THE FATE OF AN INTRANEURAL INJECTION AS DEMONSTRATED
BY THE USE OF RADIO-ACTIVE PHOSPHORUS

BY

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The history of the experimental investigation of such diseases as tetanus, rabies, and anterior polio-
myelitis has so frequently involved the intraneural injection of the specific infective agent that some
assessment of the potential spread of a small volume of indifferent material is long overdue.

The object of the present investigation was to determine the extent to which the intraneural
injection of a crystalloid substance not exceeding 0.05 ml. in volume may enter the blood stream,
the cerebrospinal fluid, and various regions of the nervous system. The choice of a radio-active
tracer substance for this purpose was made not only because its detection is possible in very high
dilution but because the curves of its concentration in both blood and cerebrospinal fluid can be plotted
 throughout the period of the experiment.

The chief consideration determining the selection of P 32 (as phosphoric acid) for this work was the
fact that, provided the blood level does not exceed 750 to 800 counts/min./100 mg. (in the rabbit)
there is no appreciable concentration of the tracer element in the spinal fluid (Table I). Further,
within a survival period of two to two and a half hours there is no significant combination of the
phosphorus with the lipoids and proteins of the nervous system as proved by the virtual absence of
activity in the tissue residue after extraction with trichloracetic acid.

0.05 ml. was found to be the least volume which contained sufficient radio-active element to produce
blood concentrations capable of estimation with no more than a probable error of 5 per cent. in a
counting time of fifteen to twenty minutes per sample, a limitation imposed by the large number of
samples taken in each experiment.

The injections were carried out at three different
levels in the left sciatic nerve of the rabbit: (1) at
the mid-femoral level (six experiments); (2) at the
upper end of the popliteal fossa (four experiments);
(3) at the level of the greater trochanter (six experi-
ments).

A series of four control animals were given
intravenous injections of 50 to 60 μc of P 32 and the
curves of concentration in blood and cerebrospinal
fluid plotted for a survival time of two hours. At
the end of this period the activities of standard
segments of the spinal cord and sciatic nerves were
estimated as a basis for comparison with corre-
sponding parts from the experimental animals
(Table I).

Technique of Injection

Under nembutal anaesthesia the left sciatic nerve was
exposed and complete hemostasis secured before
commencing the injection. The syringe used for
injection was an all-glass tuberculin syringe into the
nozzle of which the shaft of a No. 26 hypodermic needle
was cemented. In this way "dead space" in the
 syringe was eliminated and a volume of 0.05 ml. could
be delivered into a nerve without an accompanying air
bubble. After the required volume of the indicator had
been drawn up the needle tip was carefully dried.

The nerve was steadied by gripping its supporting
connective tissue web just distal to the point selected for
injection. The needle was then inserted slowly and
always in a central direction at an angle of about 30°
to the surface, until its point was thought to be well
within the nerve substance. The injection itself took
up to two minutes, depending on the degree of resistance
encountered, while a small disc of filter paper applied
to the point of entrance of the needle prevented any
leak back along the needle track. At the conclusion of
the injection the needle was left undisturbed for some
thirty seconds while a fresh disc of filter paper was laid
on the nerve at the entry point. The needle was now
slowly withdrawn and two further paper discs successively
applied, to be followed by a third which was left in situ
and the wound closed over it.

It must be admitted that these precautions, while
adequate to prevent any important modification of the
subsequent blood concentration curves, were not so
thorough as those of Abel and others (1935b) using tetanus
toxin. Nevertheless the injections described above are far
less likely to lead to leakage than the simpler procedures
employed by the majority of workers with toxins and
viruses. They have the further merit that there is
minimal disturbance of the vascular connexions of the
nerve.

The whole injection procedure was carried out under
the binocular dissecting microscope and the features of
each injection were recorded.
TABLE I
CONTROL EXPERIMENTS: SEGMENTS 1 TO 11 AS SHOWN IN FIG. 2

<table>
<thead>
<tr>
<th>No.</th>
<th>Wt. Kg.</th>
<th>P32 µc</th>
<th>Max. blood level</th>
<th>Max. C.S.F. level</th>
<th>Right sciatic nerve</th>
<th>Left sciatic nerve</th>
<th>Spinal cord</th>
<th>Survival (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.6</td>
<td>60</td>
<td>551</td>
<td>10-9</td>
<td>45:1 24:7 30:4</td>
<td>45:4 40:7 36:0 37:8 42:4</td>
<td>9 10 11</td>
<td>129</td>
</tr>
<tr>
<td>3</td>
<td>2.0</td>
<td>50</td>
<td>643</td>
<td>contam.</td>
<td>45:1 46:1 58:9</td>
<td>42:6 41:6 53:5 61:2 39:0</td>
<td>74:2 30:8</td>
<td>—</td>
</tr>
<tr>
<td>4</td>
<td>1.3</td>
<td>59</td>
<td>1042</td>
<td>Bkg.</td>
<td>43:4 47:9 38:3</td>
<td>26:6 46:3 43:8 31:3 28:3</td>
<td>56:2 18:1 10:5</td>
<td>150</td>
</tr>
</tbody>
</table>

Bkg. = Count virtually background.
contam. = C.S.F. specimen contaminated.

TABLE II
LOW SCIATIC INJECTIONS: LEVEL OF POPLITEAL FOSSA

<table>
<thead>
<tr>
<th>No.</th>
<th>Wt. Kg.</th>
<th>P32 µc</th>
<th>Max. blood level</th>
<th>Max. C.S.F. level</th>
<th>Right sciatic nerve</th>
<th>Left sciatic nerve</th>
<th>Cord</th>
<th>Type of injection</th>
<th>Max. spread</th>
<th>Survival (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R.P. 10</td>
<td>2:0</td>
<td>55</td>
<td>162-0</td>
<td>21:2</td>
<td>66:9 125:5 52:7</td>
<td>85:0 130:0 65:0 60:0</td>
<td>40:5 26:6</td>
<td>High R. Mod. D</td>
<td>2/3rds of nerve</td>
<td>162</td>
</tr>
<tr>
<td>R.P. 31*</td>
<td>2:1</td>
<td>74</td>
<td>159:0</td>
<td>27:5</td>
<td>91:7 159:2 113:2 62:9</td>
<td>85:0 90:0 65:0 50:0 25:0</td>
<td>Min. R. Slight D</td>
<td>Segment 10</td>
<td>122</td>
<td></td>
</tr>
</tbody>
</table>

R = Resistance to injection.
Min. R = Minimal resistance.
Mod. R = Moderate resistance.
D = Dilatation of nerve at injection site.
Mod. D = Moderate dilatation.
∞ = Corrected count in excess of 15,000 counts/min./10 mg.
C = Column of fluid seen to ascend nerve.

* Note R.P. 31. This injection had spread along the nerve to enter segment 9, without however causing any appreciable activity in the cerebrospinal fluid. In view of this and of the high blood level, this injection must be regarded as transitional between the two types.

COUNTS/MIN/100 MG/M.

R.P. 12. 'LOW SCIATIC' INJECTION (60 µc) OF 'BLOOD TYPE'

Fig. 1.—Blood and cerebrospinal fluid curves in a typical "blood" type injection. The blood activity reaches a peak of 122 counts/min./100 mg. after five minutes, while the maximum cerebrospinal fluid activity is 9 counts/min./100 mg.
Collection and Estimation of Samples

Blood and Cerebrospinal Fluid.—Following the completion of the injection, samples of blood were taken from an ear vein, the first at one and a half to two minutes and subsequently at intervals of ten to fifteen minutes. Some four to seven specimens of cerebrospinal fluid (each about 0.1 ml.) were obtained at intervals during the experiment by cisterhal puncture. The radio-active content of the samples which were absorbed on filter paper discs by the method of Reiss and others (1949) was estimated in a G.M. 4 “dry” counter with a Dynatron scaler.

![Diagram of the sciatic nerves and spinal cord divided into the standard segments](image)

**Fig. 2.**—Diagram of the sciatic nerves and spinal cord divided into the standard segments, to illustrate a typical “blood” type injection with spread of the indicator confined to the two distal segments of the left sciatic nerve. The values of the spinal cord segments do not exceed those in the control experiments. Note the raised level of activity (78:0) in segment 2—the lowest contralateral effect in the series.

Sciatic Nerve and Spinal Cord.—After death the two sciatic nerves were fully exposed from popliteal fossa to sacrum and each was marked off into four equal segments with a pen compass dipped in red ink. Each segment was removed with minimal connective tissue, weighed on a torsion balance, and transferred to a clean homogenizing tube. The instruments used for the removal of the tissue samples were thoroughly cleansed after each removal. 2 to 3 ml. of 25 per cent. trichloroacetic acid were added to each tube and the contents homogenized in accordance with the technique of Reiss and others (1949) for the determination of acid soluble phosphorus in the brain. The supernatant fluid obtained by centrifugation at 3,000 r.p.m. for three minutes was poured into an M.R.C. 1 “liquid” counter for estimation of radio-activity by the Dynatron counter as above.

Certain segments of the spinal cord were taken regularly from each member of the series and, like those of the sciatic nerve, numbered according to a standard plan (Fig. 2).

The spinal cord segments were dried with filter paper in order to remove cerebrospinal fluid before weighing. To standardize as far as possible the “personal equation,” each worker performed the same duties throughout the series of experiments.

Calculation of Statistical Error.—Blood and cerebrospinal fluid samples were counted for not less than ten minutes unless highly active. Low activity fluids from cord or nerve samples were counted for not less than twenty-five minutes, while those of high activity were diluted sufficiently to render “paralysis time” corrections unnecessary. The background count of both “dry” and “liquid” counters was estimated for at least thirty minutes before the commencement of counting and for periods of one to two minutes between each sample.

From the count of a particular sample and the background count, the probable error on the corrected count (expressed as counts/min./100 mg.) was calculated by the method of Wilson Wright (1947).

Observations

1. **LOW SCIATIC INJECTIONS (TABLE II)**

Four injections were carried out at this level. Three of these had certain features in common and will be considered first. In each the resistance to be overcome increased as the injection proceeded, resulting in a variable degree of dilatation, seldom uniform but appearing as a localized bulging or an area of blistering confined to one section of the nerve circumference. In no case was a column of fluid seen to shoot up the nerve. In these three experiments the concentration of P 32 in the blood stream rose rapidly to between 112 and 162 counts/min./100 mg. (Fig. 1). In two cases the maximal blood activity was attained after twenty to twenty-five minutes, while in the third (R.P. 11) a particularly rapid absorption from the injection site produced the highest blood level a minute and a half after the injection. The cerebrospinal fluid in one case (R.P. 11) showed no more activity than was accountable for by the blood level, while in the other two (R.P. 10 and R.P. 31) the slightly higher
levels of 21.2 and 27.5 counts/min./100 mg. were found.

Assay of the sciatic nerves and spinal cord (Fig. 2) showed that there had been no direct spread of the indicator beyond the limits of the nerve itself (except R.P. 31: see Table II), the concentrations in the spinal cord being attributable to the P 32 level in the general circulation (Table II). An injection of this character resulting in a localized accumulation at the injection site or at most within the extent of the nerve and a rapid passage into the blood stream without direct extension to the spinal cord or cerebrospinal fluid may be termed a "blood type" of injection.

The nature and outcome of the fourth injection (R.P. 30) at this level were in marked contrast to the preceding three. Thus there was no appreciable resistance, an absence of dilatation of the nerve, and in addition a thin column of fluid was seen to pass up the nerve with great rapidity at the instant of applying minimal pressure to the syringe plunger. It was recorded that the resistance experienced in this particular injection was the least of the whole series. The maximal blood level attained at twenty-four minutes was only 66.2 counts/min./100 mg. while the cerebrospinal fluid exhibited an activity of 1,485 counts/min./100 mg. at the termination of the experiment.

Estimation of activity in the spinal cord samples showed that a remarkable spread of the tracer substance had occurred (Fig. 3), so that blocks of tissue taken to include the thalamus and corpus striatum gave counts considerably above their "control" level. Further the segments of upper cervical cord and upper medulla were divided in the median plane into right and left halves, and there was some indication that the injected material had ascended more extensively on the ipsilateral side.

In this one case, then, an injection associated with low resistance and the absence of dilatation of the nerve gave rise to a low level of blood activity, a highly active cerebrospinal fluid, and the spread of the tracer substance along the whole length of the spinal cord and brain stem. An injection of this sort may be classed as of "cord-cerebrospinal fluid type" (abbreviation, C.S.F. type).

2. Mid-femoral injections (Table III)

Six injections were carried out at the mid-femoral level. Of these, four were found to be of the "blood type" as defined above and two of "cord-cerebrospinal fluid type." In the former group, however, there was a certain spread of phosphorus within the nerve, so that in three cases (R.P. 8, 9, and 32) all nerve segments gave high counts. Spread into the nerve below the injection site took place in all four animals even though the needle had been directed proximally. The maximal blood levels lay between 73 and 274 counts/min./100 mg., while the maximum cerebrospinal fluid activity was 26.8 counts/min./100 mg.

In the two "cord-cerebrospinal fluid type" injections (Fig. 4), while the blood level did not rise beyond 40 and 81 counts/min./100 mg., the cerebrospinal fluid showed very considerable activity (4,078 and 2,565 counts/min./100 mg.), which could only be attributed to direct extension of the tracer from the injection site. Within the spinal cord the phosphoric acid had reached the mid-thoracic level in R.P. 13 and probably a considerably higher level in R.P. 14 in view of an activity of 6,100 counts/min./100 mg. at the thoracic level.

3. High sciatic injections (Table IV)

Of the six injections carried out at this level, three were effected with minimal or very slight
resistance and an absence of distention of the nerve. They resulted in a considerable spread of the indicator, in one case (R.P. 27) as far as the basal ganglia, while the least spread (R.P. 28) was as far as spinal cord segment 9. From the behaviour of injections at lower levels in the nerve it might have been expected that a high degree of activity of the cerebrospinal fluid would be associated with this widespread penetration of the spinal cord, yet it was in fact noted that in none of these three animals did the cerebrospinal fluid activity exceed 40 counts/min./100 mg. (R.P. 27). Again the blood activity, which it was anticipated would be low, attained a level of 192.3 counts/min./100 mg. in R.P. 27.

Injections of this type featuring direct spread to the cord but not to the cerebrospinal fluid may be classed as of “cord type” (Fig. 5). The absence of the tracer substance from the cerebrospinal fluid in the face of a high concentration in the spinal cord is remarkable, particularly after two hours, during which time diffusion from cord to fluid might have been expected to take place.

The survival period of one of these animals was...
only nine minutes (R.P. 28), its death occurring at the first cisternal puncture. It is of interest that within this space of time the tracer substance had passed up to the level of the upper sciatic entry segment (10) to produce a count of 108-8 counts/min./100 mg. In view of the high activity (∞) of segment 8 of the sciatic nerve, and the low maximum blood level (86 counts/min./100 mg. after one minute) and a cerebrospinal fluid count of 32-6 counts/min./100 mg., it appeared that the activity of segment 10 must be attributed to spread of the indicator from the sciatic nerve (c.f. control range (10) 18-1—93-6: with blood range 551-1,042 counts/min./100 mg.). This fact, together with the low resistance encountered during the injection, justified the inclusion of this experiment in the "spinal cord" group.

The remaining three injections were of "blood" type, the maximal blood concentrations ranging from 45 to 148 counts/min./100 mg., the former rather low blood level probably being due in part to the injection of only 55 μc into a large animal (2.9 kg.). The cerebrospinal fluid activity in this group was rather higher than in the "blood" type injections at the mid-femoral and low sciatic levels, reaching 76-9 counts/min./100 mg. in R.P. 26 after forty minutes. In one case (R.P. 25) a slight direct extension to the spinal cord took place (234-6 counts/min./100 mg. in segment 9), suggesting that this particular injection is intermediate between "cord" and "blood" types.

Changes in the Opposite Sciatic Nerve.—Interesting and unexpected findings were noted in the uninjected sciatic nerve which was divided into the usual four segments for estimation of radio-active content. In thirteen of the sixteen experiments,
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the activity of one or more segments of the contra-
lateral nerve was considerably higher than the
average control value. Further, in eight of the
thirteen animals showing the effect, the elevated
activity of the opposite nerve segments showed a
rough correspondence to the site of the intraneural
injection, while in the remaining five cases the effect
occurred in one or both of the two distal segments.
Tables II, III, and IV show the incidence and local-
ization of the effect in the three groups of injections.
It is evident that the contralateral effect is less
marked in the “low sciatic” injections than in the
“mid” and “high sciatic” types, in which it was
noted in four out of six and five out of six cases
respectively.

Anatomical Localization of the Injection Mass.—
The resolution of intraneural injections into two
main groups on the basis of the immediate behaviour
of the injection mass has prompted a further
enquiry into the anatomical localization of the
needle point and of the indicator material in the
two types. In order to obtain precise histological
information a suspension of Indian ink was employed,
bearing in mind the much greater resistance to flow
of such a suspension compared with the crystalloid
radio-active material.

A number of injections of filtered Indian ink of particle
size 0.5-1.5 μ were made into the sciatic nerves of
anaesthetized rabbits at the mid-femoral level. A
volume of approximately 0.1 ml. of ink was introduced
through the same type of needle as was employed
in the previous tracer experiments. After a survival of
ten minutes the animals were killed and the sciatic
nerves removed, examined, fixed in formalin and em-
bedded in celloidin. Transverse sections of the nerves
were cut at 12 μ and stained with alum carmine.

Gross Appearances.—It was again noted that the
injections fell into two main groups whose local
characteristics were closely similar to those of the
tracer indicator. In the first type, the injection
encountered little resistance and a sharply defined
black column could be seen to run up the undis-
tended nerve for at least 1.5 cm. In several in-
stances a similar column of ink was seen to move in
a centrifugal direction for a somewhat lesser
distance, recalling the high activity of segments
distal to the injection site in certain of the tracer
experiments. From the close similarity between
the features of these ink injections and those of
radio-active phosphorus of “cord-cerebrospinal
fluid” type; it was considered that the low- and
high-power appearances so obtained could justifi-
bly be used to elucidate the behaviour of the
indicator in this group of tracer injections.

Low-power examination of the nerve sectioned
transversely near the apex of the ascending column
of ink showed clearly (Fig. 6A) that the ink
was confined to the centre of one of two large
fasciculi. Viewed longitudinally in the intact
specimen the ink column appeared to lie deeply in
the nerve substance, the exterior of the nerve being
unstained even at the injection site.

In the second type there was considerable resis-
tance to injection, and this was associated with
localized distention of the nerve. Beyond the
limits of the local bleb an irregular band of ink
extended for only a few millimetres and the exterior
of the nerve appeared black. These features
closely resemble those of the “blood” type phos-
phorous injections, for which it was felt they could
provide an anatomical basis.

A transverse section made through the injection
site in the fixed specimen allowed a little free ink to
escape and the binocular dissecting microscope
revealed the appearances shown in Fig. 6B. It
can be seen that the ink has stained the
exterior of the nerve but also forms a thin black
line separated from the nerve’s circumference by
the thickness of the perineurium. It seems prob-
able, then, that the injection has created a restricted
sub-perineural space along which no considerable
penetration is possible.

Microscopic Examination.—In those nerves where
the injected ink had spread freely with little resistance
it was distributed throughout the substance of the
fasciculus, producing a characteristic “wire-netting”
picture due to the presence of particles between the
individual nerve fibres (Fig 6C). This section,
taken near the injection site, shows that some ink
had penetrated into the “sub-perineural space.”
Little if any ink is to be seen in the connective tissue
of the nerve trunk.

The injections resulting in bleb formation and
little spread along the nerve are represented by
Fig. 6D, which is a section taken at the injection site.
The ink lies largely in and below the perineurium,
while a relatively small amount lies in the inter-fibre
spaces, along which low-power observations have
shown that progress is strictly limited.

It may be concluded from the above that the
“cord-cerebrospinal fluid” type of injection takes
place when the needle point lies at or near the centre
of a nerve fasciculus, from which the injection mass
moves freely in the inter-fibre spaces—the paths of
least resistance within the nerve. On the other
hand the “blood” type of injection occurs when
the needle point lies within or just below the peri-
neurium. The injected material then creates an
artificial sub-perineural space, but only when
considerable resistance has been overcome. A
variable degree of penetration of the fasciculi also
A. Sketch of cut end of nerve injected with Indian ink (×25). "C.S.F." type of injection. Section taken near apex of ascending ink column, which is seen to lie within the substance of a fasciculus and separated from the perineurium by unstained fibres. The exterior of the nerve is unstained.

B. Sketch of cut end of nerve injected with Indian ink (×25). "Blood" type of injection. Section taken at injection site showing staining of the exterior of the nerve, the presence of ink in a sub-perineural depot and a small quantity within a fasciculus due to penetration to this situation at a slightly different level.

C. Transverse section of a nerve injected with Indian ink (×75). "C.S.F." type of injection. Section taken at base of ascending ink column. Ink particles are seen to lie within the fasciculus between individual nerve fibres to present a typical "wire-netting" appearance. A thin film of ink lies under the perineurium at this level but it extends for only a few millimetres.

D. Transverse section of a nerve injected with Indian ink (×75). "Blood" type of injection. Section taken close to injection site. Considerable ink is seen to lie within the artificial sub-perineural space which is confined to a part of the nerve circumference. At this level ink particles have penetrated a fasciculus to give the "wire-netting" appearance but have not been able to ascend more than a few millimetres.
takes place, but is not sufficient to spread any large distance along the nerve.

It must be admitted that these ink injections, carried out as they were at the mid-femoral level, invariably failed to produce evidence of ink in the sub-arachnoid after an interval of several hours. Our experience with soluble dyes has been quite different and has provided confirmation of the claim made by Horster and Whitman (1931–2) that high intra-sciatic injections of dye not in excess of 0.1 may easily enter the cerebrospinal fluid as well as the spinal cord.

Discussion

It is a legitimate criticism that all too often the experimental production of a disease by intra-neural injection of toxin or virus has been regarded as evidence of the passage of that agent along neural pathways by virtue of forces inherent in the nerve itself. Further, the vascular components of the nerve, both blood and lymphatic, have, with certain notable exceptions, received inadequate consideration in the interpretation of such experimental results. It is therefore desirable before making reference to the relevant experimental work on tetanus and anterior poliomyelitis to review certain of the anatomical features of a typical peripheral nerve.

The sole neural element establishing an unbroken connexion between the periphery and the central nervous system is the axis cylinder, the structure currently believed to provide the pathway of transport for tetanus toxin and the virus of rabies, poliomyelitis, and certain other virus diseases. In this connexion it must be admitted that evidence is lacking of any centripetal flow within the axoplasm capable of conveying an infective agent from periphery to centre within the incubation period of the disease. On the contrary it has been suggested by Young (1945) that there is in fact a slow centripetal streaming of the axoplasm away from the parent cell body.

However, the possibility of a surface streaming of virus particles is provided by the experiments of McFarlane (1940), in which buffered suspensions of vaccinia virus were placed in the U tube of the Tiselius apparatus. It was observed that the particles exhibited an endosmotic streaming along the centre of the tube, associated with a movement of the buffer in the opposite direction. McFarlane has suggested (personal communication) that such a mechanism might explain the movement of virus along a sectioned peripheral nerve whose central end is dipped in a pool of virus, as in the experiments of Bodian and Howe (1940, 1941 a and b). The necessary physical conditions could exist, not inside the axis cylinder, but within the capillary layer of fluid occupying the intervals between the individual nerve fibres. It is evident that such a film must lie outside the myelin sheaths, as no spaces are known to exist between the axis cylinder and the myelin sheath nor between the myelin sheath and the neurilemma (De Renyi, 1932).

The sleeve-like outer coat of the nerve, the perineurium, is separated from the contained fasciculi by the no more than potential sub-perineurial space. This latter is often referred to as a true space from the appearances seen in paraffin sections, where shrinkage is largely responsible for its production, whereas celloidin sections fail to reveal any interval in this situation. The existence of connective tissue septa between perineurium and endoneurium represents a barrier to any physiological flow in either a centrifugal or a centripetal direction. Nevertheless the possibility remains that material introduced by direct injection may find the sub-perineurial space to be one of the pathways of least resistance within the nerve and therefore analogous to the inter-muscular fascial planes as a potential avenue of fluid movement (compare the fascial spaces in the palm).

That the endoneurial spaces do not establish any physiological communion with the subarachnoid space has been established by Weed (1914), Iwanow (1927), Elman (1923), and Brierley and Field (1948). Thus neither crystalloid nor particulate substances when introduced into the cerebrospinal fluid under physiological pressure conditions will pass farther along a spinal nerve than the proximal pole of its dorsal root ganglion. On the other hand Sullivan and Mortensen (1934) showed that in the rabbit coloured brominol oil introduced into the cisterna magna after removal of a corresponding volume of cerebrospinal fluid could be seen to pass some distance along the major peripheral nerves, both by x-ray examination and by dissection. Some twelve to twenty hours were required to obtain this effect, and at necropsy the oil in the sciatic nerve at the level of the greater trochanter was seen to lie both within and around the fasciculi. Deposits of the coloured oil also occurred free in the tissues surrounding the nerve. Such findings are in sharp contrast to those obtained with both Weed's crystalloid mixture and with indian ink suspensions, and suggest that an oily medium is better able to pass through the cul-de-sac of the sub-arachnoid space around the nerve roots, but once again they emphasize the need for a precise study of the anatomy and properties of the various membranes in this situation. Under the artificial conditions of intraneural injection, Key and Retzius (1875) were the first to show that an injection mass could be
made to pass from a peripheral nerve into both the cerebrospinal fluid and the substance of the spinal cord. This finding was again noted by Horster and Whitman (1931–2), and has been confirmed in the present investigation.

The blood supply of the sciatic nerve of the rabbit has been described in detail by Adams (1943), who pointed out that a nerve is never supplied by a single artery but by a number of vessels, each of which divides into ascending and descending branches before penetrating the perineurium to enter the inter-fascicular connective tissue. Here a rectangular network of branches is formed from which the capillary plexuses are derived, and these alone extend into the endoneurium. The vessels of a nerve are not end arteries but form a longitudinal arterial network. It can thus be seen that a nerve is a richly vascular structure affording a considerable capillary bed from which injected substances may be absorbed.

Defrise (1930) has elucidated many of the problems relating to the lymphatic drainage of nerves, and in addition to a rich plexus lying on the epineurium has described communications between this plexus and lymphatics lying within the nerve substance in the inter-fascicular planes. The existence of such intraneural lymphatics has always been denied, and this finding of Defrise has not been confirmed by Zhdanov (1931).

Any consideration of the ultimate fate of a substance injected into a nerve must take into account the anatomical factors enumerated above, and it is clear that a nerve cannot be regarded as a route leading solely to the central nervous system, but rather as a tubular system of neural units containing potential spaces and affording opportunities for absorption into the general blood vascular and lymphatic systems closely similar to those in any other tissue of the body.

The well-known work of Key and Retzius (1875) provided the first evidence that intraneural injections of dyes could reach the subarachnoid space of the spinal cord and brain, and these workers drew attention to the lack of uniformity of spread in any group of injections. Thus in some instances Key and Retzius noted the “oft wunderbare Leichtigkeit” with which intraneural injections of dyes could be carried out in the perineural lymph sheaths, that is, in the endoneurial spaces, while in other cases there was little appreciable spread of the injection mass. Comparable observations were made by Horster and Whitman (1931–2) when carrying out intraneural injections of trypan blue. It was recorded that in those animals (rabbits) where staining of the spinal cord or of the cerebrospinal fluid was obtained, the injection was made with the greatest of ease, and the impression was gained that the injection was passing into natural preformed pathways in which only a minimal pressure existed. In certain cases, however, the injection produced a local bleb, the dye only spreading up and down for a short distance without reaching the cord.

The “cerebrospinal fluid” types of injection described in the present series of experiments may be regarded as corresponding to those of Key and Retzius and of Horster and Whitman, in which the dye stuff could ultimately be detected in the spinal fluid. The “blood type” of injection associated with resistance to injection and distention of the nerve probably corresponds to those injections of the earlier workers in which there was bleb formation together with minimal spread of the dye. Clearly, then, the position of the needle point in the nerve is an important factor determining the spread of an injection and is largely a fortuitous circumstance. Attempts to produce at will one or other type of injection are in most cases unsuccessful.

A further and obvious factor is the volume of the injection employed and here the present investigation has shown that so small a volume as 0.05 ml. of a crystalloid material may on occasion run from the lower part of the sciatic nerve up to the basal ganglia under even minimal injection pressure. It is probable, therefore, that injection volumes used by many workers may spread widely in the nervous system and produce relatively high concentrations in even remote parts. Thus Yuien (1928), injecting 0.5 ml. of trypan blue into the sciatic nerve of a rabbit at the mid-femoral level, observed the passage of the dye along the ventral roots of the nerve into the spinal cord and cerebrospinal fluid. The conclusion reached from these and similar experiments with Weed’s solution was that the flow of nerve lymph was centripetal in motor nerves and centrifugal in sensory nerves, a conclusion completely without justification in view of the large quantity of dye injected. This objection applies with even greater force to the experiments of Yuien and Sato (1929), who injected the heroic volume of 15 ml. of “Dekalin” in a central direction into a peripheral nerve of a rabbit. The injection was carried out “slowly under a little pressure,” and the stain was observed to travel largely in the subperineural space to the subarachnoid space. This was taken to demonstrate that dyes made up in a non-aqueous medium could travel with greater rapidity than those dissolved in water.

Turning now to those experiments in which infective agents were introduced into the body by intraneural injection, the literature of the experimental investigation of tetanus reveals frequent
inattention to the complex nature of a peripheral nerve and the importance of the factors of injection volume, injection pressure, and position of the needle point.

Of the many workers who have contributed to the development of the theory of axonal transmission of the toxin, Marie and Morax (1902) are well known. A single dose of 3 g. of tetanus toxin (equivalent to about 1,000 lethal doses) was injected into the gastrocnemius muscle of a pony which died less than three days later. The first development of tetanic symptoms in the injected limb was adduced as evidence for the passage of the toxin along the peripheral nerves to the related spinal cord segments. Nevertheless these workers admitted that in a given weight of the sciatic nerve the content of toxin is exactly what calculation showed should be present if the total dose were equally distributed in every organ and tissue.

Meyer and Ransom in the same year, from a series of experiments in which the toxin was injected intraneurally, concluded that its absorption from the nerve took place via the neural lymphatics. The latter delivered the toxin into the general blood stream, from which it could only gain access to the central nervous system along motor nerves from their endings in muscle.

Further intraneural injections of tetanus toxin were carried out by Horster and Whitman (1931-2) employing an injection volume of 0.04 to 0.1 ml. These experiments indicate clearly the importance of the factors of injection pressure and the position of the needle point, and closely parallel the earlier investigation by the same workers in which trypan blue in similar volumes was injected into the sciatic nerve. Thus in five out of fifteen experiments the toxin could be identified in cerebrospinal fluid (cisternal puncture) very soon after the original injection. In the remaining cases the toxin never appeared in the liquor and was found to have passed only a short distance up and down the nerve. Abel’s (1935) criticism of these experiments is that “... when Horster and Whitman did not succeed in driving the toxin into the lumbar cerebrospinal fluid at once by syringe pressure, the cisternal fluid never contained any of it later, even when the authors waited almost three days for signs of its appearance” (p. 105); and further, when the toxin fails to spread far from the injection site, “... it cannot be carried into the central nervous system by forces naturally inherent in the nerve or by its lymphatics.”

In a discussion of the intraneural channels available for fluid movement Abel draws attention to the lack of evidence in support of any centripetal flow within the axis cylinder, while in relation to the question of fluid flow within the tissue spaces (“lymphraume”) Abel writes, “The tissue pressure at peripheral points is not of sufficient magnitude to move solutions through such narrow spaces, frequently interrupted as they are by tissue barriers. These minute spaces are energized by molecular forces only (surface energy and diffusion) and it remains to be proved that such forces can compass the astonishing feat of transporting a poison for long distances, as in the sciatric nerve of a horse, and through intervening cell barriers to the central nervous system, in any period of time that could ever come into consideration under experimental conditions or in the naturally occurring disease, tetanus” (p. 107).

In order further to discredit the concept of neural transport, Abel and others (1935) carried out a series of intraneural injections of tetanus toxin using a volume of only 0.025 to 0.083 ml. and with most stringent precautions to prevent leakage. It was recorded that in no case were symptoms of central tetanus seen to occur, the animals remaining normal for twenty-four days except for a slight local rigidity which was attributed to undetected leakage of toxin. In the absence of any movement of the toxin along the nerve to the central nervous system no symptoms were produced, whereas a similar dose injected intramuscularly produced typical local tetanus, so that, “We must conclude that intraneurally injected toxin, when its local escape has been avoided, is conveyed in its entirety away from the nerves by their lymphatic trunks to the venous system” (p. 320). The failure of the dose of toxin used, to produce symptoms on entry into the blood stream was considered to be due to its relatively low rate of absorption: “... an intraneural injection of toxin is exactly equivalent to a slow intravenous injection of an equal dose” (p. 332).

The scope of Abel’s work is too large for review here, but a series of convincing arguments are put forward to support the view that in tetanus the muscular rigidity is due to the direct local action of the toxin on the normally innervated muscle and that the spread of the disease to the “general” form is explicable solely by dissemination of the toxin in the blood stream. Once again, “The history of science bears testimony to the fact that it is not easy to dislodge from the mind a theory that has long been whole-heartedly accepted.” (Abel and others, 1938, p. 399.)

Any review of the origin of the current theory of axonal progression of the virus of anterior poliomyelitis would be out of place in this communication; and attention must be confined to an examination of certain of those experiments involving intraneural injection of the virus, the results of which have been adduced as support of this theory. In
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this connexion the well-known experiments of Hurst (1930) must be re-considered in the light of the observations here presented. The intra-sciatic injection of 0-5 ml. of a virus suspension, together with deliberate trauma of the nerve by the injecting needle, was found to be a certain method of producing the disease in the monkey. The progress of the virus was found to be from the lumbar cord to the opposite motor cortex, the cervical region and the ipsilateral cortex giving evidence of virus at a later date. It was considered that this behaviour would be expected were the pyramidal fibres the transmitting structures. It is now clear that the passage of the injection mass to the lumbar region is a highly probable consequence of so large an injection, carried out as it was in the upper half of the sciatic nerve. Further, the deliberate trauma occasioned by moving the needle point up and down within the nerve almost certainly ensures that the subsequent injection shall be of the "cord-cerebrospinal fluid" or "cord" type rather than the localized "blood type," a finding recorded on several occasions when trauma to the nerve with the needle point was deliberately associated with an injection of radio-active phosphorus.

The proved spread of the tracer injection along the whole length of the spinal cord and brain stem to the basal ganglia at once raises the question whether such a tracer injection in a monkey with well-developed crossed pyramidal tracts would not in fact tend to follow the endoneural spaces of these bundles to give higher levels of activity in the opposite hemisphere. If this were so, the initial distribution of virus throughout the length of the central nervous system may well be explicable solely as the result of injection pressure, while its subsequent concentration in the brain stem and spinal cord enlargements and in the motor cortex may be related to the particular affinities for virus of the cells of these regions.

The distribution throughout the neuraxis of a proportion of intra-sciatic injections of 0-05 ml. of tracer substance suggests that extra-axonal pathways are concerned, and of these the endoneural spaces of peripheral and central nervous system are most probable. This is supported by the peri-axonal "wire-netting" appearance of sections of nerve injected with Indian ink and also by the rapidity of movement of the radio-active indicator. Thus a distance of 35 to 40 cm. is traversed in a period of 120 to 140 minutes, a velocity far greater than has ever been suggested for intra-axonal progression. Further information concerning the actual velocity of the tracer movement is obviously desirable and could be obtained from similar injections carried out with shorter survival periods. It is unfortunate that facilities have not been available for the completion of such experiments which alone can demonstrate the true speed of crystalloid spread in the nervous system.

When a comparison is made between an intra-neural injection of virus and one of a crystalloid, allowance must of course be made for the heterogeneous particulate nature of the former, which could impair its ability to traverse capillary spaces and so reduce the speed and extent of its spread. However in spite of this, endoneural spaces must still afford the main avenue for rapid progression of the virus suspension consequent upon the act of injection. Such a mechanism of distribution is in fact that described by Römer (1913), who, in relation to intra-cerebral injections of virus, stated that, "Should these observations be confirmed they would seem to indicate that the active agent spreads along the neurone, or at least, spreads more quickly in this direction than in any other. It is to be supposed that in doing this it passes along the lymph channels accompanying the neurone, although this would presuppose a considerable degree of isolation of these channels" (p. 99).

In this connexion it is interesting to note that O'Leary and others (1932) observed that the functional activity of nerve fibres, as measured by the cathode ray oscillographic technique, remains normal until demonstrable changes occur in the nerve cell. They therefore suggest that the virus may travel between the nerve fibres, in which case Hurst's (1930) results after sciatic inoculation may be attributable to the passage of virus along the path of least resistance between the pyramidal fibres, rather than within them. Further, Howe and Bodian (1942), in their discussion of the mechanism of intraneural virus spread, state: "... in fact it is possible that instead of being adsorbed on the surfaces of the axones, virus particles may move along interfaces in a gel at velocities greater than is possible by simple diffusion" (p. 24). If this suggestion and that of McFarlane (vide supra) are substantiated, we may well witness the abandonment of the original hypotheses of Di Vestea and Zagari (1887, 1889) in favour of a mechanism which differs in no important respect from that of Römer whose "perineural lymph spaces" are the capillary intervals whose physico-chemical properties are now under discussion.

The considerable blood absorption from a proportion of intraneural injections raises the question whether similar absorption may take place from the central end of a divided nerve when dipped in the tracer indicator. The problem is important in view of the technique adopted by Bodian and Howe.
(1940), in which the sectioned monkey sciatic nerve was dipped in a pool of poliomyelitis virus for ten minutes. This was found to be a very reliable method of producing the disease. The possibility of absorption into the blood was not considered by these authors, for whom the pathway of virus to the central nervous system lies solely within the nerve. However, the work of Lennette and Hudson (1936) and German and Trask (1938) suggests that modifications in the blood-brain barrier may permit circulating virus to invade the central nervous system and indicates that spread by the blood is a problem that even now merits further enquiry.

The central end of the cleanly sectioned rabbit sciatic nerve was inserted in one limb of a U tube containing radio-active phosphoric acid as described by Bodian and Howe (1940).

Preliminary results have shown that there is in fact appreciable absorption into the blood to produce activities of 55 to 60 counts/min./100 mg.; after fifteen minutes' contact with the indicator. Caution must, however, be exercised in carrying over these results obtained with a crystalloid material to the problem of virus absorption in similar experiments.

**Significance of the Contralateral Effect.**—The mechanism responsible for the heightened activity in the contralateral nerve cannot be elucidated in full from the present experiments, designed as they were to solve a different problem. The most that can be said is that a particular segment of nerve contains more radio-active phosphorus than would be expected from the maximum blood level of the experiment. It is clear that the source of this phosphorus can only be the blood stream, as the possibility of streaming from the injected nerve or from the spinal cord is excluded by the occurrence of normal counts in the segments between the cord and the region of increased activity. Presumably there is some change in the peripheral blood-nerve relationship associated with either an increased vascular permeability or an increased phosphorus interchange between blood and nerve.

A survey of the features of all the injections concerned leads to the suggestion that trauma may be the most important single factor in the production of the effect. It is noteworthy that the contralateral activity was least in an injection that was recorded as "the easiest of the whole series" and most in an injection where trauma with the needle point was deliberately inflicted. If this should prove to be the case, the mechanism involved is most likely to be nervous and of reflex type.

A contralateral change of this type appears to resemble the observations of Barnes and Truea (1942). These workers noted that after the application of a tourniquet to one limb of a rabbit "in some of the animals there was . . . a well-marked spasm of the femoral artery of the uninjured limb, the spasm beginning at a level corresponding approximately with the site of the tourniquet on the other limb."

**Summary**

1. A technique for introducing 0·05 ml. of radio-active phosphorus (as phosphoric acid) into the sciatic nerve of the rabbit is described.

2. Of the sixteen injections performed, four were at the apex of the popliteal fossa, six at the mid-femoral level, and six just above the greater trochanter.

3. The injections fell into two groups according to their immediate local features and to the subsequent distribution of the indicator in blood, cerebrospinal fluid, and the nervous system. The outcome of any one injection was purely fortuitous.

4. The following features distinguished a "cord-cerebrospinal fluid" type of injection:
   i. Minimal resistance to injection.
   ii. Absence of dilatation of the nerve.
   iii. A column of fluid moving rapidly in a central direction.
   iv. Spread of the indicator throughout the nerve.
   v. Spread of the indicator into the spinal cord.
   vi. Entry of the indicator into the cerebrospinal fluid.
   vii. Relatively little absorption into the blood stream.

5. The following features distinguished a "blood" type of injection:
   i. Considerable resistance to injection.
   ii. Variable dilatation of the nerve.
   iii. Absence of an ascending fluid column.
   iv. Limited spread of the indicator within the nerve.
   v. No spread of the indicator into the spinal cord.
   vi. No entry of the indicator into the cerebrospinal fluid.
   vii. Considerable absorption into the blood stream.

6. "High sciotic" injections resembled the "cord-cerebrospinal fluid" injections except that there was no appreciable entry of the indicator into the cerebrospinal fluid, while blood activity was relatively high. The term "cord" type was applied to these injections.

7. Changes were reported in the opposite sciatic nerve in which the activity of one or more segments was considerably higher than the average control values.

8. Indian ink injections were employed to provide an anatomical explanation of the localization of needle point and injection mass in the two types. It was concluded that the "cord-cerebrospinal fluid" and "cord" types of injection could be attributed to the penetration of a fasciculus by the needle point whence spread took place along the
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inter-fibre spaces—the pathway of least resistance within the nerve. In the “blood” type injection the needle point lay within or just under the perineurium, the injection mass creating a limited subperineural space with variable but slight penetration of the fasciculus.

9. The employment of intraneural injections in the experimental investigation of tetanus and poliomyelitis is discussed in the light of these observations.

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