves show a greater average distal excess in the former (5.1 mm.) than in the latter (4.1 mm.), a fact which may be correlated with the size difference between these nerves. Even in the India ink experiments it was noted that the distal shift tends to be more marked in smaller nerves.

B. Crystal injections. In 51 experiments, crystals of $K_4Fe(CN)_6$ were introduced into the nerves. This method eliminates the variables of direction and pressure of injection. Accordingly, results were more uniform than in the preceding series. Of the 51 cases, four (8 per cent) showed equal spread in both directions; forty-three (84 per cent) greater spread distad, exceeding the proximal spread by an average of 5.4 mm., while only four (8 per cent) showed proximal excess, averaging 2.3 mm.

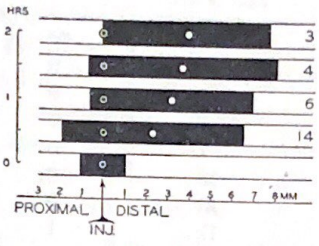

Fig. 1

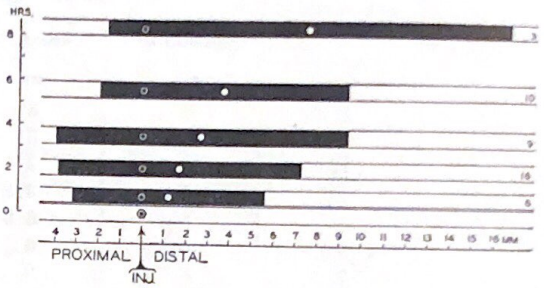

Fig. 2

Fig. 1. Distribution of $K_4Fe(CN)_6$ injected in solution into live nerve in situ. Number of cases in each class given at right margin.

Fig. 2. Distribution of $K_4Fe(CN)_6$ in nerve in situ after introduction in crystalline form. Numbers at right margin indicate number of nerves in each class.

The time course of the distal shift is illustrated in figure 2. The graph was obtained by breaking the 43 positive cases up into 5 groups according to time, as indicated in the figure. Just as in the fluid injection tests, the diffusion field does not continue to expand appreciably after the first or second hour. The shift of the whole field in distal direction, however, may continue for several hours, as evidenced by the progressive increase with time of the distance between the center of the stained zone (white circle) and the injection point (black circle). Unlike the preceding series A, convection had not come to a stop even after six hours. (The disproportionately great shift in the $7\frac{1}{2}$–9 hr. group cannot be considered as typical because of the small number of cases.) Later the stain begins to fade; five cases examined 16 hours after injection showed no more stain left.

This series thus confirms the existence of proximo-distal fluid convection in live nerve, particularly if series A and B, based on a total of 128 cases, of which 98 (i.e., 77 per cent) were positive, are taken in conjunction.

C. Experiments with cut nerves. Nerves were first treated as in the preceding series, but after injection were severed at levels either proximal or distal to the site of injection, or both. The cuts were placed far enough from the point of injection to allow adequate space for diffusion. The results of 53 experiments reveal the following facts. The total length of the diffusion field is of the same order as in the intact nerves of series A and B; it is longer after implantation of a crystal than after fluid injection. The distal asymmetry, however, has failed to develop except in two series, comprising ten cases, in which the injection had been pointed in the distal direction (average distal excess, 3.5 mm.). Evidently, nerves still contain the mechanism of proximo-distal convection even after transection. But the operation of this mechanism was in most cases suppressed by cutting the nerve. The location of the cut did not seem to matter, and a single cut was as effective as complete isolation.

III. *Tests with radioactive tracer substances. Method.* By the use of radioactive isotopes, the diffusion field can be plotted out quantitatively. For practical purposes, the radioactivity of any given segment of the injected nerve is a direct function of the concentration of the injected substance in that segment, provided allowance is made for the natural decay of radioactivity. Our choice of appropriate substances was determined primarily by considerations of experimental expediency. Some of the selected substances were highly toxic, but since only short-term experiments were contemplated, this fact was of no consequence.

Three substances were used: $Na^{24}Cl$ (half-life of 14.8 hrs.), $Cu^{64}Cl_2$ (half-life of 12.8 hrs.), and practically insoluble $Cu^{64}S$ (half-life of 12.8 hrs.).

The salts were deposited in the nerve as solid pellets made by first pulverizing the crystals and then compressing the powder in the finely drawn out tip of a glass pipette (0.15 to 0.3 mm. wide). A glass thread fitting the bore of the pipette served as plunger to force the pellet out, after the mouth of the pipette had been thrust through the sheath and eased into the interior of the nerve. Each pellet was from 1 to 2 mm. long, corresponding to cca. 0.1 to 0.4 mgm. of substance. The direction of injection was alternately up and down, so that for a given lot of nerves any possible asymmetry from this source cancels out. Even without this precaution, errors due to the direction of injection are highly improbable, since the mass is introduced in the solid state. The nerve was superficially dry at the time of injection, and special care was taken to avoid the escape of substance to the surface. The sodium chloride and copper chloride powders dissolved promptly.

The experiments were done either in situ, with or without additional interventions (nerve transection, ligation of blood supply), or in vitro. In the former case, the wound was closed and the animals were allowed to recover from nembutal anesthesia. In the latter case, the nerves were excised and kept in moist chambers at 37°C., stretched out horizontally along silk threads, glass rods, or on paraffin-coated glass plates. In vitro experiments required particular caution to prevent surface contamination by capillary spread between nerve and supports.

After a lapse of from $1\frac{1}{2}$ to 48 hours, the injected nerves were dissected, if in

situ, or removed from the moist chambers, if in vitro. From there on, both groups were treated alike. All nerves were first dried on glass plates, then cut into smaller segments of equal length. The injection site, always clearly discernible, served as landmark. A piece of nerve measuring 5 mm. to either side of this point was excised; it included the original injected zone plus a safe margin. This piece will be designated as "center piece" or "O." Moving from it in both directions, the rest of the nerve was then cut with a clean sharp blade into 5 mm. fragments (10 mm. in the early series), consecutively labelled as P_1, P_2, P_3 . . . and D_1, D_2, D_3 . . . for the proximal and distal direction, respectively. Thus, identical index (e.g., P_2 and D_2) indicates conjugated samples located symmetrically and equidistant from the injection point.

Because of the small sizes involved, several nerves were used for each experiment. After cutting, all fragments from the same levels were lumped in dry depression slides: one slide received all P_1 fragments, another all D_1s, a third the P_2s, etc. Owing to the unequal length of the different nerves, the terminal segments were often fewer in number; allowance for this fact was then made in the calculation of the average radiation per unit.

Blood and tissue samples from treated and untreated control animals, as well as solutions to be tested for radioactive contents, were evaporated in depression slides and then assayed in the dry state.

Radioactivity was measured with a Geiger-Müller counter equipped with an automatic recording device. Each depression slide, containing the lumped samples from the same level of all nerves used in the particular experiment, was exposed separately. Position and distance from the counting tube were rigorously kept constant. The counting period was usually 5 minutes. In order to make corrections for radioactive decay unnecessary, conjugated pairs were always counted in immediate succession. Thus the order of counting was mostly O-P_1-D_1-P_2-D_2-P_3-D_3-P_4-D_4, etc. These nerve segments are strictly comparable only within the stretch over which the size and structure of the nerve remain unchanged. This was true of all but the most remote levels (6 and 7), as D_6 and D_7 lacked a few small peripheral branches leaving the common trunks at D_5. Counts at the extreme levels therefore favor the proximal segments.

The "background" of stray radiation in the room was determined before and after the nerve samples were exposed and at 20 to 30 minute intervals in between. The mean of 147 background recordings over a four months period was 21.95 ± 0.39 quanta/min. ($\sigma = 1.74 \pm 0.28$). This however includes a two weeks' period during which the counter went out of order and registered 2–3 quanta per minute above average. Outside of this period the maximum fluctuation observed during any one day was ± 3 quanta. All values quoted in the tables express actual radiation from the specimens, i.e., total radiation minus the average of background readings taken over the course of the given experiment.

In the following description, experiments in rats and guinea pigs are treated jointly; they are designated by the letters "R" and "GP," respectively. In the graphs, the abscissa represents the axis of the nerve, with the assayed segments

arranged serially in proximo-distal order. The ordinates give the counter readings per minute for each level. These readings are the sum of the background radiation and active radiation from the specimen. Background radiation is recorded in the graphs as a band between the maximum and minimum background readings obtained in the course of the experiment. All values above this band express radiation actually issuing from the sample; they are represented as bars. The varying height of these bars reflects the distribution (concentration) of the substance in the nerve. Differences of concentration between segments located symmetrically with respect to the injection site are represented in the graphs by the black portions of the higher columns.

The following report is based on a total of 35 experiments (23 in situ, 12 in vitro) comprising 193 nerves.

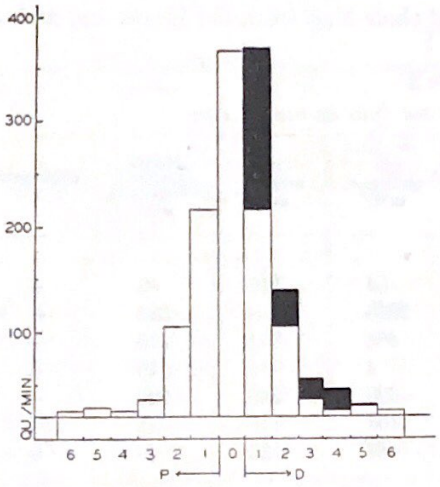

Fig. 3

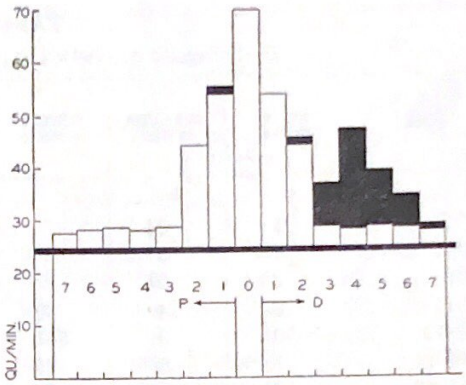

Fig. 4

Fig. 3. Distribution of radioactive $Na^{24}Cl$ in 10 rat nerves (R6-10), 3 hours after injection.

Fig. 4. Distribution of radioactive $Na^{24}Cl$ in 6 rat nerves (R15-17), 18 hours after injection.

A. Injections of $Na^{24}Cl$. This group consists of 7 experiments with 33 nerves in situ, and one experiment in which the nerves had been cut and which will be discussed in a later chapter. The results of two typical experiments are reproduced in the graphs, figures 3 and 4.

Experiment R6-10 (fig. 3), in which the left and right sciatic nerves of five rats had been injected was terminated after 3 hours. At this time the substance had diffused up and down the nerve and assumed the distribution shown in the graph. It can readily be seen that concentrations are consistently higher at the distal levels than at the corresponding proximal levels up to and including level 4 (25 mm. from injection point). More substance has spread in the distal than in the proximal direction.

Experiment R15-17 (fig. 4) in which the right and left sciatic nerves of 3 rats

had been injected—with the pellet in all six cases being deposited proximal to the puncture—was continued for 18 hours. The distribution of radioactive substance in the nerve after this period reveals the following facts:

1. The concentration is still highest at the original injection site, and falls off rather sharply to either side. Since sodium chloride with its high solubility must have long since diffused out from the region, it is obvious that some of the radioactive sodium had become fixed in less soluble form in the tissue, thus preserving some of the shape of the original concentration gradient.

2. There is no significant difference between the proximal and distal fragments at levels 1 and 2.

3. The distal segments 3, 4, 5 and 6 contain radioactive sodium greatly in excess of the amount present at the corresponding proximal levels. The low intensity and relative constancy of the radiation from P_3 through P_7 suggest that these parts of the nerve have obtained their Na^{24} from the blood and not by

TABLE 2

Distribution of $Na^{24}Cl$ injected into nerves in situ

EXP.	NO. OF NERVES	DURATION OF EXP.	CENTER PIECE O	ΣP QUANTA/ MIN.	ΣD QUANTA/ MIN.	DISTAL EXCESS D−P QUANTA/ MIN.	ASYMMETRY (D > P)
		hrs.					
R 1 L.....	1	3½		64	146	82	+
R 2–4.....	4	3		2936	3318	382	+
R 3.....	2	2½		584	854	270	+
R 5.....	2	24	30	4	14	10	+
R 6–10.....	10	3	347	320	546	226	+
R 11–14.....	7	5	75	107	148	41	+
R 15–17.....	6	18	46	87	123	46	+

direct diffusion from the center piece. Distally, this condition is not reached until D_7. Only the zone from P_2 to D_6 contains concentrations that can be ascribed to direct diffusion. As the center of this area lies in D_2, it is evident that the whole diffusion field has shifted distad by at least as much as the distance between levels O and D_2, i.e., 10 mm. This value does not express the true rate of the shift, because, as stated in point (1), after 18 hours we are no longer plotting the original diffusion field but only its fixed residue.

The remaining five experiments gave essentially similar results. They are summarized in table 2. This summary treatment ignores the distribution gradients and merely compares the total amount of radioactive substance found distally to the injection point with that contained in the corresponding proximal stretch of nerve. Radiation intensities varied greatly from experiment to experiment, owing to differences in the initial potency and the degree of decay of the different radioactive preparations at the times they were used. The very low count of R5 is explained by the long duration of the experiment (24 hrs.), allowing for the washing out and excretion of the injected substance. Otherwise the results are uniform. All tests show a distinct excess of substance in the distal portions, rising to as much as several hundred quanta per minute.

B. Injections of $Cu^{64}S$. Four experiments with 19 nerves were carried out in this series. In line with the low solubility of the salt, no radiation was detected in any nerve segments except the ones containing the original injected mass. Only minute traces could be detected in the blood of these animals. Radiation intensities in the center pieces of the four groups were 19232, 9644, 1583 and 1726 quanta per minute after 6, 25, 42 and 48 hours, respectively. The contrast between these high values and the absence of activity in even the next adjacent segments not only proves the complete lack of diffusion in these cases, but provides a crucial test for the general reliability of the technique, since any traces of contamination due to handling would have produced positive counts.

C. Injections of $Cu^{64}Cl_2$. This substance, which owing to its bluish tint could easily be recognized after its deposition in the nerve, did not dwindle as rapidly

TABLE 3

Differential distribution of radioactivated salt in nerve segments nearest to injection site

EXPERIMENT	NO. OF NERVES	DURATION OF EXP.	INJECTED PIECE (0) QUANTA/ MIN.	P_1	D_1	DISTAL DIFFERENTIAL		ASYMMETRY D>P...+ D=P...= D<P...−
						D_1-P_1 quanta/ min.	Percentage	
		hrs.					*per cent*	
GP 1– 2...	3	4	2040	221	289	68	31	+
GP 6– 7...	4	5	6144	689	829	140	20	+
GP 12–13...	4	6	7099	829	708	−121	17	−
GP 4– 5...	4	18	544	17	25	8	insign.	=
R 32–40.....	10	4	5760	1464	458	−1006	220	−
R 41–43.....	4	16	3908	107	109	2	insign.	=
Total.....	29		25495	3327	2418	−909	38%	−

as did $Na^{24}Cl$. Consequently, it was still visible at the end of an experiment as a distinct blue spot. Since the radiation intensities of the center piece and the adjacent P_1 and D_1 segments were too high to be shown on the same scale with the much weaker radiations of the more distant levels 2, 3, 4, etc, they were omitted from the graphs and are given in tabular form later (table 3).

There were 6 experiments comprising 29 nerves in which $Cu^{64}Cl_2$ was injected into nerves in situ with no further interference. Two sample cases are shown in the graphs (figs. 5 and 6), both of 4 hours' duration, the first with 3 nerves in guinea pigs, the second with 10 nerves in rats. The longer nerves of guinea pigs yield more 5 mm. segments for testing, and being more fasciculated than rat nerves, they offer interfascicular spaces for liquid transfer. Yet, there were no essential differences between the two groups. Both show a spectacular excess of radioactivity in the distal segments over the corresponding proximal ones. In experiment GP 1–2 (fig. 5), the presence of directly diffused substance (i.e., not carried through the blood) is demonstrable between levels P_5 and D_{10}. It presumably extended beyond D_{10}, where nerve samples were not assayable for

anatomical reasons. The geometric center of the radioactive area, accordingly, lies between D_2 and D_3, which corresponds to a total shift of the diffusion field down the nerve of cca. 15 mm. in 4 hours. In experiment R32-40 (fig. 6), the distal spread has likewise gone beyond the limits of the nerve stretch dissected for assaying, but by extrapolation from the graph it may be estimated to have extended into D_9, which would bring the approximate center to D_2, corresponding to a distal shift of cca. 10 mm. in 4 hours.

Of the remaining 4 experiments, done with 16 nerves and ranging from 5 to 18 hours, the 18-hour one (G.P. 4–5) gave very low counts (compare table 3) with a correspondingly small, though definite, distal overbalance ($\Sigma D = 70$; $\Sigma P = 46$). Evidently, most of the substance had disappeared by this time. The results of the other three experiments (GP 6–7, 5 hr.; GP 12–13, 6 hr.;

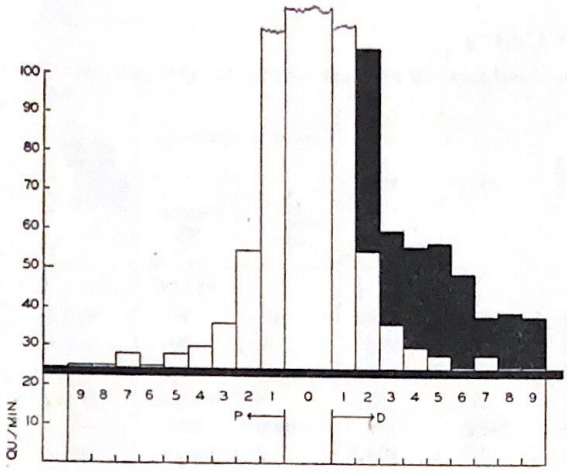

Fig. 5

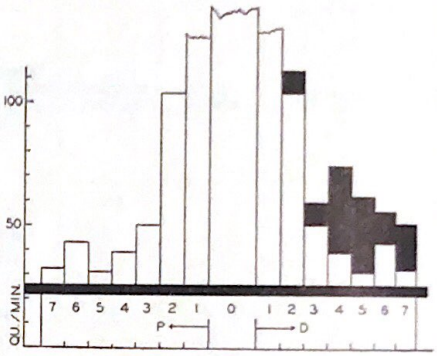

Fig. 6

Fig. 5. Distribution of radioactive $Cu^{64}Cl_2$ in 3 guinea pig nerves (GP 1–2), 4 hours after injection.

Fig. 6. Distribution of radioactive $Cu^{64}Cl_2$ in 10 rat nerves (R32-40), 4 hours after injection.

R 41–43, 16 hr.), all essentially alike, have been lumped into a single graph, figure 7. The marked distal shift of the diffusion field for the whole lot is evident.

In contrast to these fully consistent results with segments from levels 2 and beyond, the counts from segments P_1 and D_1 were erratic. They are given in table 3. There was no significant difference between P_1 and D_1 in some cases; others showed a positive distal, and still others a positive proximal differential. A discussion of this fact will be given later.

D. Injections combined with other interventions. The following experiments constitute an attempt to elucidate the mechanism of the proximo-distal convection effect.

Stoppage of blood circulation. On a previous occasion (Weiss, 1943a), the possibility was discussed that endoneurial fluid might be propelled by the

arterial pulse. In order to test this hypothesis, the right and left sciatic nerves of two guinea pigs were injected with $Cu^{64}Cl_2$ as usual, and then circulation was stopped by ligating the aortae near the heart. The nerves were left in situ for 5 hours and then dissected and assayed. The results are shown in figure 8. Evidently, a shift of the diffusion field distad had occurred. This result disproves the assumption that the pulse wave, or any other circulatory factor, furnishes the driving force for the endoneurial shift of fluid. It also shows that death of the animal does not immediately stop the shift. According to table 1, however, the shift no longer occurs a few hours after death.

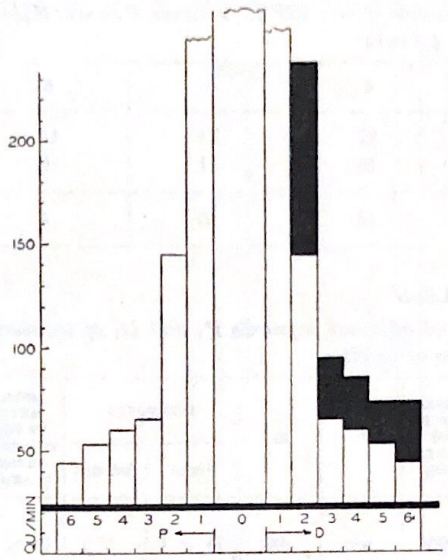

Fig. 7

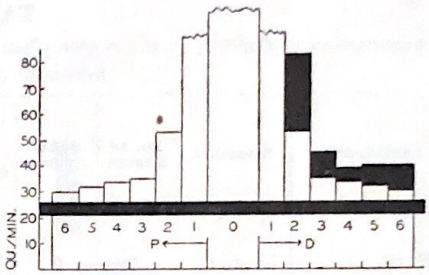

Fig. 8

Fig. 7. Distribution of radioactive $Cu^{64}Cl_2$ in 12 nerves, 6–16 hours after injection.

Fig. 8. Distribution of radioactive $Cu^{64}Cl_2$ in 4 nerves of guinea pigs with arrested circulation, 5 hours after injection.

Injection of transected nerves. In this series, 16 sciatic nerves in 4 groups were first transected far proximally and distally and then injected about half way between the cuts with $Cu^{64}Cl_2$, but otherwise left undisturbed. Assays after 4 to 6 hours gave the following results. One animal (GP 11) had died shortly after the injection and 6 hours later showed no significant asymmetry of the diffusion field within levels 2, 3, 4 (transections in P_5 and D_5). Two series (8 nerves) showed a definite distal shift (table 4) and one series (6 nerves) showed a distal surplus at levels 3 and 4, but a distal deficit at level 2. (For counts of segments P_1 and D_1, see table 5.) The results prove that a distal shift may still occur after the continuity between the injected stretch and the rest of the nerve has been interrupted. However, the absence of the effect in one case and its partial reversal in another are perhaps significant in view of a similar variability noted in transected nerves tested with Prussian Blue (series II C), as well as in the completely excised nerves described in the following.

Injection of excised nerves in vitro. This series includes 6 experiments with 52 nerves, injected with $Cu^{64}Cl_2$ in vitro and then incubated in moist chambers for from $1\frac{1}{2}$ to 5 hours. The results were not consistent. In the shortest experiment (4 rat nerves; $1\frac{1}{2}$–2 hr.), there was no significant asymmetry (R27–30; table 5), conceivably because of insufficient time. Another experiment (R1–10; 10 nerves, 4 hr.) showed a proximal surplus from P_1 through P_4, but in this set the nerves had been hung across horizontal threads in chain-like fashion and the alternation between sagging and supported parts may have affected diffusion.

TABLE 4

Distribution of $Cu^{64}Cl_2$ injected into transected nerves in situ (GP 10, 2 nerves, 6 hours; R44–46, 6 nerves, 4 hours)

LEVEL	2	3	4	5	6
D	488	45	27	21	14
P	58	24	14	11	10
D − P	430	21	13	10	4

TABLE 5

Concentration of $Cu^{64}Cl_2$ in the center piece and adjacent segments P_1 and D_1 of transected nerves in situ or in vitro

EXPERIMENT	CONDITION	NO. OF NERVES	DURATION	CENTER PIECE (O) QUANTA/MIN.	P_1	D_1	ASYMMETRY		ASYMMETRY IN REST OF NERVE BEYOND P_1 AND D_1
							Sense	Amount	
			hrs.						
GP 10	in situ	2	6	9963	808	236	D < P	572	D > P
R 37–39	in situ	6	$4\frac{1}{2}$	2443	605	530	D < P	75	D > P
R 44–46	in situ	6	4	21484	2302	1998	D < P	304	D > P
GP 15–26	in vitro	12	$3\frac{1}{2}$	56449	2015	1585	D < P	430	D > P
R 1–10	in vitro	10	4	14812	615	111	D < P	504	D < P
R 11–18	in vitro	8	4	90604	9577	2892	D < P	6685	D > P
R 19–26	in vitro	8	$4\frac{1}{2}$	20012	182	257	D > P	75	D > P
R 27–30	in vitro	4	$1\frac{1}{2}$	5432	11	13	D = P	2	D = P
R 55–64	in vitro	10	5	17045	4942	1721	D < P	3221	D < P

A third experiment (R55–64; 10 nerves, 5 hr.) showed a marked proximal surplus in P_2 and P_3, and a very slight distal surplus in D_4 and D_5. The remaining three experiments (12 guinea-pig nerves, $3\frac{1}{2}$ hr.; 16 rat nerves; 4–$4\frac{1}{2}$ hr.) produced a very pronounced distal shift (fig. 9). In one of these experiments (R11–18; table 5), a very potent salt preparation was used, and this accounts for the unusually high values in this group.

Taking these results in conjunction with those of the preceding section and those of section II C, two facts emerge. First, proximo-distal convection is still demonstrable in completely isolated nerve fragments, and second, this shift may in certain cases be abolished or even wholly or partly reversed as a

result of transection. Whether the transected nerves are left in situ or are transferred into moist chambers of body temperature, seems to have no influence on the results.

The counts at levels P_1 and D_1 adjoining the center piece are given in table 5. In 7 out of 9 experiments with transected nerves in situ and in vitro, the concentration of the diffused substance was lower in D_1 than in P_1. A careful check of our procedures has convinced us that this paradoxical relation is to be ascribed to a slight, but systematical, error in the cutting of the nerve samples under the binocular microscope. The coincidence between the injection point and the null point of the measuring scale was established by binocular vision, while the

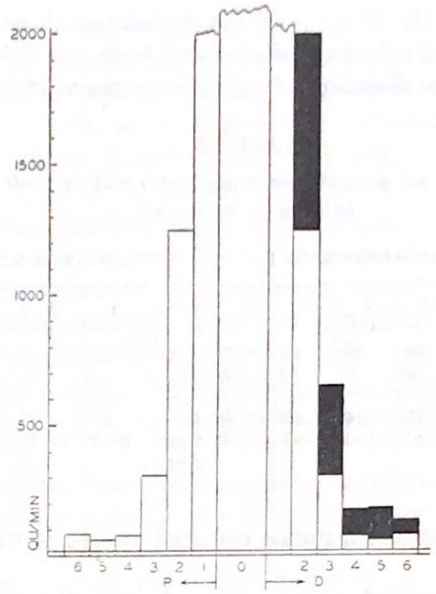

Fig. 9. Distribution of radioactive $Cu^{64}Cl_2$ in 28 excised nerves, $3\frac{1}{2}$–$4\frac{1}{2}$ hours after injection.

5 mm. marks were viewed with the right eye only. This produced a parallactic shift of 0.5 mm. to the right from the injection point, enough to give the observed asymmetry in the center piece, where the concentration gradient is very steep. This error is negligible for the more distant segments with gentler concentration slopes.

Nerve ends in pools. This series was designed to test possible polarity in the leaking of substance from cut nerve ends and in the diffusion into a nerve through its cut surface. The experiments could not be carried beyond the exploratory state and deserve repetition on a larger scale.

Three experiments with 14 nerves were set up to examine polarity of leakage. The nerves were excised and injected with $Cu^{64}Cl_2$. They were then propped up in their middle portions, with the ends dipping into separate pools of Ringer's solution in depression slides. The arrangement was as nearly symmetrical as

Origin, composition and destination of the "endoneurial fluid" (Weiss, 1943a) are still unknown. It may be in communication with the subdural cerebrospinal fluid and receive contributions from blood capillaries, nerve fibers and epineurial lymphatics.

The normal rate of "endoneurial flow" cannot be safely calculated from our measurements, because it is perceptibly altered by the very indicators used to measure it. For instance, it is slowed by ferrocyanide injection (figs. 1 and 2), presumably due to an astringent effect on the endoneurial connective tissue. The isotope markers, on the other hand, tended to become bound to the tissue in less soluble compounds, thereby losing mobility. Even one day after an injection, the isotope, introduced in very soluble compounds, was still found near the injection site in relatively high concentrations. A general estimate of the rate of flow can, nevertheless, be obtained from experiments of short duration. The total shift of the diffusion field distad is given by the distance between the original injection point and the center of distribution of the marker substance at the end of the experiment. This shift, calculated from the graphs, figures 5 and 6, amounts to 10–15 mm. in 4 hours, or an average of cca. 3 mm. per hour. The same value has been found during the first hour after ferrocyanide injection (fig. 1), while after the implantation of ferrocyanide crystals, the rate was slightly less (cca. 1 mm. per hr.). These values, obtained by very different methods of injection and assaying and with different chemicals, are sufficiently in agreement to permit us to set the rate of endoneurial flow in the nerves studied as of the order of from one to a few millimeters per hour (one to several inches per day).

The mechanism of propagation and the motive force of this endoneurial flow are obscure. In an earlier paper (Weiss, 1943a), the hypothesis had been considered that the pulse wave might serve as motor. However, the observation that the flow may continue after stoppage of circulation, as well as in completely excised nerves, disposes of that hypothesis. The latter cases, at the same time, prove that the mechanism must lie and operate right in the nerve itself. In excised nerves, the distal convection effect was much less regular, and if present, usually weaker. This suggests that some uncontrollable by-product of transection may interfere with the mechanism of propagation; e.g., occlusion of the nerve interior by closure of the epineurium over the wound; superficial drying of the wound; presence or absence of blood clots; tufting of cut nerve fibers.

Pending further research, the problem remains a matter for speculation. It seems that a simple polarized convection of fluid in such a system as the interstices of nerve at the observed rate can be explained either by electrical

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neither the neurilem, nor the nervous substance, nor the vessels, but a sheath belonging to each fiber bundle ("filet"). I (i.e., Ranvier) have demonstrated since (Recherches sur l'anatomic et la physiologie des nerfs, Arch. de physiol., 1872, p. 439) that the injected mass which penetrates into a nerve fascicle spreads between the different constituent tubes and may extend longitudinally between them to great lengths without reaching the enveloping perineurium, and consequently the injection of nerve filaments does not imply the existence of a circumscript canal in their interior" (pp. 770–771).

polarity or by mechanical propulsion. There is no crucial evidence in our experiments either for or against the assumption that the transport may be of electrophoretic nature (Yuien and Sato, 1929). One would have to postulate a steady and rather high electrostatic potential along the axis of the nerve as motive force. However, information on this latter point is scanty. Mendelssohn (1885) has described a longitudinal potential gradient in resting nerve. But we are unaware of any confirmatory evidence that would have been published since. Burr, in a private communication, informs us that exploratory tests on two uninjured sciatic nerves of guinea pigs in situ showed potential differences between two levels 8 mm. apart of the order of a few millivolts, with the distal level positive to the more proximal one. The "demarcation potential" after transection could be shown to be composed of the injury potential and the basic longitudinal potential of the intact nerve. Suggestive as these observations are, they are evidently not yet sufficient to support an electric theory of endoneurial flow.

Mechanical propulsion in narrow spaces may result from ciliary activity, which is definitely absent in nerve, or from peristaltic contractions. A slight peristalsis in nerve fibers, consisting of a rhythmic local dilatation and contraction wave propagated centrifugally, could readily produce the observed flow. In fact, the model suggested previously (Weiss, 1943a) for the collateral propulsion of endoneurial fluid by the pulse wave, is pertinent to any sort of peristaltic wave. It illustrates how a succession of such waves in closely packed tubular spaces would maintain a steady flow of liquid in the interstices, provided the whole system is contained in a rather rigid sheath as is the case with nerve.

The notion of peristalsis in mature nerve fibers deserves serious consideration. Some recent discoveries on the growth and behavior of constricted nerve fibers may have an intimate bearing on the case, as follows. Extensive observations on the damming, ballooning, telescoping and coiling of nerve fibers proximal to a constriction, briefly mentioned in previous publications (Weiss, 1943b, 1944a, 1944b), but not yet reported in full, have led to the conclusion that axonal substance is constantly moving at a very slow rate in centrifugal direction, not only in the growing phase, but in the stationary condition of the functional mature fiber. It may be difficult to explain this phenomenon otherwise than by some sort of peristalsis. A semblance of peristalsis can be discerned in some of Speidel's motion pictures of living axon sprouts. However, it has never been seen or suspected in the mature nerve fiber. If it should prove to be a fact, it could account for the endoneurial flow, too.

How the endoneurial fluid is disposed of peripherally, remains to be determined. Its biological significance is likewise still a matter of conjecture. Since it bathes all nerve fibers, its composition and physico-chemical properties are evidently of prime importance to the normal maintenance and functioning of nerve. Its possible rôle in nerve regeneration has been mentioned on previous occasions (Weiss, 1943a, 1943b).

The direct demonstration of centrifugal endoneurial flow in this paper confirms the interpretation of edema in constricted nerves advanced on an earlier occasion

(Weiss, 1943a; Weiss and Davis, 1943). It can readily be seen that any narrowing of the endoneurial channels must lead to accumulation of fluid between the nerve fibers at the proximal side of the constriction with resulting distention of the nerve. A similar edema often arises at the blind end of an unconnected central nerve stump, where further fluid transport is blocked by the connective tissue cap forming over the wound. Since such terminal edemas commonly form the basis for the development of large bulbous neuromas (Weiss, 1943a), it would seem indicated in amputations to insure conditions that will least interfere with the continued drainage of the nerve fluid into the surrounding tissues.

Denny-Brown and Brenner (1944) have contended that "edema" in constricted nerves is due to ischemia and may be present on both sides of a constriction. The latter statement is meaningless in view of the fact that the authors use the term "edema" for both the collection of interstitial fluid between nerve fibers and for the pathological swelling of nerve fibers themselves, which is a wholly different process. To avoid further confusion, the reader may be referred to microphotographs of typical cross sections of nerves proximal and distal to a constriction, reproduced in figure 4 of Weiss and Davis (1943) and figure 5 of Weiss (1943a), showing the strict confinement of interstitial edema to the proximal side. The notion that constriction edema in nerve is of vascular origin, has been advocated by Denny-Brown and Brenner as a matter of opinion, without experimental verification. It can hardly be accepted in the face of the experimental facts cited by Weiss (1943a) as disproving it. The alternative interpretation of nerve edema as dammed up endoneurial fluid was correctly labelled by Denny-Brown and Brenner as a "hypothesis." The facts reported in the present paper, however, remove it from that category.

Our demonstration of descending convection in the endoneurium seems to be at variance with earlier claims (see the introductory pages) according to which substance injected into nerve trunks spreads toward the cord. However, the two sets of data are hardly comparable. While we have used hundreds of animals, most earlier authors based their conclusions on a few, and not always consistent, cases. Where we used minimal amounts of substance (cca. 0.1 cu. mm.), exercised meticulous care to avoid major nerve disruption, controlled or wholly eliminated pressure and directiveness in the act of injection, and assayed the spread of the test substances quantitatively, they injected several thousand times as much, thereby causing profound disturbances of the nerve structure, took no adequate precautions against pressure artifacts, and judged their results by much cruder criteria. In those earlier experiments, so much violence was done to the nerve that the delicate phenomenon of endoneurial flow could not possibly have manifested itself.

While our experiments contain no indications that fluid traffic between fascicles and inside fascicles would occur in opposite directions, it should be borne in mind that the choice of small and poorly fasciculated nerves for our experiments means that our conclusions are strictly applicable to the endoneurial, i.e., intrafascicular transport only.

SUMMARY

1. The hypothesis of a steady proximo-distal movement of fluid in the endoneurial spaces of peripheral nerves, first suggested by observations on nerve edema (Weiss, 1943a), was subjected to an experimental test, comprising a total of 420 limb nerves of rats and guinea pigs.

2. To test fluid transport in nerve, marker substances were deposited between the nerve fibers with the least possible damage to the continuity and structure of the nerve. In contrast to earlier experiments of this kind, only minute amounts of substance were used (*ca.* 0.1 cu. mm.) and care was taken to eliminate all possible artifacts due to the pressure and orientation of the injection. Test substances were introduced either in solution or in solid form. After periods varying from less than one hour to a few days, the distances to which the substance had spread up and down the nerve were determined. With the use of radioactive tracers, the actual shape of the concentration gradients could be determined. These assays gave the following results.

3. Diffusion in nerves injected several hours after death proceeds symmetrically, that is, for equal distances up and down from the injection point.

4. In live nerves, no matter what method is used, an excess of substance is found distal to the site of injection, indicating a progressive transport of the injected substance in the distal direction, with endoneurial fluid serving as vehicle.

5. India or Chinese ink injections (46 expts.) showed the distal shift but were not fully conclusive because of technical difficulties.

6. Injection of potassium ferrocyanide which was later precipitated to Prussian Blue by ferric chloride (162 cases) proved, on the whole, the presence of a proximo-distal shift in intact nerves at a rate of from 1 to 3 mm. in the first hour, gradually slowing down, presumably because of an astringent action of the test substance. In transected nerves, the convection effect is more or less disturbed.

7. Injection of radioactive isotopes ($Na^{24}Cl$, $Cu^{64}Cl_2$) made it possible to follow the distribution of the injected material quantitatively with the aid of a Geiger-Müller counter. The results obtained with 193 nerves thus studied have proved conclusively the gradual shift of the diffusion gradient in the distal direction, indicating a proximo-distal flow of the endoneurial fluid at the rate of a few millimeters per hour. This flow may continue after the stoppage of circulation and even in completely excised nerves, although nerve transection often introduces uncontrollable disturbances.

8. The origin and fate of the endoneurial fluid and the mode and motive force of its displacement down the nerve are unknown. An electrical and a mechanical concept (peristalsis of nerve fibers), both wholly tentative, are briefly discussed in the text.

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