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(duplicate)

II : 10 ppm
III : 50
IV : 100
V : 500

approx. 25 gms/day/rat
" wt. 815 Gms = 0.815 kg.

Species: 5000 ppm

5 Gms / 1000 Gms Food
: 5 Gms / 100 Gms Food
= 1.25 Gms / 25 Gms Food

25 Gms food

5000 ppm Rats:
approx. 0.125 Gms daily
sum. wt. approx. .8 kg
∴ around 0.156 Gms/kg/day.

CHRONIC PERIPHERAL NEUROPATHY PRODUCED BY LEAD POISONING IN GUINEA-PIGS* †

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In 1880 Gombault (10) described peripheral nerve changes resulting from lead poisoning in guinea-pigs which had been fed small doses of white lead mixed with their food for many months. Although the animals showed no evidence of paralysis, extensive histological changes were found in the nerve trunks. Gombault teased out single nerve fibres so that several internodes of the same fibre could be examined. In this way he was able to show that the myelin over one internodal segment degenerated while internodes at either side remained normal. This was the first description of the process now known as segmental demyelination. Gombault also demonstrated that recovery occurred by the formation of several short segments along a length of nerve which previously consisted of a single internodal segment. Although he did not use silver stains to demonstrate the axis cylinders, Gombault assumed that they remained in continuity through the demyelinated segments. In addition to segmental demyelination which affected many fibres, Gombault found a few fibres showing axonal degeneration.

Changes in nerve conduction associated with segmental demyelination have recently been produced and studied in experimental animals; for example, in diphtheritic neuropathy (20, 21, 22, 16, 15, 27) and experimental allergic neuritis (4, 12). The present work was undertaken since, if Gombault's observations could be confirmed, it seemed that lead poisoning might produce a chronic demyelinating neuropathy suitable for further experimental study.

An investigation has, therefore, been made of nerve conduction velocity in guinea-pigs during lead administration and the results have been correlated with the histological changes found.

METHODS

Administration of Lead Acetate: Male guinea-pigs, aged from a few days to 2 years, were given repeated doses of a 50 per cent solution of lead acetate by stomach tube. The optimum dose to produce neuropathy proved difficult to select. With large doses the animals died before developing neuropathy, whereas small doses might be continued for many months without electrophysiological or histological evidence of peripheral nerve damage.

Initially the technic of Gombault (10), Prévost and Binet (29), and Doinikow (5) was followed. Lead acetate was given in doses varying from 0.2 to 3.2 g./kg. from 1 to 5 times a week. Each animal was assessed clinically, weighed several times a week, and the

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haemoglobin level was estimated every 2 or 3 weeks. When an animal began to lose weight, or when the haemoglobin level fell below 5 g./100 ml. lead acetate was stopped for 1 or 2 weeks, and subsequently continued at the same or at a reduced dose. With greater experience it proved possible to select a dose which could be given without interruption and which produced neuropathy in a proportion of animals. Young animals survived much larger quantities of lead acetate than adult animals. A dose of 1 g./kg. administered 5 times a week was used in young animals, while a dose of 0.5 g./kg. given 3 times a week was given to adults.

Electrophysiological Methods: Serial measurements of motor nerve conduction velocity in the fibres supplying the small muscles on the plantar surface of the hind foot were made in anaesthetised animals, by a method similar to that described by Kaeser and Lambert (16). Light anaesthesia was produced with intraperitoneal pentobarbital "Nembutal" (30 mgm./kg.), which was then deepened with a halothane and oxygen mixture inhaled through a facial mask, as described by Marley and Payne (25). To prevent heat loss during the experiment, the animal was examined lying on a thick pad of cotton wool and heat was provided from a lamp. The intramuscular temperature in the thigh was measured at the end of each experiment, and varied between 35° and 39°C. When the intramuscular temperature was also measured in the foot it was found to be 1 to 1.5°C. lower than that in the thigh.

The arrangement of stimulating and recording electrodes is illustrated in Figure 1. The proximal stimulating cathode was a steel needle inserted through the skin to lie near the sciatic nerve in the upper thigh. The distal stimulating cathode was a steel clip with

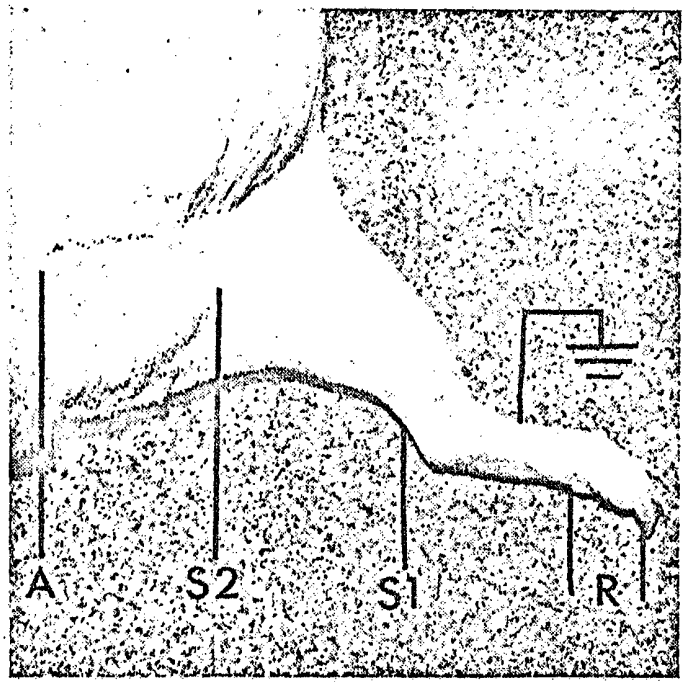

FIG. 1. Arrangement of stimulating and recording electrodes. A = anode, S1 and S2 = cathodes, R = recording electrodes.

its points just penetrating the skin over the course of the posterior tibial nerve at the medial side of the ankle. Dissection with the clip *in situ* showed that the nerve, which lies deep to the Achilles tendon at this site, was not damaged by the clip. The anode was another steel clip attached to the skin over the back.

The stimulus consisted of a condenser discharge with a time constant of 20, 50 or 100 μsec. delivered through a 1:1 isolating transformer. The output impedance was less than 1 kilohm. Stimulus voltage was continuously variable up to 300 volts, and was adjusted to be supramaximal for the motor fibres, except when weak shocks were used to study responses of single motor units.

The muscles on the plantar surface of the guinea-pig's hind foot consist of 6 interosseous and 2 lumbrical muscles. For recording muscle action potentials stainless steel needles were used, potentials being recorded between an active electrode over the bellies of the interosseous muscles and a remote electrode over the lateral toe. Kaeser and Lambert (16) recorded potentials from a needle inserted through the muscle mass. In the present experiments it was found that satisfactory recordings could be obtained from a needle with its tip just penetrating the skin. Using this technic the muscles were not damaged and many serial recordings could be made from the same animal. In a few cases individual motor units were recorded through a coaxial needle electrode inserted into the muscles.

Muscle action potentials were amplified through conventional RC coupled amplifiers and displayed on one beam of a cathode ray oscilloscope, the second beam displaying a time scale. The stimulus was set to repeat at 1 second intervals. The distance between the two cathodes was measured on the skin, and used for the calculation of conduction velocity. In a few animals, the nerve was dissected and measured directly; its length was within 5 mm. of the skin measurement in the intact animal.

Histological Methods: One to 2 centimetre lengths of peripheral nerve were gently stretched on card frames and fixed in 10 per cent formol saline or Flemming's solution. Nerves fixed in formol saline were examined in 2 ways. At least one nerve from every animal was stained with 1 per cent osmium tetroxide and teased apart in glycerine using the technic described by Thomas (1955). Single fibres were prepared under a dissecting microscope at a magnification of ×100 and at least 100 fibres were inspected. Abnormal fibres were preserved by mounting in Canada balsam and later examined at higher magnification. In this way several internodal segments of a single fibre could be examined. Other nerves were embedded in paraffin and longitudinal sections cut at 5 μ. These were stained by the Holmes silver impregnation method combined with luxol fast blue-cresyl fast violet (24).

Nerves fixed in Flemming's solution were embedded in paraffin, transverse sections were cut at 5 μ, and the sections stained by the modified Weigert method to demonstrate myelin sheaths (11).

Spinal cords were removed within 1 hour of death and fixed in 10 per cent formol saline for at least a week. The lumbar region of the cord was embedded in paraffin, and transverse sections were cut at 15 μ. These were stained with haematoxylin and eosin, or cresyl violet.

RESULTS

Clinical Effects of Lead Acetate

Seventy-two male guinea-pigs were poisoned. There were 30 young animals, aged between 2 and 3 weeks at the beginning of the experiment, 3 animals aged 7 weeks to 3 months, and 39 'adult' animals, aged more than 3 months.

In young animals, the first sign of toxicity was failure to grow normally. The weight of such animals might remain constant for several weeks, whereas healthy young guinea-pigs gain approximately 100 g. per week up to a body

weight of 1.0 to 1.2 kg. The poisoned animals all started to gain weight within a few days of stopping lead acetate.

A sign of more serious significance in both young and adult animals was actual loss of weight. If dosing was interrupted when an animal began to lose weight, then it might recover so that lead could again be given after an interval of 1 or 2 weeks. Some animals, however, did not recover when lead was stopped, but continued to lose weight; 28 animals died in this way. In such cases the immediate cause of death was obscure and no gross lesions were demonstrated at autopsy. The animals would become severely dyspnoeic and unable to move about their cages. The weakness was not usually due to peripheral neuropathy, since histology of the nerves in a number of these animals was subsequently shown to be normal; nor did they necessarily die with severe anaemia.

Most of the animals developed blood changes during poisoning. Punctate basophilia of the erythrocytes was common, and was seen after 2 days' poisoning in one animal and after a week in a number of others. Anaemia then usually developed, and in many of the animals the haemoglobin level fell from the normal range of 12 to 14 g./100 ml. to less than 7 g./100 ml. The lowest recorded haemoglobin level was 3.8 g./100 ml. A fall of 7 g./100 ml. during the first 2 weeks of poisoning occurred in some animals on high doses of lead acetate. Although the haemoglobin level gave an indication of the severity of poisoning, it was a less sensitive indicator of whether an animal was likely to die than loss of weight. For example, dosing was continued for a few months in several animals after the haemoglobin level had fallen to 5 g./100 ml.

Although loss of weight was the usual indication of severe poisoning, there were in the series 21 animals which died suddenly after variable amounts of lead had been given, without having previously shown any of the manifestations described above. Three of these animals were found at autopsy to have had pneumonia, but the immediate cause of death could not be ascertained in the others.

In 13 animals, death was related to an anesthetic administered when conduction velocity was being recorded. Eight animals died during the anaesthetic and 5 within the next 48 hours, during which time they had appeared ill and weak.

Ten animals in the series were killed for histological examination when still apparently healthy.

Convulsions were seen in 9 animals and were often precipitated by noise or movement. They were not always of serious significance; 5 of the animals lived for several weeks after a series of fits. During an attack the animal would run rapidly round its cage, then fall to the ground with clonic movements of the limbs for about 30 seconds, following which it would lie inert for several minutes and then appear to recover completely. These attacks were similar to those described previously in guinea-pigs suffering from lead poisoning (29, 33).

In cachectic or moribund animals it was usually impossible to decide whether or not neuropathy was present, and it has already been mentioned that some animals with gross cachexia and weakness were later found to be without histological evidence of peripheral neuropathy. However, there were 9 animals in which clinical evidence of neuropathy could be detected at a time when they appeared otherwise healthy. In 7 of them the neurological deficit was mild. The animals were able to run, but they appeared clumsy and had some difficulty in righting themselves from a supine position. They were slow to withdraw their hind limbs when these were stretched out. An example of the abnormal posture is shown in Figure 2. Histological abnormalities of peripheral nerves were later shown to be present in all of the nerves that were examined from these animals.

Two other animals appeared to have more severe paralysis. Their hind limbs became stiff and fixed in a position of flexion at the hips and knees, so

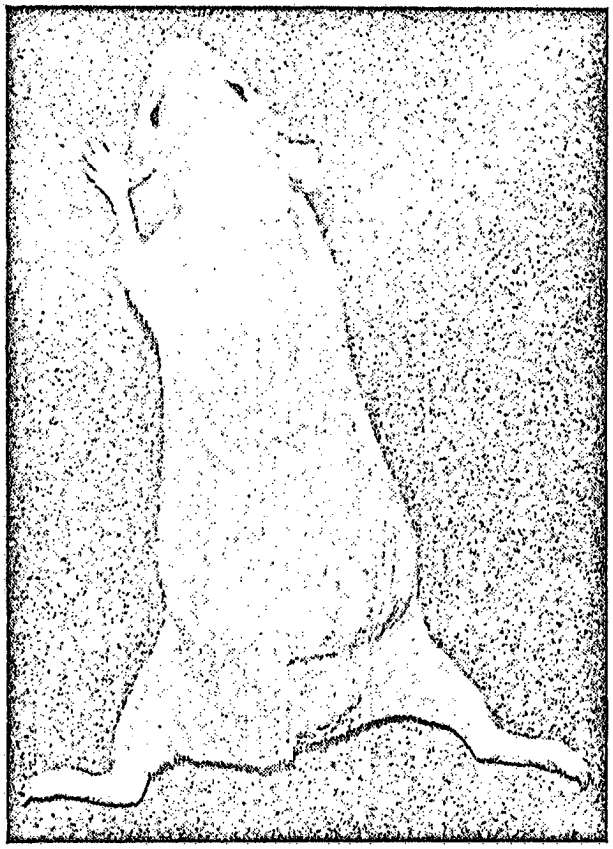

FIG. 2. Abnormal posture of hind limbs in a poisoned animal (GPC 29).

that it was not possible to straighten them. They used their fore limbs to run and dragged their hind limbs along the ground. Although both of these animals were later found to have histological abnormalities of their peripheral nerves, these were not more severe than in many other animals, and it seemed possible that there was upper motor neuron involvement as well. Unfortunately examination of the spinal cord was not carried out in either of these animals.

Electrophysiological Studies

Maximal Conduction Velocity

a. *Control Animals:* 191 estimations of maximal motor nerve conduction velocity are available for 119 healthy animals, with ages ranging from a few days to 2 years. Seventy-four animals were examined personally and the remainder by others working in the same laboratory and using the same technic.

Figure 3 shows the muscle action potentials recorded from a control guinea-pig aged 4 months. Each record consists of 5 consecutive traces superimposed. In this case the conduction velocity for the fastest fibres between hip and ankle was 47 m/sec. The small inconstant potential following the main action potential in Figure 3 has a longer latency following stimulation at the ankle than in the thigh, indicating that it is either a spinal reflex or an F wave similar to that described by Magladery and McDougal (23) in human subjects.

Conduction velocities for all of the control animals are shown in Figure 4 plotted against age. It can be seen that the velocity at birth was below the adult range, and gradually increased with age up to 4 to 5 months, thereafter remaining constant. The mean velocity for animals up to 2 weeks of age was 30 m/sec. and for animals over 20 weeks was 51 m/sec. (S.D. 4.7). The value for adult animals is similar to that reported by Kaeser and Lambert (16), who found a range of 48–62 m/sec. using a comparable technic.

In order to determine the reproducibility of estimations of conduction

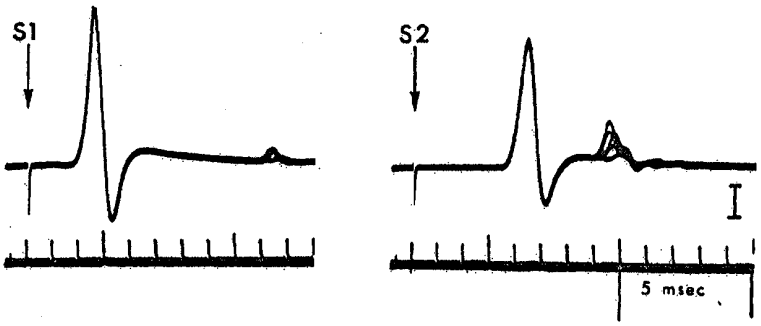

FIG. 3. Muscle action potentials from a control animal produced by supramaximal nerve stimulation at ankle (S1), and in thigh (S2). Five consecutive traces superimposed. Calibration—1 mV.

velocity, several adult animals were examined repeatedly over the course of 6 or 9 months. The results varied by no more than 8 m/sec. in the same animal on different occasions.

b. *Lead Poisoning:* Motor conduction velocity was measured in 40 animals during chronic lead poisoning. The lowest value recorded in each animal is shown in Figure 4, plotted against the age of the animal at the time the examination was made. It can be seen that conduction velocity was below the control values in 17 animals, and that in 9 animals the velocity was less than 30 m/sec. In one animal, not shown in Figure 4, the sciatic nerve appeared to be inexcitable in the thigh, although a small muscle action potential was obtained when the posterior tibial nerve was stimulated at the ankle.

Figure 5 shows the muscle action potentials recorded from 3 animals during lead poisoning. The upper pair of records was obtained from guinea-pig GPC 57, aged 15 weeks at the beginning of the experiment and given 0.5 g/kg. lead acetate 3 times a week for 19 weeks. The animal had lost no weight and showed no clinical paralysis. The maximal conduction velocity between thigh and ankle was 26 m/sec. In the case of GPC 38, the action potentials shown in Figure 5 were recorded after 14 weeks of intermittent lead dosage. At this time the animal was 17 weeks old; its growth was retarded, but it did not become paralysed. Conduction velocity was 24 m/sec. Guinea-pig GPC 27, aged 24 weeks was given lead acetate intermittently in a dose of 0.5 to 1 g/kg. for 22 weeks. At this time the abnormal posture shown in Figure 2 was present, and it can be seen that conduction velocity was 16 m/sec.

Conduction velocity in the right and left hind limbs was measured on the same occasion in 10 animals. In 4 animals, velocities were within the normal range on both sides. The results in 6 animals with reduction of conduction velocity are shown in Table 1, from which it can be seen that the findings on each side were similar.

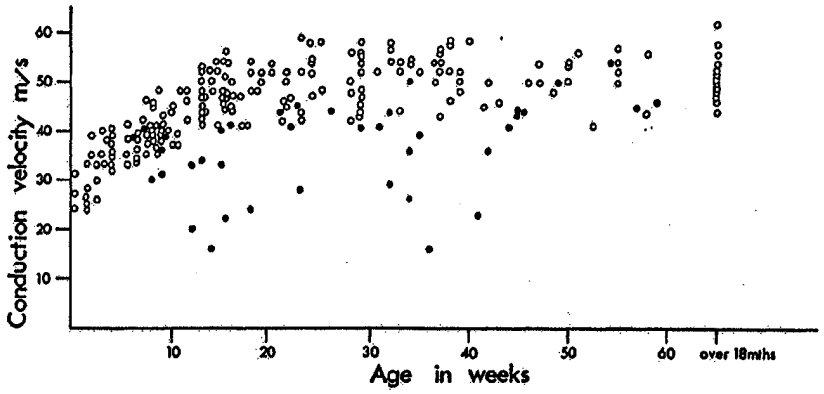

FIG. 4. Maximal motor nerve conduction velocity in relation to age. Open circles are from control animals and filled circles from lead-poisoned animals.

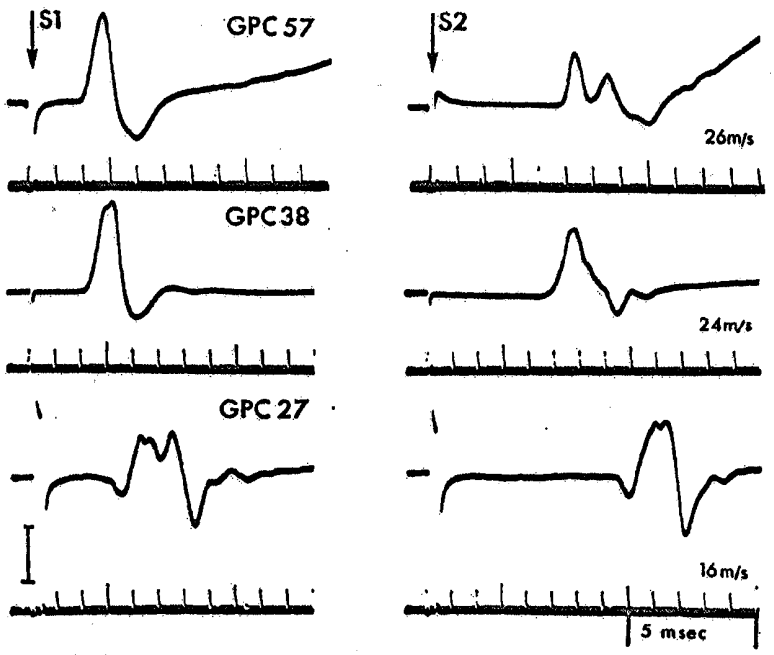

FIG. 5. Muscle action potentials evoked by supramaximal nerve stimulation at ankle (S1) and in thigh (S2) in 3 lead-poisoned animals. Calibration mark = 2 mV for GPC 57, 1 mV for GPC 38, 500 μV for GPC 27. Conduction velocity in metres *per* second given for each animal.

TABLE 1

Comparison of Findings in the Right and Left Hind Limb of Poisoned Animals with Abnormal Nerve Conduction Velocity

Animal	Conduction velocity (m/sec.)	
	Right	Left
GPC 8	34	38
GPC 10	Inexcitable	Inexcitable
GPC 29	38	34
GPC 57	33	34
GPC 77	36	34
GPC 95	22	21

Late Components of the Muscle Action Potential

Dispersed muscle action potentials may be recorded from healthy animals that have been kept in small cages for several months due to slow conduction in the plantar nerves in the hind foot, only mild changes being seen above the ankle (7). In lead-poisoned animals dispersion could sometimes be shown to

be due to slowing of conduction in the leg. The records from guinea-pig F12 shown in Figure 6 illustrate this point. In Figure 6a a long duration muscle action potential can be seen, recorded through belly-tendon electrodes with supramaximal stimulation of the sciatic nerve in the thigh. In order to determine the conduction velocity over a short segment of nerve in the thigh, a second needle was inserted through the skin to lie beside the sciatic nerve, 1.2 cm. distal to the first; a concentric needle electrode was used for recording from the interosseous muscles as illustrated in Figure 6b. By using a weak shock to the sciatic nerve, a single motor unit with prolonged latency could be activated (fig. 6b). For this motor unit, conduction velocity in the thigh was only 8 m/sec., whereas conduction velocity in the fastest surviving motor

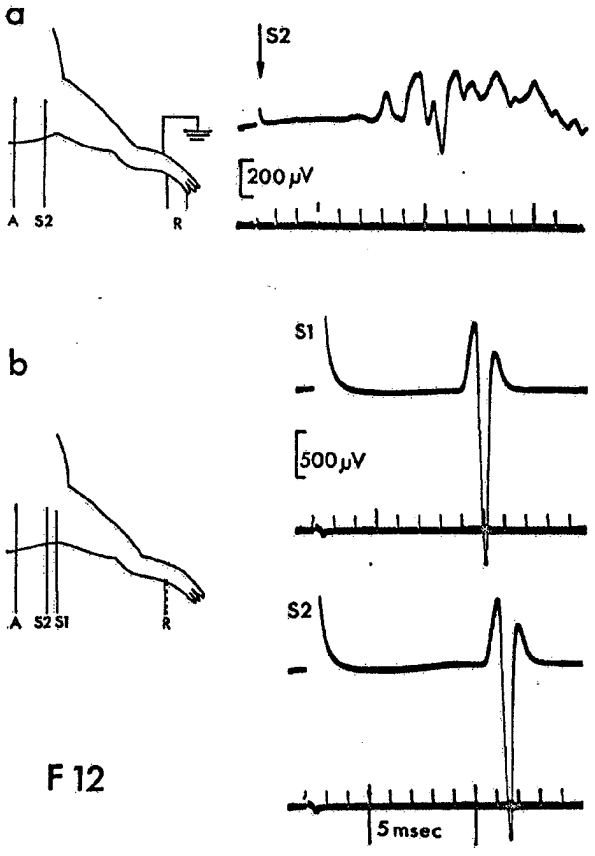

FIG. 6.(a). A prolonged polyphasic muscle action potential evoked by supramaximal stimulation of sciatic nerve in thigh in a lead-poisoned animal. Belly-tendon recording electrodes. (b). Single motor unit action potentials evoked by weak shocks to sciatic nerve at two levels in thigh in the same animal, recorded through a coaxial needle electrode. Conduction velocity 8 m/sec. for this motor unit.

TABLE 2

The Number of Animals with Normal and Abnormal Conduction Velocity after Different Durations of Poisoning

Duration of poisoning (weeks)	No. of animals examined	No. of animals with reduced conduction velocity	Percentage with reduced velocity
Young animals			
2-8	12	4	33
9-15	13	8	62
16-22	6	2	33
Adult animals			
2-8	3	0	0
9-15	6	1	17
16-22	18	5	28

fibres was 16 m/sec. Thus, it may be assumed that the dispersion of the action potential was due to pathological and unequal slowing of conduction velocity in different nerve fibres in the thigh and leg.

Time Course of Electrophysiological Abnormalities in Poisoned Animals

The original intention was to record conduction velocity at frequent intervals in each animal during poisoning, but since some of the animals died during or shortly after an anaesthetic was given, conduction velocity was measured at longer intervals. Detailed information about the development of abnormal conduction is, therefore, not available for more than a few animals.

However, it has been possible to obtain some indication of the time-course of development of slowing of conduction by comparing the results for all the animals. Young and adult animals have been treated separately, since different dosage schedules were used in the two groups. The number of animals with normal and abnormal conduction velocities after different durations of poisoning are shown in Table 2. In some animals conduction velocity was measured during 2 or 3 of the periods shown. It can be seen that one-third of the young animals, but no adult animals developed slowing of conduction velocity after less than 2 months poisoning; abnormal conduction velocity was found in only 1 adult animal within 4 months. The larger doses of lead acetate given to young animals probably account for the earlier onset and higher proportion of young animals with neuropathy.

Figure 7 shows serial conduction velocities for one young and one adult animal. Guinea-pig GPC 95, aged 2 to 3 weeks at the beginning of the experiment, was given 1 g/kg. lead acetate 5 times a week. It can be seen that conduction velocity fell below the control range after 5 weeks poisoning and was markedly reduced (22 m/sec.) when the animal was killed after receiving lead acetate for 13 weeks. Guinea-pig GPC 29 was aged 14 weeks at the beginning of the experiment, and lead acetate was given in a dose of 0.5 or 1 g/kg 2 to 4 times a week for 14 weeks, except for one period of 2 weeks when dosing was stopped because the haemoglobin level was below 6 g/100 ml.

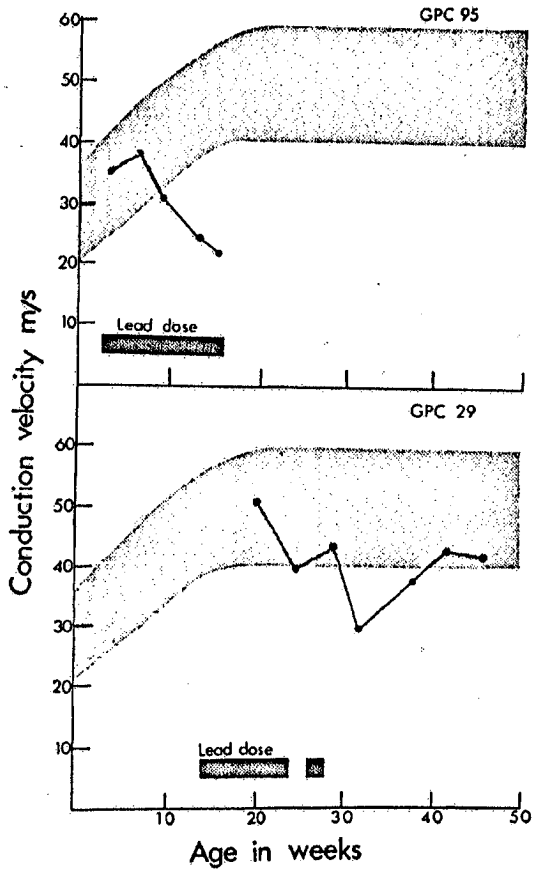

FIG. 7. Conduction velocities calculated from serial records during lead poisoning in a young animal (GPC 95), and an adult animal (GPC 29). The shaded areas represent the range of conduction velocity in control animals of different ages.

After 14 weeks, dosing was again stopped because of a low haemoglobin level. One week later the maximal conduction velocity was normal, but when re-measured after a further 3 weeks it had fallen to 29 m/sec. It was decided to allow the animal to recover and no more lead acetate was given. Maximal conduction velocity gradually returned to within the control range, although remaining below the first value that was obtained for this particular animal.

Histological Studies

Histological examination was carried out on peripheral nerves from 8 control animals and 52 animals which had been given lead acetate for periods ranging from a few days up to a year. The sciatic nerve in the thigh was examined in all animals and in 25 of them other peripheral nerves (posterior

tibial, sural or radial) were also examined in order to assess the distribution of the lesions.

Peripheral nerves from the 8 control animals were entirely normal. Nerves from 21 of the 52 lead-poisoned animals were also normal, but the remainder showed varying degrees of abnormality.

Type of Abnormality in Lead-Poisoned Animals: Most of the poisoned animals showed both segmental demyelination and axonal degeneration of peripheral nerves.

Nerves undergoing segmental demyelination showed characteristic changes when single fibres were stained by osmium tetroxide. Examples of different stages of demyelination are illustrated in Figures 8 and 9. A normal node of Ranvier is shown in Figure 8a and may be compared with the widened node in Figure 8b. This widening of the node has been emphasized by earlier writers as the earliest stage of segmental demyelination (38, 3). A longer length of demyelinated nerve is shown in Figure 8c and is labelled 1. The same partly demyelinated segment is shown at higher magnification in Figure 8d. As Gombault pointed out, demyelination need not necessarily spread to involve a whole internode, but recovery may occur with remyelination of part of an internodal segment. An example of this is shown in Figure 8c and is labelled 2. This thinly remyelinated segment is shown at higher magnification in Figure 8e, the Schmidt-Lantermann incisures being clearly visible. Intercalated internodes of this sort are similar to those described by Lubinska (19).

If demyelination does not stop at the stage shown in Figure 8c, it may spread to involve a whole segment. When this occurs, several short segments are formed during recovery. This process is illustrated in Figure 9. A single fibre is shown at low magnification in Figure 9a–d, consecutive parts of the

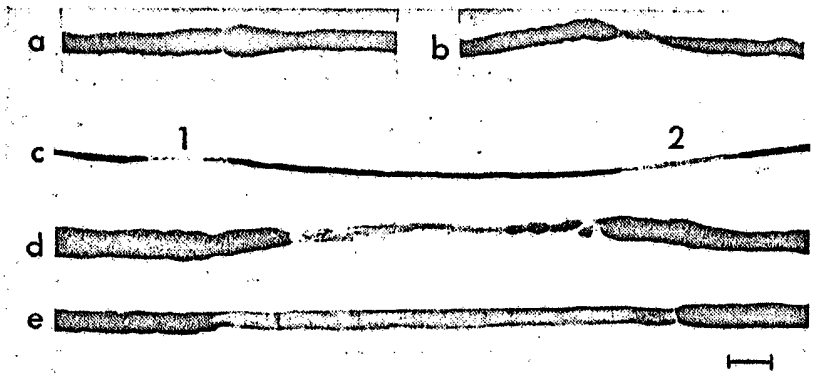

FIG. 8. Teased single fibres stained with 1 per cent osmium tetroxide to illustrate various stages of segmental demyelination. a. Normal node of Ranvier; b. Widened node of Ranvier from poisoned animal (F12); c. Fibre from poisoned animal (GPC 28) showing at 1, a short length of demyelination; at 2, remyelination of a previously demyelinated region; d. Demyelinated region 1 shown at higher magnification c. Remyelinated region 2 shown at higher magnification Calibration—20 μ for a, b, d, e; 75 μ for c.

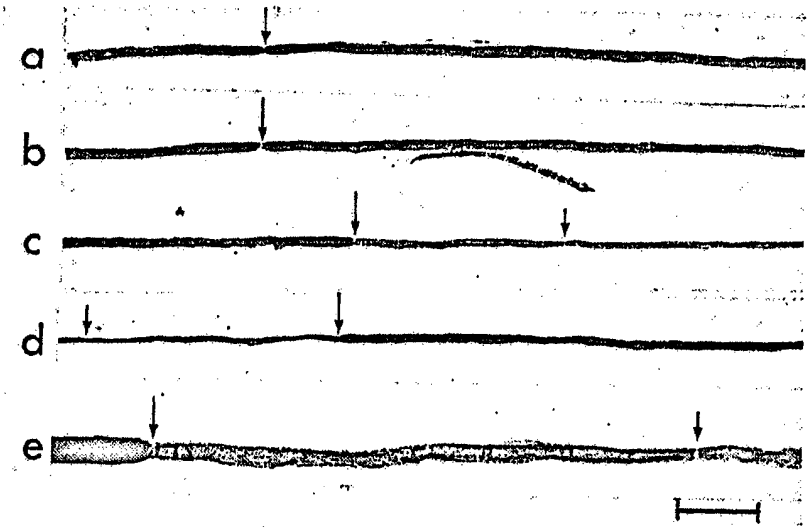

FIG. 9a–d. Consecutive lengths of a single fibre from a poisoned animal (GPC 38) stained with 1 per cent osmium tetroxide. Original nodes are indicated by long arrows, and new nodes in remyelinating segment are shown by short arrows in (c) and (d). (e) One of the newly formed short internodal segments is shown at higher magnification. Calibration—100 μ for a–d; 40 μ for e.

same fibre being mounted one below the other. Long arrows over the nodes of Ranvier emphasize the regular spacing of the nodes. One complete segment (shown in fig. 9c and d) is in the process of remyelination, and short arrows mark the points where two new nodes have been formed. One of the new segments from Figure 9c is shown at higher magnification in Figure 9e.

Different stages of segmental demyelination and remyelination were usually found in the same animal and often in different parts of the same fibre. Short remyelinated segments such as those shown in Figures 8e and 9 were more commonly seen than the totally demyelinated appearance shown in Figure 8d. This may be related to the fact that the poisoning is of long duration.

The appearance of axonal degeneration in a single fibre is shown in Figure 10a. The fibre is abnormal throughout its length and large ovoids of degenerating myelin may be seen. Two ovoids from this fibre are shown at higher magnification in Figure 10b. This appearance is indistinguishable from the changes occurring during Wallerian degeneration following nerve section.

When fibres regenerate following axonal degeneration they have uniformly shorter internodal segments in relation to their diameter than normal nerves (37). An example of a segment from a regenerating fibre is shown in Figure 10c. Fibre diameter and internodal distance were measured for 10 adjacent segments of this fibre. The mean of three measurements of diameter was taken for each segment and was between 4 and 5 μ for all of them. Inter-

nodal distance for the 10 segments ranged from 0.15 to 0.28 mm. In normal sciatic or lateral popliteal nerves the internodal length of a fibre with diameter of 4 to 5 μ would be expected to be between 0.4 and 0.6 mm (8). It is, therefore, clear that the fibre illustrated in Figure 10c is regenerating. In addition, a residual fragment of myelin debris can be seen attached to the fibre.

When longitudinal sections of peripheral nerves are stained by a silver method with luxol fast blue-cresyl fast violet, axons can be seen as well as myelin sheaths. The fibre between the arrows in Figure 11a is undergoing segmental demyelination, and the darkly staining axon can be seen in continuity across the figure. Normal myelin sheaths show a prominent neurokeratin network, and this can be seen where the intact myelin sheath surrounds the axon on the right of the figure. At the centre and left of the figure there is no myelin sheath around the axon, but many small globules of degenerating myelin are present. The fragmentation of axons as well as of myelin sheaths in axonal degeneration is shown in Figure 11b. Several large ovoids of degenerating myelin can be seen in the middle of the figure and remains of the axis cylinder can be identified as darkly staining bands within the myelin ovoids. The loss of continuity of the axon makes it certain that this fibre is in the process of axonal degeneration rather than segmental demyelination.

Degree of Abnormality in Individual Animals: Nerves from 31 of the 52 poisoned animals were histologically abnormal. The results are shown in Tables 3 and 4 for the young and adult animals respectively. Segmental demyelination alone was present in 8 animals, and axonal degeneration alone in 5. Both types of pathological change were present in the same nerve in the remaining 18 animals. Axonal degeneration alone was usually seen in animals that died after receiving large doses of lead acetate over a short period (less than 2 months). Segmental demyelination without axonal degeneration was

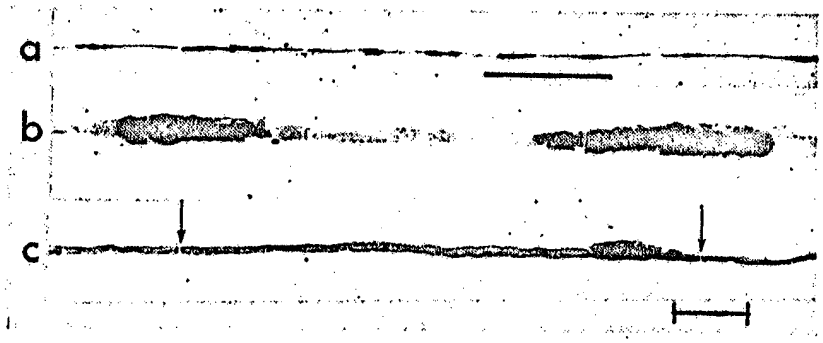

FIG. 10.(a) Single fibre from a poisoned animal (GPC 38) stained with 1 per cent osmium tetroxide to show axonal degeneration; (b) Part of fibre indicated by a line in (a) at higher magnification to show two myelin ovoids; (c) Internodal segment in a regenerating fibre from poisoned animal (GPC 38). Arrows indicate new nodes. Residual myelin debris is present beside fibre. Calibration—175 μ for a, 30 μ for b, 40 μ for c.

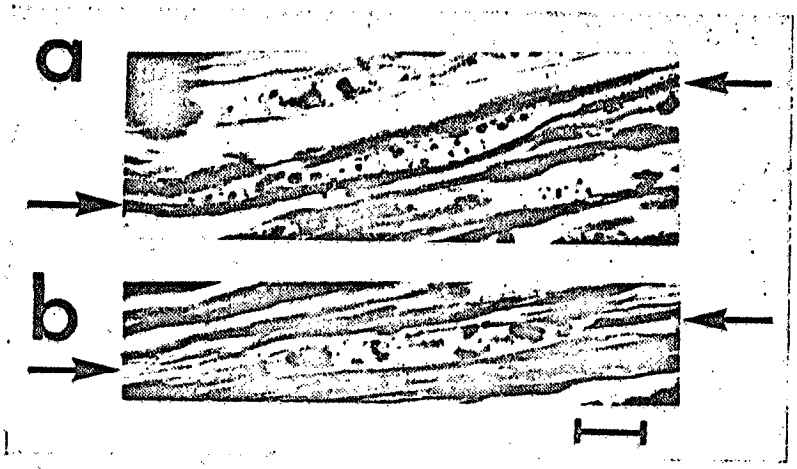

FIG. 11. Longitudinal sections of posterior tibial nerve from a poisoned animal (F12), stained by Holmes silver method with luxol fast blue-cresyl fast violet. (a) The fibre between the arrows shows segmental demyelination. A normal myelin sheath surrounds the axon at the left of the figure; in the middle and on the right myelin debris surrounds an intact axon; (b) The fibre indicated by the arrows shows axonal degeneration. Remains of axis cylinder can be seen within myelin ovoids. Calibration—20 μ.

TABLE 3

Conduction Velocity and Peripheral Nerve Histology in Young Animals, Aged 2 to 3 Weeks at Beginning of Experiment

Animal	Duration of poisoning (weeks)	Dose of PbAc (g/kg.)	Lowest conduction velocity (m/sec.)	Segmental demyelination	Axonal degeneration
GPC 8	19	37.6	34	+++	—
GPC 9	12	44.8	20	Not obtained	
GPC 10	13	58.0	Inexcitable	—	+
GPC 12	40	60.5	28	Not obtained	
GPC 14	28	55.4	34	+	+
GPC 32	6	30.0	36	—	++
GPC 38	15	36.5	24	+++	+
GPC 41	48	56.7	50	+	—
GPC 42	1½	13.5	—	+	—
GPC 80	13	54.0	33	+	+++
GPC 82	7	27.0	30	+++	++
GPC 95	13	47.5	22	++	+++
GPC 96	4	16.0	29	+++	+
GPC 97	4	16.0	39	—	+
GPC 98	13	54.0	40	++	++
F 11	12	32.0	41	+	+
F 12	10	27.0	16	+++	++

+ = Any fibres abnormal; ++ = moderate number of abnormal fibres; +++ = many or most fibres abnormal.

TABLE 4

Conduction Velocity and Peripheral Nerve Histology in Adult Animals (Age is Given for the Beginning of the Experiment)

Animal	Age (weeks)	Duration of poisoning (weeks)	Dose of PbAc (g/kg.)	Lowest conduction velocity (m/sec.)	Segmental demyelination	Axonal degeneration
GPC 23	10	26	35.5	39	++	+
GPC 27	14	22	24.0	16	++	++
GPC 28	14	28	32.2	36	+++	+
GPC 29	14	38	21.5	29	Not obtained	
GPC 35	9	20	28.0	41	++	+
GPC 46	12*	21	16.2	53	+	—
GPC 54	13*	18	14.0	41	+	+
GPC 57	15	39	55.5	26	+	—
GPC 58	19	7	17.0	—	—	+++
GPC 59	15	29	29.0	41	+	+
GPC 63	15	43	50.2	50	+	—
GPC 65	29	1½	4.0	—	+	+
GPC 71	25	18	27.0	—	+++	+
GPC 75	30	29	34.0	56	++	—
GPC 77	12	20	45.0	36	Not obtained	
GPC 78	12	4	8.5	—	—	+
GPC 93	35	31	37.0	45	+++	+
GPC 100	21	6	9.5	—	+	—
GPC 101	21	46	64.0	23	Not obtained	

* Approximate age estimated from weight.

\+ = Any fibres abnormal; ++ = moderate number of abnormal fibres; +++ = many or most fibres abnormal.

more commonly seen in animals that had received smaller doses of lead over a long period. Six of the 8 animals in which only segmental demyelination was found had been poisoned for between 4 and 11 months. The relation between the duration of poisoning, dose and type of pathological change was, however, not exact, and there was considerable individual variation in the findings in animals on similar dosage schedules.

Although axonal degeneration was present in 23 animals, regenerating fibres of the type shown in Figure 10c were rarely found. In most of the animals sufficient time had elapsed for axonal regeneration to have occurred and our failure to find it suggests that this type of change was usually accompanied by irreversible neuronal damage.

Distribution of Lesions: The sciatic nerve was examined in all the 52 poisoned animals. In addition, the posterior tibial nerve was examined in 23 animals, the radial nerve in 1, and the sural nerve which contains only cutaneous sensory nerve fibres in 6 animals. In each animal, the changes were similar in degree in the different nerves examined, indicating that the pathological process was diffuse.

Fibre Diameter: Transverse sections of posterior tibial nerves, fixed in Flemming's solution and stained to show myelin sheaths, were used for measurement of fibre diameter. Photographs were taken at a magnification of ×250 and enlarged to a final magnification of ×1000. The external diameter of all the myelinated nerve fibres was measured to the nearest 1 μ by matching against circles in a perspex sheet, as described by Thomas and Fullerton (35).

Posterior tibial nerves from a control animal and from a lead-poisoned animal are shown in Figure 12. The fascicles marked by arrows in Figure 12

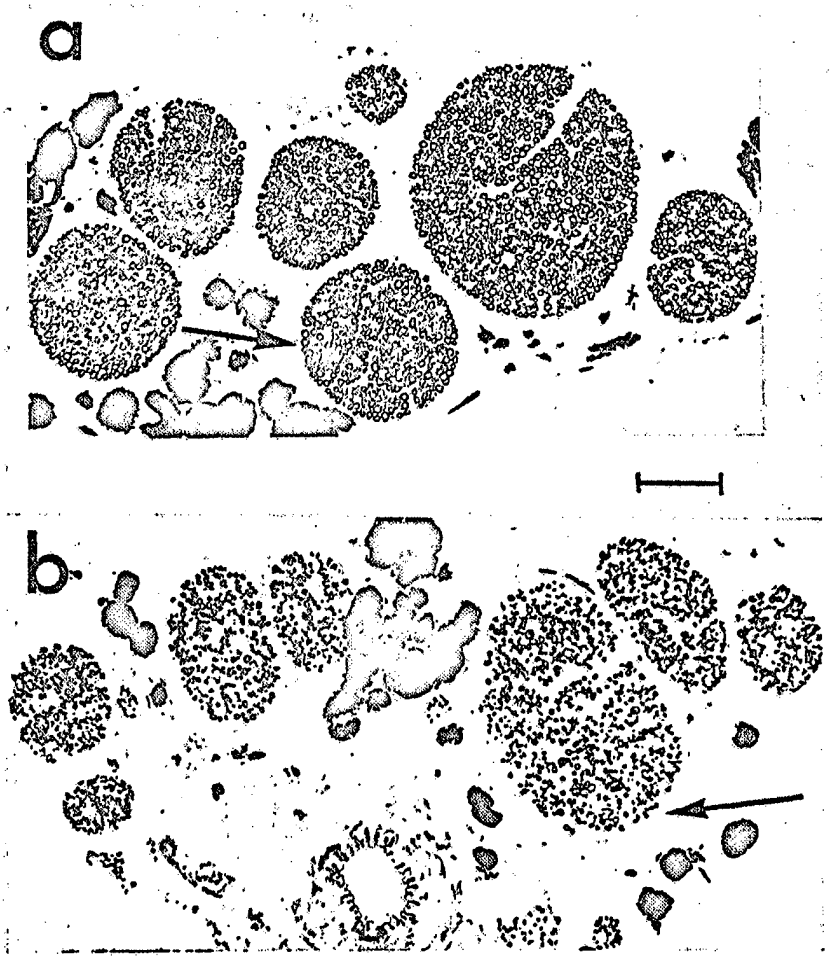

FIG. 12. Transverse sections of posterior tibial nerves fixed in Flemming's solution and stained by the modified Weigert method. (a). Control nerve; (b) Poisoned animal F12; Calibration—100 μ.

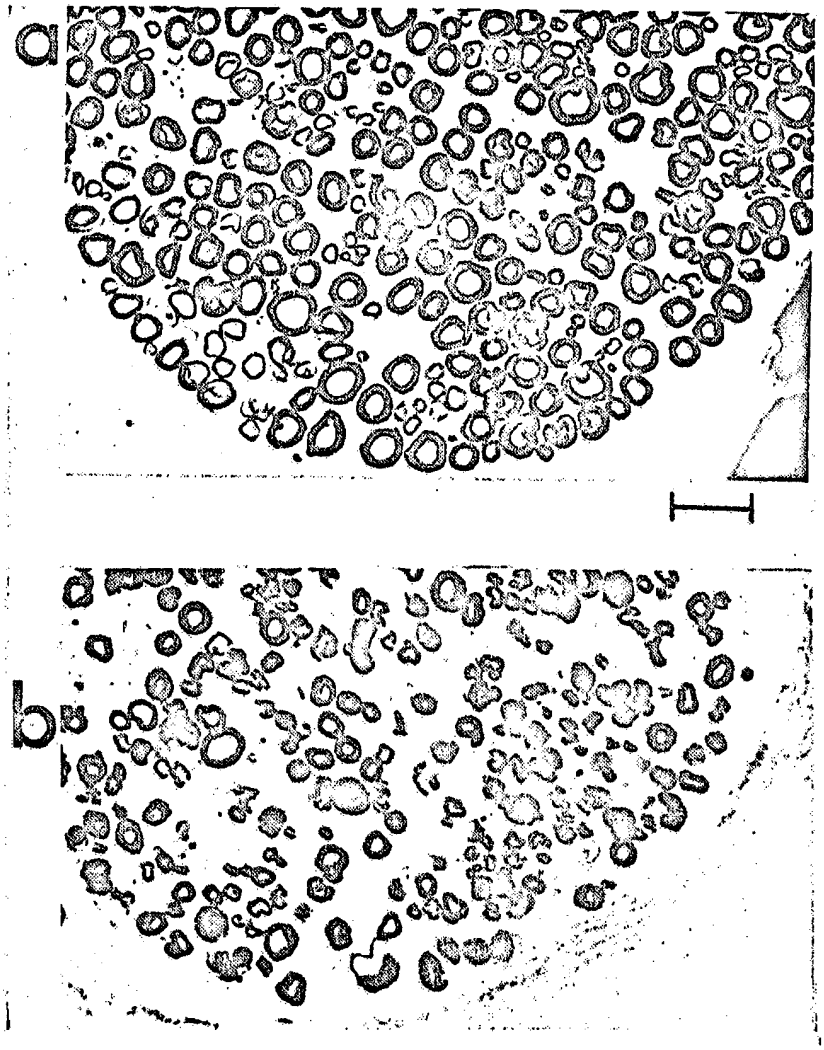

FIG. 13. Fascicles marked by arrows in Fig. 12 at higher magnification to show loss of large myelinated fibres in poisoned animal; (a) Control nerve; (b) Poisoned animal F12; Calibration—25 μ.

are shown at a higher magnification in Figure 13. It can be seen that there is a diffuse reduction in the number of nerve fibres in the poisoned animal.

Histograms of fibre diameter for 2 control animals and 2 lead-poisoned animals (F12 and GPC 38) are shown in Figure 14. Their ages were between 13 and 18 weeks at the time of death. It can be seen that in the normal nerves there was a bimodal distribution of fibre diameter with peaks at 4 μ and 8 μ.

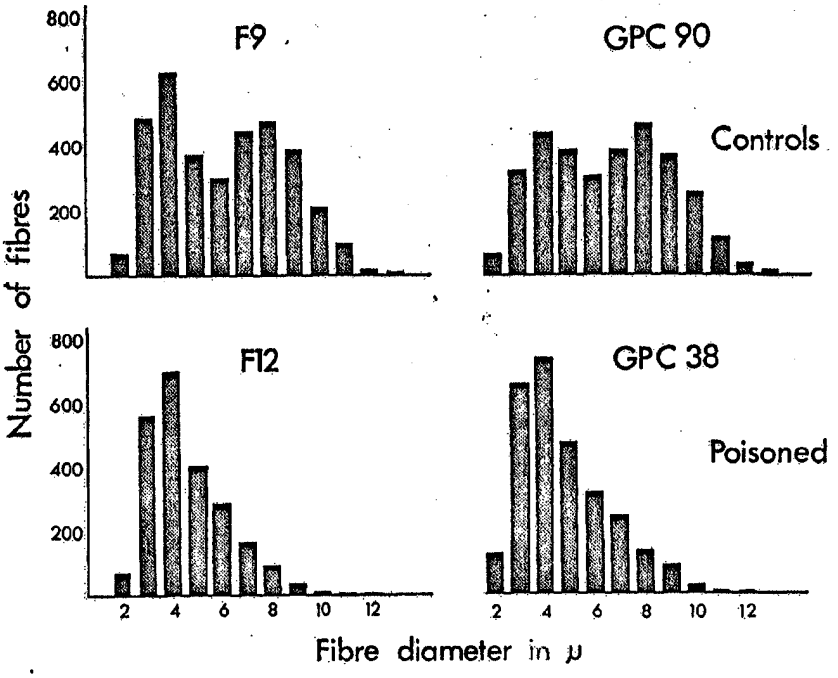

FIG. 14. Histograms showing distribution of diameters of all myelinated fibres in the posterior tibial nerves of 2 control animals and 2 lead-poisoned animals.

In the poisoned animals there was a marked reduction in large diameter fibres but no decrease in the number of small diameter fibres.

It might be suggested that the histograms indicate that large diameter fibres are more severely affected than those of small diameter. However, fibres originally of large diameter will appear of smaller diameter if the section is taken through a thinly remyelinated segment. This would cause an apparent decrease in large diameter fibres and increase in small diameter fibres in the histogram. The nerves of guinea-pigs F12 and GPC 38 did in fact contain a number of remyelinated segments. From the data available, therefore, it is not possible to be certain that large diameter fibres are more severely affected by the poison than those of small diameter.

Spinal Cord: Lumbar segments of the spinal cord were examined from 3 control animals and 8 lead-poisoned animals, all of which had shown some axonal degeneration in the peripheral nerves. In spite of this no definite abnormality of anterior horn cells could be found.

DISCUSSION

The present experiments have confirmed Gombault's (10) observation that guinea-pigs with chronic lead poisoning may show histological changes of segmental demyelination as well as axonal degeneration in their peripheral

nerves. The clinical findings in the two studies were also similar, in that none of Gombault's animals became paralysed, and in the present experiments only a few of the animals showed signs of weakness. The remainder appeared to have normal power and coordination of the limbs, even when profound electrophysiological and histological changes were present in the nerves. The absence of severe paralysis in animals with lead poisoning is in contrast to the findings in guinea-pigs suffering from diphtheritic neuropathy studied in the same laboratory (27). These latter animals were often severely paralysed and unable to walk or stand. Diphtheritic and other experimental neuropathies have been produced by a single dose of a toxic agent and the nerve lesions all develop simultaneously. By contrast, lead neuropathy only occurs after exposure to the toxin for many weeks or months, and different stages of demyelination and remyelination are often present at the same time in different nerve fibres. This may account for the preservation of normal function of the limbs.

The electrophysiological studies described in the present work have shown that motor nerve conduction velocity was reduced below the control range in a number of guinea-pigs given lead by mouth for long periods. The question of whether this was due to actual slowing of conduction in individual fibres or whether it was due to selective failure of conduction in the most rapidly conducting fibres must be considered. The study was restricted to motor nerves, and, therefore, has the advantage that fibres with a relatively narrow range of conduction velocities were investigated. Thus, long latency responses such as those shown in Figure 6 could not be due to unmasking of the more slowly conducting fibres present in a normal nerve. This is obvious from the fact that the muscle action potentials evoked by nerve stimulation in a normal animal would have ended before the time of onset of the motor unit potential shown in Figure 6b.

Slowing of nerve conduction has previously been demonstrated in other experimental neuropathies in which segmental demyelination occurs; for example, in diphtheritic neuropathy (22, 16, 27), experimental allergic neuritis (4, 12) and in local lesions produced by pressure (26). It seems probable that the slowing of conduction in lead neuropathy is also associated with segmental demyelination, since it can be seen from Tables 3 and 4 that histological changes of segmental demyelination were found in the nerves of all the animals which showed marked reduction of conduction velocity. In animals in which axonal degeneration but no segmental demyelination was found in the nerves, marked reduction of conduction velocity was not seen.

Conduction is slow in nerve fibres that are regenerating following axonal degeneration (2), but it is highly unlikely that conduction in regenerating fibres accounts for the slowing of conduction in lead neuropathy for two reasons. In the first place it has already been mentioned that such fibres were very rarely found in lead-poisoned animals; furthermore, slowing of conduction was present in some of the young animals too soon after the onset of poisoning for regeneration to have occurred.

The histological changes of segmental demyelination were present in some

nerves for which maximal conduction velocity was normal. This presumably indicates that only a proportion of the fibres in the nerve trunk were affected, some surviving fibres with a normal velocity being present. There is no doubt that teasing of single fibres was a much more sensitive method of histological examination than preparation of stained longitudinal sections of the nerves. Not only were the early stages of demyelination more easily seen in teased fibres, but the late stages of remyelination could only be recognized by comparing internodal distances over several successive segments of a single fibre.

Previous experimental work on chronic lead poisoning suggests that the type of pathological change is not necessarily the same in different species. Segmental demyelination has been described in guinea-pigs given lead by mouth by several authors, some axonal degeneration being also noted (29, 33, 5). On the other hand, axonal degeneration without segmental demyelination has been described in rabbits given lead by mouth or injection (5, 31, 36). Rats have been found to be more resistant to the toxic effects of lead salts than other species, showing only scanty peripheral nerve changes (29). This observation has been confirmed during the present work. Seven rats were given large doses of lead acetate for 6 to 9 months. Only a few fibres undergoing axonal degeneration were seen in nerves from 3 animals, the others being normal. Cats, on the other hand, seem to become paralysed rapidly when exposed to lead salts (18). The predominant change appears to be in the central nervous system, but scanty changes of segmental demyelination and axonal degeneration have been described in peripheral nerves (31, 6).

There has been considerable controversy concerning the pathological changes associated with lead palsy in man, and in view of the species difference in experimental animals, the present experiments in the guinea-pig are not necessarily relevant. It has been suggested that the lesion in man is a myopathy rather than a neuritis (30, 14). However, the evidence for this is poor, and is based mainly on experiments on isolated frog nerve and muscle tissue (1, 30, 32). In fact, axonal degeneration in peripheral nerves has been found on a number of occasions in patients dying from lead poisoning (9, 13, 17, 28). Segmental demyelination has not been described, although specifically looked for on at least two occasions (17, 28).

SUMMARY

Seventy-two guinea-pigs were poisoned with repeated doses of intragastric lead acetate. During poisoning growth was inhibited in young guinea-pigs; in both young and adult animals weight loss, anaemia, and convulsions were seen. Only 9 animals developed paralysis of the hind limbs, which was usually mild.

Motor nerve conduction velocity was estimated in 40 animals, and was found to be reduced in 17 of them. In some cases maximal velocity fell from the normal range of 40 to 60 m/sec. to less than 20 m/sec. In a few single motor nerve fibres velocities as low as 8 m/sec. were recorded.

The peripheral nerves were examined histologically in 52 animals and abnormalities were found in 31. The most common pathological change was a mixture of segmental demyelination and axonal degeneration (18 animals). In 8 animals only segmental demyelination was found, and in 5 animals axonal degeneration was the sole finding. Markedly reduced conduction velocity was only seen in those animals in which segmental demyelination was present.

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