Various Syn. 1085

ARCH. NEUROLOGY & PSYCHIATRY. 17:444-465, Jan.-June 1927

LEAD STUDIES

XIV. EXPERIMENTAL STUDIES OF LEAD PALSY*

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The mechanism by which lead produces paralysis has not been studied thoroughly. Those investigations which have been reported have been almost entirely of a clinical and pathologic nature. Recent contributions to the chemistry and physiology of muscular contraction[1] and of the behavior of lead within the organism have suggested new methods for further study of the location and mechanism of the action of lead on nerve and muscle tissue, and the experiments described in this paper are the result of this newer knowledge.

LITERATURE

Among the older investigators, Gusserow[2] was the first to obtain evidence suggestive of the direct action of lead on muscle. He reported that relatively large amounts of lead may be recovered from the muscles of rabbits with lead poisoning, but his observation was questioned by Heubel[3] and has never been confirmed. These experiments, however,

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1. Fletcher, W. M.: The Survival Respiration of Muscle, J. Physiol. **23**: 10, 1898-1899; The Relation of Oxygen to the Survival Metabolism of Muscle, ibid. **28**:474, 1902; The Influence of Oxygen on the Survival Respiration of Muscle, ibid. **28**:354, 1902: The Osmotic Properties of Muscle and the Modifications in Fatigue and Rigor, ibid. **30**:414, 1903-1904; Lactic Acid Formation, Survival Respiration and Rigor Mortis in Mammalian Muscle, ibid. **47**:361, 1913-1914. Fletcher, W. M., and Hopkins, F. G.: Lactic Acid in Amphibian Muscle, J. Physiol. **35**:247, 1906-1907. Hopkins, F. G.: The Chemical Dynamics of Muscle, Bull. Johns Hopkins Hosp. **32**:359 (Nov.) 1921. Hill, A. V.: The Mechanism of Muscular Contraction, Physiol. Rev. **2**:310, 1922. Meyerhoff, O.: Die Energiewandlungen im Muskel: I-VI. Arch. f. d. ges. Physiol. **182**:232 and 284, 1920; **185**:11, **188**:114, **191**:128, 1921; **195**:22, 1922. Embden, G., and Laquer, F.: Ueber die Chemie des Lactacidogens, Ztschr. f. physiol. Chem. **93**: 181, and **113**:1, 1916-1917. Embden, G., and Adler, E.: Ueber die physiologische Bedeutung des Wechsels des Permeabilitätszustandes von Muskelfasergrenzschichten, Ztschr. f. Physiol. Chem. **118**:1, 1922.

2. Gusserow, A.: Untersuchungen ueber Bleivergiftung, Arch. f. path. Anat. **21**:443, 1861.

3. Heubel, E.: Pathogenese und Symptome der chronischen Bleivergiftung, Berlin, A. Hirschwald, 1871.

drew attention to the possibility that lead might affect muscular activity. Muson[4] is reported to have immersed frogs in water containing lead and to have produced palsy of the hind limbs, as far as could be judged from voluntary effort and reflex response. Harnack[5] injected lead triethyl acetate into the dorsal sac of frogs and observed rapid exhaustion of muscular contractility and, in a short time, complete loss of excitability. He thought that muscle rather than nerve or nerve-ending was affected because direct stimulation of muscle evoked no stronger response than did stimulation through the nerve, and also because similar changes in muscular activity could be obtained with curarized frogs after injection of lead triethyl acetate. Since the toxicity of this compound depends not only on lead, but also on the triethyl group, these experiments cannot be taken as criteria of the action of lead on muscular tissue.

One of the phenomena observed by Harnack in the muscles that he considered poisoned with lead was delay in relaxation after stimulation. The same retardation of relaxation was seen by Cash,[6] who treated frogs with lead acetate. However, his work was entirely uncontrolled and most of his positive results were obtained at a warm temperature which in itself may affect the activity of muscles from cold-blooded animals. In well controlled experiments on frogs, Mellon[7] determined the effect of lead on fatigued muscle. To fatigue the gastrocnemius of one side, he stimulated it for several days and then injected lead acetate into the dosal sac. After excision, the previously fatigued muscle was able to perform much less work than that of the other side. All these investigators suggested that lead produces a definite physiologic lesion in muscle tissue.

Dozzi[8] raised the question whether lead might not affect isolated nerve as well as muscle. He used both nerve-muscle preparations (sciatic-gastrocnemius) from the same frog and immersed one nerve in physiologic sodium chloride solution and the other in isotonic lead acetate solution. In the nerve exposed to lead, the excitability, as judged by muscular response to minimal nerve stimulation, diminished markedly and finally disappeared completely. These experiments, however, can hardly be considered of much value because the lead solution was so

4. Muson, J. J.: Lead Poisoning in Frogs, New York M. J., July, 1877; quoted in Centralbl. f.-d. med. Wissensch. 16:480, 1878.

5. Harnack, E.: Ueber die Wirkungen des Bleis auf den tierischen Organismus, Arch. f. exper. Path. u. Pharm. 9:152, 1878.

6. Cash, J. T.: The Contraction of Frog's Muscle After Administration of Lead, Arch. f. exper. Path. u. Pharm. 59: (supp. vol.); 93:106, 1908.

7. Mellon, R. R.: The Relation of Fatigue to Paralysis Localization in Plumbism, Arch. Int. Med. 12:399 (Oct.) 1913.

8. Dozzi, L.: Studi sull'azione del piombo: azione del piombo sulla eccitabilità dei nervi motori, Sperimentali 66:666, 1912.

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concentrated (27 mg. of lead per cubic centimeter); the acidity, although not mentioned by Dozzi, must necessarily have been greater than is physiologic, and finally, the lead solution contained no balancing ions. This summary indicates that further knowledge of the action of lead on the function of the neuromuscular system is needed. Some of the investigations in this laboratory have suggested a means of studying the subject further. In experiments dealing with the effect of lead on red blood cells,[9] it was found that lead alters the surface of the corpuscle so that its permeability to water is changed. The question arises, therefore, as to whether lead may not act similarly on the surface of muscle and thus change its permeability. Various workers have studied the passage of substances through the surface of muscle in both directions under different conditions, and before describing our experiments it may be well to review briefly some of their results. Fletcher,[10] who studied the effect of fatigue and rigor on the intake of water by the isolated muscle of frogs, observed the development of marked variations of permeability from the normal. In his early work he also investigated the carbon dioxide output of muscle.

Experiments on the production and diffusion of lactic acid have been performed on isolated muscles by Fletcher, Hopkins,[11] Hill[12] and Meyerhoff.[13] In determinations of the diffusion of inorganic phosphates from muscle during rest and activity, Embden and his co-workers[14] have shown that inorganic phosphate is an intermediary product of glucose metabolism which diffuses in increased amounts during muscular activity. Since the quantity of inorganic phosphate in the muscle does not increase, the process of its formation is apparently reversible; Embden therefore concluded that the increased diffusion of inorganic phosphate is due to greater permeability of the cell membrane during activity and not to the production of more phosphate. Of the various methods of studying the changes in permeability of muscle, Embden's is the most satisfactory for our purpose, because of the ease of determining quantitatively the inorganic phosphate in solution; it has proved valuable in studying the effect of lead on the permeability of the surviving muscle of the frog.

METHOD OF STUDY

The technic of the experiments carried out in this laboratory was as follows:

9. Aub, J. C.; Reznikoff, P., and Smith, D. E.: Lead Studies. III. The Effect of Lead on Red Blood Cells, J. Exper. Med. 40:151, 173 and 189 (Aug.) 1924.

10. Fletcher (footnote 1, sixth reference).

11. Hopkins (footnote 1, seventh reference).

12. Hill (footnote 1, eighth reference).

13. Meyerhoff (footnote 1, ninth reference).

14. Embden et al. (footnote 1, tenth and eleventh references).

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Both gastrocnemius muscles were removed from pithed frogs with as little injury as possible. They were handled only by the bone or tendon; they were treated in exactly the same way, and all manipulations were as nearly simultaneous as the procedure permitted. Each was placed in a beaker of known weight, containing 15 cc. of Ringer's solution,[15] and the weight of the muscle was determined. Both were kept in the solution through which oxygen was bubbled constantly to prevent rigor (Fletcher). After standing for one hour at room temperature, the muscles were raised above the fluid, carefully washed with Ringer's solution, and transferred to a second beaker containing fresh Ringer's solution. This procedure was repeated hourly until the phosphate content of the solution became low and practically constant. After this stage had been reached, in most cases within two or three hours after excision, one muscle was placed in Ringer's solution containing lead chloride (0.05 mg. of lead per cubic centimeter) and allowed to stand for one hour at 30 C.; the other was kept in the regular Ringer's solution under identical conditions. In a few experiments the muscle was exposed to lead before the lowest value was reached. The muscles were then washed and placed in fresh Ringer's solution hourly as before, at room temperature. The Ringer's solution in each beaker was analyzed for inorganic phosphate, corresponding solutions from the control and "leaded" muscles being examined at the same time. In these determinations, a modification of the Bell-Doisy[16] method was used. No preliminary protein precipitation was necessary, and because the quantity of phosphate present was small the results were determined more accurately in Nessler tubes than in a colorimeter. The entire volume of fluid was compared with standards which were prepared for each test from stock "blood standard" phosphate solution. Relatively small differences in the phosphate content of these standards produced such marked differences of color that there was an error of only between 0.0005 and 0.0010 mg. of phosphate for each reading. The Ringer's solution containing lead was not analyzed, because the phosphate that diffused from the muscle combined with the lead as an insoluble lead phosphate and could not be determined by this method.

RESULTS

Before the results of these experiments are presented in detail, it is necessary to point out that the tests were made at different times of the year, whenever animals could be obtained, and that therefore frogs of different species and of varying weights had to be used. Consequently, too much stress cannot be laid on an exact quantitative interpretation of the results. However, the figures show definitely that the diffusion of inorganic phosphate (mg. of phosphorus per gram of muscle per hour) increases markedly after "leading." Of thirty-five experiments, thirty-four demonstrated this clearly. Control muscles underwent no such

15. The Ringer's solution contained: sodium chloride, 0.6 per cent; potassium chloride, 0.03 per cent; calcium chloride, 0.02 per cent. No phosphate or carbonate was added because lead forms insoluble phosphate and carbonate. The p_H was kept at 6.5, since lead hydroxide precipitates in more alkaline solutions.

16. Bell, R. D., and Doisy, E. A.: Rapid Colorimetric Methods for the Determination of Phosphorus in Urine and Blood, J. Biol. Chem. 44:55 (Oct.) 1920.

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change, except for a relatively slight increase in diffusion following stimulation in some experiments, but the diffusion curve followed the same course as that in Fletcher's carbon dioxide experiments, which was characterized by an immediate rapid decline succeeded by a gradual decrease in the rate of diffusion. Figures 1 and 2 show graphically the results of two striking experiments in which the increase in the diffusion of phosphate after exposure to lead is marked. The data from the

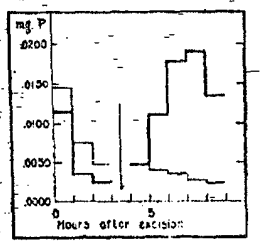

Fig. 1.—Effect of lead on the rate of diffusion of inorganic phosphates from muscle. The heavy line represents the rate from "leaded" muscle and the light line that from the control muscle. The arrow represents the period of exposure to lead (0.05 mg. of lead as lead chloride per cubic centimeter of Ringer's solution).

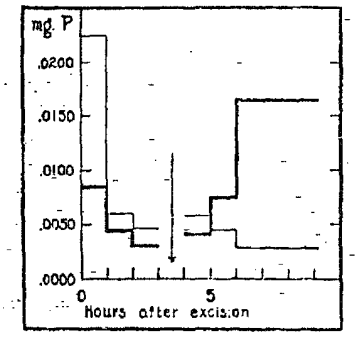

Fig. 2.—Effect of lead on the rate of diffusion of inorganic phosphates from muscle. The heavy line represents the rate from "leaded" muscle and the light line that from the control muscle. The arrow represents the period of exposure to lead (0.05 mg. of lead as lead chloride per cubic centimeter of Ringer's solution).

thirty-four positive experiments, collected in table 1, demonstrate variations in the degree of rise in phosphate diffusion; in the interval between the addition of lead and the appearance of the maximal increase; in the duration of the increase and in the return of the rate of phosphate diffusion to the minimum level.

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TABLE 1.—*The Diffusion of Inorganic Phosphate from Normal and "Leaded" Muscle**

Number of Experiment	Content of Ringer's Solution in Mg. of Phosphorus per Gm. of Muscle per Hour: Minimum Diffusion from "Leaded" Muscle	Corresponding Value of Control Muscle	Maximum Diffusion from "Leaded" Muscle after Exposure to Lead	Corresponding Value of Control Muscle	Per Cent Variation in Phosphate Diffusion: "Leaded" Muscle	Control Muscle in Corresponding Time	Duration of Increased Diffusion from "Leaded" Muscle: Before Return to Normal, Hours	Without Return to Normal, Hours	Development of Maximum Diffusion after "Leading," Hours	Duration of Maximum Diffusion, Hours
1	0.002	0.010	0.011	0.011	+450	+10	...	4	4	1
2	0.003	0.012	0.026	0.008	+770	−50	...	4	3	2
3	0.005	0.006	0.017	0.006	+240	0	...	4	2	2
4	0.003	0.013	0.015	0.008	+400	−40	...	4	3	1
5	0.003	0.004	0.011	0.006	+270	+50	...	4	3	1
6	0.004	0.007	0.010	0.005	+150	−30	...	4	2	1
7	0.003	0.005	0.015	0.004	+400	−20	...	5	2	3
8	0.003	0.004	0.007	0.004	+130	0	...	5	3	2
9	0.002	0.003	0.008	0.003	+300	0	...	5	3	2
10	0.004	0.006	0.014	0.006	+250	0	...	5	2	1
11	0.005	0.006	0.017	0.004	+240	−30	9	..	3	−1
12	0.005	0.007	0.011	0.008	+120	+10	7	..	3	1
13	0.007	0.009	0.015	0.009	+110	0	...	7	6½	1
14	0.008	0.004	0.018	0.015	+130	+30	...	7	6½	1
15	0.008	0.008	0.010	0.004	+ 50	−50	4	..	3	1
16†	0.003	0.005	0.016	0.003	+430	−40	....	5	3	3
17	0.002	0.005	0.019	0.003	+850	−40	...	5	4	1
18‡	0.001	0.002	0.002	0.002	+100	0	...	4	4½	..
19§	0.006	0.007	0.023	0.005	+250	−30	...	4	2½	2
20#	0.003	0.004	0.020	0.003	+570	−30	...	8	4	−2
21‡	0.004	0.006	0.008	0.004	+200	−20	...	8	8	..
22#	0.005	0.006	0.016	0.005	+250	−20	...	8	7	1
23§	0.002	0.003	0.008	0.003	+300	0	...	7	4½	4½
24‡	0.003	0.003	0.011	0.003	+270	0	....	6½	7	..
25‡	0.001	0.001	0.013	0.002	+1200	+100	...	6½	6½	..
26#	0.011	0.009	0.024	0.013	+120	+40	....	6¼	3	1
27#	0.006	0.005	0.020	0.003	+230	−40	7½	..	2	1
28#	0.002	0.005	0.012	0.002	+ 50	−60	....	9½	7½	1
29‡	0.005	0.008	0.017	0.003	+ 40	0	....	8½	7¼	..
30‡	0.006	0.009	0.019	0.012	+220	+30	....	5¼	2	1
31‡	0.002	0.005	0.023	0.004	+1050	−20	....	6	5½	1
32‡	0.008	0.005	0.021	0.009	+150	+10	...	4	4	..
33‡	0.010	0.010	0.024	0.011	+140	−30	...	3	2	1
34‡	0.013	0.016	0.027	0.014	+110	−10	...	4	4	..

* Average increase of phosphate diffusion from "leaded" muscle, +320 per cent. Average decrease of phosphate diffusion from control muscle in corresponding period, −10 per cent.
† Still maximal at last determination.
‡ Muscles stimulated. Experiment stopped while diffusion was maximal.
§ Muscles stimulated. Still maximal at last determination.
# Muscles stimulated.

TABLE 2.—*Increase of Diffusion of Inorganic Phosphate from "Leaded" Muscle**

Per Cent Increase after "Leading"	Number of Experiments
Less than 100	3
Between 100 and 200	9
Between 200 and 300	10
Between 300 and 400	2
Between 400 and 500	4
Between 500 and 600	1
Between 600 and 700	0
Between 700 and 800	2
Between 800 and 900	1
More than 1,000	2

* Average change of phosphate diffusion from "leaded" muscle, +320 per cent. Average change of phosphate diffusion from control muscle, −10 per cent.

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A summary of these results (table 2) shows that the average rate of diffusion increased, after exposure to lead, 320 per cent above the lowest value. In only three cases was this increase less than 100 per cent. In nine experiments it was between 100 and 200 per cent of the minimum, in ten between 200 and 300 per cent, and in two more than 1,000 per cent. During the same period the average rate of phosphate diffusion from the control muscles decreased 10 per cent. In the composite curve of all the experiments (fig. 3), the marked difference in phosphate diffusion between the "leaded" and control muscles is clear. It should be pointed out that in some of the experiments (ten) the low value, before the beginning of the rise caused by lead, occurred late and that therefore the composite chart, showing the average diffusion for each hour, does not give as good an indication of the degree of change as can be obtained from the study of the individual experiments in table 1. The length of time elapsing after the removal of the muscles from the lead solution before the diffusion of phosphate became maximal

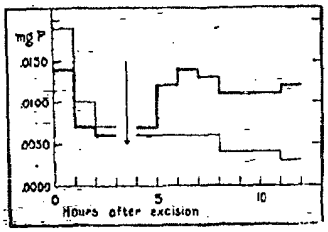

Fig. 3.—Composite chart showing effect of lead on the rate of diffusion of inorganic phosphates from muscle. The heavy line represents the rate from the muscle exposed to lead solution and the light line that from the control. The arrow represents the period of exposure to lead (0.05 mg. of lead as lead chloride per cubic centimeter of Ringer's solution).

varied from two (seven experiments) to eight hours (one experiment). In the majority of cases (ten experiments), the peak was reached within three hours. Of the twenty-seven experiments which were continued after the peak was reached, eighteen showed a sustained maximal diffusion for only one hour, and six for two hours. In the other three, the diffusion remained maximal until the experiment was terminated—three hours in two cases, and four and one-half hours in one case. In only four experiments did the rate of diffusion return to the low control value during the course of the observations. In one case the curve did not return to its original low level even nine and one-half hours after exposure to lead.

Factors other than exposure to lead may alter the rate of phosphate diffusion. Injury increases the permeability of muscle to inorganic phosphate; but in this series of experiments the results obtained in the

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controls indicated that this was negligible. That stimulation increases the rate of diffusion was shown by Embden; but the degree of increase is never of the same magnitude as that caused by lead. In twenty-nine experiments with normal muscles, stimulation increased the diffusion so little that the figures usually fell well within the limits of experimental error. Increases of between 0.001 and 0.002 mg. of phosphorus per gram of muscle per hour followed only eight of 100 stimulations; and only once did the output increase as much as from 0.008 to 0.016 mg. In this case, stimulation was so frequent that rigor probably resulted. In most of our experiments, rigor, which of course increases the rate of diffusion of phosphate markedly, was avoided by providing a constant supply of oxygen and by preventing undue fatigue. Although the relationship between rigor and the muscular changes caused by lead cannot be considered in detail here, it will be of interest to remember that rigor is supposed to be due to the acid products of fatigue. Examination of muscles after exposure to lead demonstrates that they are shrunken, of rubbery consistency and lusterless—in fact, comparable in appearance to muscles in rigor.

Other observations merit consideration. If a "leaded" muscle is treated with ammonium sulphide and examined under the microscope, all the lead appears on the surface and none can be demonstrated deep in the muscle. Another fact is also of interest in this connection. When a muscle is exposed to lead, there is a change in the reaction of the surrounding solution, in one case from p_H 6.5 to 4.8; in a second experiment to p_H 5.5, and in a third to p_H 6.1. Such an increase in acidity agrees with the results of our work on the effect of lead on red blood cells, which show that soluble lead salts unite with the inorganic phosphate to form insoluble lead phosphate with the liberation of free acid. To determine the effect of acid on the permeability to inorganic phosphate, acid Ringer's solution was added to muscles. In one case in which the p_H of the Ringer's solution was 4.5, the permeability of the treated muscle remained practically like that of the control, but in two experiments in which the p_H of the Ringer's solution was 3.5, the rate of phosphate diffusion increased markedly at once. The maximum increase was brief, but throughout the experiment the rate of diffusion remained constant and did not decrease as did that of the control. In one case it remained twice as high as the original rate before exposure to acid. The maximum increase in diffusion due to acid was 140 per cent in one of those experiments, and in the other 520 per cent. This type of experiment, however, is not comparable to those in which the muscle is exposed to a lead salt, for in the latter acid is produced in the tissue of the muscle and the buffer phosphates must be diminished by their interaction with lead. These acid experiments offer further evidence that when lead salts act on isolated muscle the reaction that probably occurs is similar to that demonstrated with red blood cells.

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Such a striking change in the permeability of the surface of muscle to inorganic phosphate suggests that lead may have a demonstrable effect on the activity of isolated muscles. To determine this, experiments were performed in which nerve-muscle preparations (sciatic-gastrocnemius) were removed from pithed frogs and placed in Ringer's solution in specially constructed chambers. One muscle was exposed to lead (0.05 mg. of lead per cubic centimeter) and the other was kept in Ringer's solution as a control. They were stimulated both directly and through their nerves, and the rate of onset of fatigue and the degree of recovery were noted. The apparatus illustrated in figure 4 was devised for these procedures.

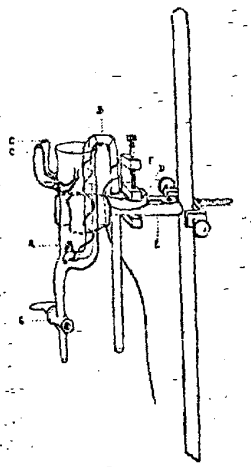

Fig. 4.—Nerve-muscle stimulation and diffusion chamber.

The muscle is attached by the Achilles tendon to the platinum hook *A*, which is fastened to the bottom of the oxygen inlet tube *B*. The chamber itself, made of glass, is 8 cm. high and 2.5 cm. wide. About 2.5 cm. from the top two glass tubes, *C*, *C* (4 mm. in diameter) are sealed into the wall of the chamber. Through these, platinum wires run into the chamber so that when the tubes are filled with mercury they may serve as stimulating electrodes for the nerve. The oxygen inlet tube runs through the cork *D* which is held firmly against the chamber *E* by means of a heavy screw clamp *F*. Wound about the platinum hook *A* and the oxygen inlet tube is a fine insulated wire which permits the hook to serve as one of the muscle stimulating electrodes. The other muscle electrode consists of a platinum S-shaped hook run through the knee joint and attached to one end of a fine copper wire which has been made soft by heat. The other end of this wire is fastened to an ordinary muscle lever for recording muscular contractions. At the bottom of the muscle chamber a stopcock *G* is inserted, through which Ringer's solution may be admitted and removed by gravity pressure. With such an apparatus, the muscle may be con-

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tinually bathed in Ringer's solution which can be changed at regular intervals and through which oxygen may be bubbled constantly, so that both the rate of phosphate diffusion and the character of muscular contractions can be readily determined.

In each experiment two such chambers were used, one for the control and the other for the "leaded" muscle. The electrodes were connected in series so that both nerves or both muscles were stimulated simultaneously by the same current. This permitted direct and controlled comparison of the two muscles, and made unnecessary an exact regulation of the stimulating current in the different experiments. The recording levers (the ordinary straw variety) were of equal length; they were always afterloaded, and were usually weighted with from 5 to 10 Gm. In any given experiment the same weight was applied to both levers at the same distance from the fulcrum. The stimulating electrodes were attached to the secondary post of a standard student's induction coil. A metronome made and broke the primary current at regular intervals. In the secondary circuit a rocking key, which permitted the current to be switched to either nerve or muscle, was inserted. In almost all experiments the stimuli were maximal, and in every case control records were made before lead was added. Usually nerve and muscle were stimulated alternately.

Thirteen experiments were performed with tetanic stimulation. In eight cases the control muscle remained in good condition, although the "leaded" muscle became fatigued rapidly. In most experiments it did not regain its normal condition. Figure 5 illustrates one experiment in which the muscle was stimulated directly and the rate of phosphate diffusion was determined hourly (fig. 6). A description of the experiment follows:

a. m.	
8:58	In first Ringer's solution
9:48	Nerve stimulated, 1
9:50	Muscle stimulated, 2
9:58	In second Ringer's solution, 20.3 C.
11:02	Upper muscle in Ringer's solution, lower in lead solution, 31.5 C.
p. m.	
12:08	In third Ringer's solution, 20.9 C.
12:12	Muscle stimulated, 3
12:18	Muscle stimulated, 4
12:24	Muscle stimulated, 5
12:38	In fourth Ringer's solution, 20.8 C.
12:48	Muscle stimulated, 6
1:38	In fifth Ringer's solution, 20.9 C.
2:03	Muscle stimulated, 7
2:19	Muscle stimulated, 8
2:38	In sixth Ringer's solution
3:02	Muscle stimulated, 9
3:25	Muscle stimulated, 10
3:31	Muscle stimulated, 11
3:38	Removed to sixth Ringer's solution, 20.7 C.

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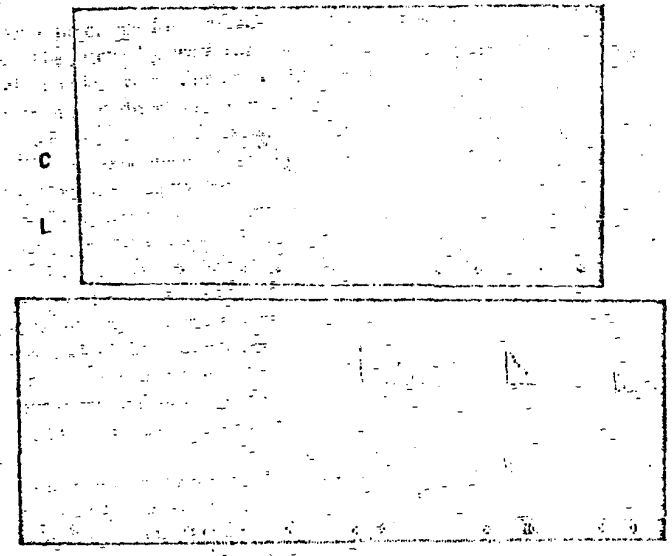

Fig. 5.—Muscular response to intermittent tetanic stimulation before and after "leading": *C*, tracing made by the control muscle; *L*, tracing made by the "leaded" muscle (0.05 mg. of lead as lead chloride per cubic centimeter of Ringer's solution). The figures indicate the periods of stimulation; the crosses represent readjustment of the after-loading of the muscles.

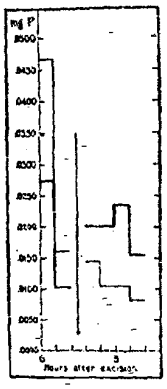

Fig. 6.—Variations of the rate of inorganic phosphate diffusion from normal and "leaded" muscles during tetanic stimulation. The heavy line represents the rate from the "leaded" muscle (0.05 mg. of lead as lead chloride per cubic centimeter of Ringer's solution) and the light line that from the control muscle.

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This experiment demonstrates several interesting facts. The "leaded" muscle became fatigued much more rapidly than the control (3 and 4) and failed to recover (5). Apparently lead alone cannot cause immediate injury to the muscle, for the "leaded" muscle gave excellent initial contractions after removal of the lead-Ringer's solution (3), but fatigue followed a series of such contractions rapidly. This occurred in all experiments and demonstrates that the action of lead on

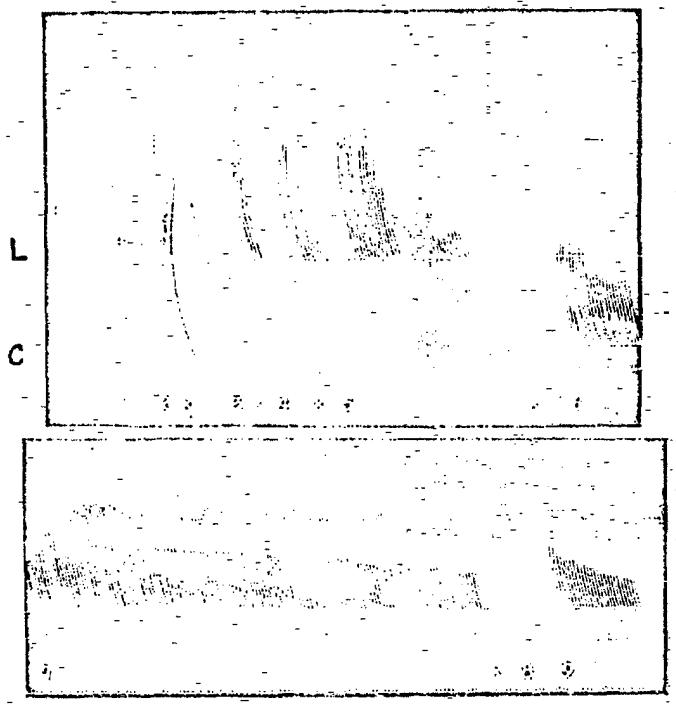

Fig. 7.—Muscular response to make and break stimulation: *L*, the tracing made by the "leaded" muscle (0.05 mg. of lead as lead chloride per cubic centimeter of Ringer's solution); *C*, that of the control.

muscle is manifested only after muscular activity occurs. Comparison of the diffusion curve and the muscle tracing shows that the greatest quantity of phosphate diffuses during the first, second and especially the third hour after exposure to lead (third, fourth and fifth Ringer's solution) when the muscle is most fatigued. The slight return of contraction evident at 9, 10 and 11 occurs simultaneously with a decrease in the rate of diffusion.

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In five of the thirteen experiments, no difference in the rate of onset of fatigue in the control and "leaded" muscles could be seen. In these cases the muscles performed an excessive amount of work, and rapid fatigue ensued. In none of these tests, however, did the control muscle fatigue more rapidly than the "leaded" muscle.

Since control muscles seemed to be fatigued so readily with tetanic stimulation, a series of experiments (ten) was performed with make and break stimulation. The results showed definitely that after an initial period of sustained uniform contraction, the "leaded" muscle became fatigued much more rapidly and completely, and recovered with much greater difficulty than the control in every case. Figure 7 illustrates one of these experiments, in which the levers were weighted with only 2.5 Gm., so that an excessive amount of work was not performed. These investigations, therefore, indicate that lead interferes markedly with the function of isolated muscle. Stimulation produces fatigue much more rapidly after exposure to lead, while recovery, slight at best, in most cases does not occur at all.

EFFECT OF LEAD ON NERVE

Because of the clinical designation of lead palsy as a peripheral neuritis as well as of the occurrence of pathologic lesions in peripheral nerves, the impression is prevalent that lead acts specifically on peripheral nerve. The only experimental study of this problem reported in the literature was made by Dozzi and has already been shown to be of little value. The action of lead on isolated nerve has been investigated in this laboratory by means of the Adrian[17] narcosis chamber. In these experiments the standard procedure was to excise the two nerve-muscle preparations of a frog, place the sciatic nerve of each in a narcosis chamber, and between the point of stimulation and the muscle to treat one with normal Ringer's solution as a control and the other with a Ringer's solution, of the same hydrogen ion concentration, which contained lead chloride. The muscles were placed in moist chambers, and the nerves were stimulated by the electrodes of the two narcosis chambers connected in series. In four experiments the conductivity, as judged by muscular response, was exactly the same in both nerves. In one of these the strength of lead used was 0.05 mg. of lead (as lead chloride) per cubic centimeter of Ringer's solution; in two, the concentration was 0.1 mg., and in the fourth the nerve was treated with 0.23 mg. of lead per cubic centimeter of Ringer's solution for one hour, and then for another hour with Ringer's solution containing 0.46 mg. per cubic centimeter, the largest quantity soluble in Ringer's solution of p_H 6.5. In none of these experiments could any difference between

17. Adrian, E. D., and Lucas, K.: On the Summation of Propagated Disturbances in Nerve and Muscle, J. Physiol. 44:68, 1912.

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the conductivity of the "leaded" and control nerves be detected. At the end of the test, the nerves were always placed in a solution of ammonium sulphide to determine whether there had actually been a deposition of lead; the invariable black discoloration of the "leaded" nerves indicated that this had occurred. As far as can be judged from this work, therefore, lead produces no decrement in nerve conduction.

To check these results an attempt was made to determine the action currents of both nerve and muscle as a delicate index of function by the string galvanometer, lent by Dr. Alexander Forbes and Miss Anne Hopkins, who assisted us in these experiments. In one of a pair of nerve-muscle preparations the nerve, and in the other the muscle, was exposed to lead. Because a great number of stimulating and leading-off electrodes must be placed in a small space for such work, there was great difficulty in avoiding artefacts. Further complications were introduced by the necessity of performing all manipulations simultaneously on both "leaded" and control structures. In one test a distinct action current was seen in the "leaded" nerve, but not in the "leaded" muscle. This experiment was, however, unsatisfactory because both muscles were not fatigued at the same time. Another experiment in which this defect was avoided demonstrated distinctly that after fatigue the action current of the "leaded" muscle diminished rapidly and soon disappeared entirely, while that of the control muscle returned to normal within a short while and persisted until the end of the experiment. Similar, undiminished action currents were obtained from both normal and "leaded" nerves. Thus, the distinct inhibitory action of lead on isolated muscle and the lack of deleterious action on isolated nerve are confirmed.

PALSY IN VIVO

In the explanation of lead palsy, as it occurs in life, the experiments already described serve merely as preliminary and suggestive observations which must be correlated with the production of palsy in living animals if they are to have any important practical significance. The ample evidence that, in life, lead paralysis develops in muscles that are fatigued [18] links the problem of lead palsy with the chemistry of muscular fatigue and suggests a method of investigation in intact animals.

18. Meyer, M.: Die Elektrizität in ihrer Anwendung auf praktische Medizin, Berlin, 1854, quoted by Weill. Moebius: Ueber einige ungewöhnliche Fälle von Bleilähmung, Centralbl. f. Nervenheilk. **1**:6, 1886; quoted by Stieglitz: Eine experimentelle Untersuchung ueber Bleivergiftung mit besondere Berücksichtigung der Veränderungen am Nervensystem, Arch. f. Psychiat. **24**:49, 1892. Weill, G.: Zur Frage von der Lokalization der Bleilähmung, Inaug. Diss. Strassburg, 1892. Edinger, L.: Der Anteil der Funktion an der Entstehung von Nervenkrankheiten, Wiesbaden, Bergmann, 1908, p. 67. Teleky, L.: Zur Kasuistik der Bleilähmung, Deutsche Ztschr. f. Nervenh. **37**:234, 1909. Oliver, T.: Lead Poisoning, London, H. K. Lewis, 1914, p. 148.

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Attempts were made to produce palsy in the frog. Lead was injected frequently into the dorsal sac, and one leg was fatigued by intermittent stimulation over the sciatic nerve for several days. In most of the experiments there were no differences between fatigued and control muscles and when any difference could be found there was always a question of injury to tissue. There are two objections to this method: first, lead salts administered subcutaneously are precipitated at the site of injection and are therefore only slightly and slowly absorbed; second, the mere response to electrical stimulation probably results in neuromuscular junction fatigue without necessarily causing fatigue in the muscle comparable to that obtained by lifting weights.

Consequently, an attempt was made to produce palsy in mammals by fatiguing the extensor muscles of one limb by voluntary contraction. At first rabbits were used, but they did not prove as satisfactory as cats which later were used exclusively. A lead carbonate suspension was injected into the lungs of some animals through the trachea, and others were kept as controls. In order to produce unilateral fatigue, weights varying from 70 to 100 Gm. were attached with adhesive plaster to the dorsal surface of the right forepaw of all animals. These rested on cotton pads, and since they apparently did not disturb the animals they were removed only to allow occasional inspection of the limb. The animals were exercised daily in a revolving drum (lent by Dr. M. J. Rosenau) to produce a marked degree of fatigue in the extensor muscles of the right forepaw by constant raising of the weights. When a distinct difference between the weighted limb and the other became apparent, the animals were allowed to rest for twenty-four hours with the weights removed. Then, under urethane anesthesia, the musculospiral nerves (radial) and the extensor muscles were exposed and submitted to various physiologic tests. The threshold of these muscles to nerve stimulation, and in some experiments to direct stimulation, was determined. Attempts also were made in some cases to obtain action currents, and to demonstrate differences between the degree of fatigue in the weighted and unweighted muscles following stimulation, but because of difficulties inherent to the experiments, these usually proved unsuccessful. In all experiments the electrodes were connected in series, so that both sides were stimulated equally and simultaneously.

Before the experimental results are considered in detail, a brief description of the typical behavior of the animals may be of interest. After the administration of lead, all the cats lost weight rapidly, and within one or two weeks showed distinct lead lines and became easily fatigued by exercise. The weighted foot showed distinct signs of weakness after about two weeks of exercise. This was manifested by difficulty in extending the foot when the weight had been removed for twenty-four hours, and was especially marked if the animal was held

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by its neck and permitted to reach for the top of a window ledge. Some of the animals limped decidedly in walking. When the weights were still attached, the contrast between the "leaded" and control animals was even more striking. Those suffering from plumbism could not lift the weighted paw without great effort and then only with the aid of the upper leg muscles, while the control animals lifted both paws with equal ease. After the animals had been run in the drum, these differences were accentuated. The "leaded" cats tired much more quickly and to a greater degree than did the normal animals and exhibited a striking fatigue of the weighted limb, which usually completely prevented extension of the paw. They took shorter steps with the weighted limb while

TABLE 3.—*Threshold Determinations in Rabbit 489*

Stimulation of	Voltage of Primary Current	Stimulation with	Threshold Stimulus Position of Secondary Coil	
			Left Side, Cm.	Right (Weighted) Side, Cm.
Nerve	2	Break	10	6.25†
Nerve	3	Break	13	8.5
Muscle	3	Break	13	8.5
Muscle*	3	Break	11	7.75
Muscle	3	Break	12.25	8.0

* Poles of secondary reversed.
† The lower the figure, the greater the current.

TABLE 4.—*Threshold Determinations in Rabbit 493*

Stimulation of	Voltage of Primary Current	Stimulation with	Threshold Stimulus Position of Secondary Coil	
			Left Side, Cm.	Right (Weighted) Side, Cm.
Nerve	3	Break	13	13
Muscle	3	Break	8.75	9.25

running and seemed to lean toward the left. This description fits nearly all of the animals, and individual variations will be mentioned in the protocols. The threshold experiments were performed on one "leaded" rabbit and its control and on four "leaded" and two control cats. Of the "leaded" cats, one showed little limitation of extension in the weighted paw before the experiment. One died in convulsions and three of inanition before differences between the weighted and unweighted limbs seemed sufficiently marked to warrant a determination of their thresholds. Two animals died during the operation.

PROTOCOLS

EXPERIMENT 1.

Rabbit 489, "Leaded."—Lead carbonate was injected intratracheally three times. Two hundred milligrams of lead, as lead acetate, was given by the stomach tube five times. The weight attached to the right forepaw was

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60 Gm. The animal was run in the drum for fifty-two days (7.3 miles). Marked stippling of red blood cells was present during this period.

The nerve was too short to permit the recording of action currents. No fatigue record was obtained, as the animal was in too poor condition.

Rabbit 493, Control.—This animal was treated in every way like 489 except that it received no lead.

The most striking feature of this experiment is the similarity between the threshold of the unweighted muscle of the "leaded" animal and that of both unweighted and weighted muscles of the control, when stimulated by nerve with uniform current. In the "leaded" rabbit, the threshold of the weighted muscle to stimulation through the nerve was much higher. There is the same relative difference between thresholds when stimulation is applied directly to the muscle. In the control, the slight difference between the two sides probably falls within the limits of normal variation. The difficulty of applying the muscle electrodes,

TABLE 5.—*Threshold of Radial Nerves and Brachioradialis Longus Muscles in Cat 480*

Stimulation of	Voltage of Primary Current	Stimulation with	Threshold Stimulus Position of Secondary Coil		
			Left Side		Right (Weighted) Side, Cm.
			Cm.	Rotation	
Nerve	4	Break	13	85 degrees	9.5
Muscle	4	Break	12.5		9.0

however, to exactly corresponding points of the muscles detracts from the value of the results obtained from muscle stimulation.

EXPERIMENT 2.

Cat 480, "Leaded."—Lead carbonate was injected intratracheally once. A 60 Gm. weight was attached to the right forepaw. The animal was run in the drum for fifteen days (2.3 miles). The body weight fell from 2.7 to 2.2 Kg. After an interval of seven days the animal was again exercised for eight days (0.42 miles). The body weight was from 2.2 to 2.3 Kg. The weight was changed to 100 Gm. and the animal was run for two days (0.18 miles). The weight was again changed to 60 Gm., and the animal was run for five days (0.44 miles). The body weight was 2.5 Kg. One hundred and fifty milligrams of lead was given by stomach tube twice. With a weight of 80 Gm. attached, the animal was run for five days (0.73 miles). The body weight was 2.7 Kg. The total length of the run was thirty-five days (4 miles).

As the animal became unconscious under urethane anesthesia, the right forepaw was held completely flexed.

An attempt to record action currents from the nerve and muscle of each limb proved unsuccessful because of artefacts; this spoiled the preparation for fatigue tests.

Cat 492, Control.—This animal was treated in every way like 480 except that no lead was administered. Under anesthesia both forepaws were equally active and were held in the same position.

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Since the threshold for stimulation through the nerve was the same on both sides, stimulation was not applied directly to muscle.

In this experiment the difference between the right (weighted) and left paws after exposure to lead is similar to that in the experiment with the rabbits. The threshold of the left, unweighted muscle is apparently normal, similar to those of the control, even after exposure to lead.

EXPERIMENT 3.

Cat 115, "Leaded."—Lead was given intratracheally once. The weight placed on the right forepaw was 70 Gm. The animal was exercised in the drum for a

TABLE 6.—*Threshold of Radial Nerves and Brachioradialis Longus Muscles in Cat 492*

Stimulation of	Voltage of Primary Current	Stimulation with	Threshold Stimulus Position of Secondary Coil			
			Left Side		Right (Weighted) Side	
			Cm.	Rotation	Cm.	Rotation
Nerve	4	Break	13	80-85 degrees	13	80-85 degrees

TABLE 7.—*Threshold Determinations in Cat 115*

Stimulation of	Voltage of Primary Current	Stimulation with	Threshold Stimulus Position of Secondary Coil			
			Left Side		Right (Weighted) Side	
			Cm.	Rotation	Cm.	Rotation
Nerve	4	Break	13	85 degrees	12.5	
Nerve	4	Break	13	85 degrees	13	30 degrees
Nerve	4	Make and break	13	85 degrees	9.5	
Nerve	4	Make and break	13	45 degrees	10.75	
Muscle	4	Break	11		7.5	
Muscle	4	Make and break	7.5		5.5	
Muscle	4	Make and break	7.5		5.5	
Muscle	4	Make and break	7.5		5	

period of forty-two days, during which it rested for an interval of fourteen days (ran 3.3 miles). Body weight during this time fell from 2.8 to 2.5 Kg. and then gradually rose to 3.1.

Records of action currents could not be taken because the string galvanometer was out of order.

In making the fatigue curves the levers were loaded with 30 Gm., and the stimuli (break shocks) were maximal. No difference in the rate of fatigue of the right and left sides could be demonstrated. Neither side was completely fatigued.

Although the difference between the weighted and unweighted muscles is not as striking as in the previous experiments, it is distinct and constant. As this animal had been exercised for only twenty days, the muscles were probably little fatigued. The failure to demonstrate a

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difference in the fatigue curves of the two sides may be due to insufficient weight on the muscles.

Experiment 4.

Cat 107, "Leaded."—Lead was injected intratracheally once. The animal was run in the drum for seventeen days (2.6 miles). One hundred and fifty milligrams of lead was then given by stomach tube on four successive days and a weight of 70 Gm. was attached to the right forepaw. The animal was then run again for fourteen days (2.7 miles). After the first administration of lead, the body weight of the animal fell from 2.5 to 2 Kg. Just before the second administration the weight had risen to 2.3 Kg., but it again fell to 1.9 after the last day of exercise. The animal was weak and in a poor condition.

Table 8.—*Threshold Determinations in Cat 107*

Stimulation of	Time of Stimulation	Voltage of Primary Current	Stimulation with	Threshold Stimulus Position of Secondary Coil: Left Side Cm.	Left Side Rotation	Right (Weighted) Side Cm.	Right (Weighted) Side Rotation
Nerve	2:50 p.m.	2	Break	13	75 degrees	13	45 degrees
Nerve	3:00	2	Make and break	13	45 degrees	13	5 degrees
Nerve	3:05	2	Break	13	75 degrees	13	20 degrees
Nerve	4:15	2	Break	13	85 degrees	13	20 degrees
Nerve	4:17	2	Make and break	13		11	
Nerve	4:45	2	Break	13	45 degrees	12.25	
Nerve	5:35	2	Break	12.25		12.25	
Nerve	6:45	2	Break	11		11	
Nerve	7:45	4	Break	11		10	
Nerve	8:17	4	Break	9		6.25	

Table 9.—*Threshold Determinations in Cat 119*

Stimulation of	Voltage of Primary Current	Stimulation with	Threshold Stimulus Position of Secondary Coil: Left Side Cm.	Left Side Rotation	Right (Weighted) Side Cm.	Right (Weighted) Side Rotation
Nerve	2	Break	13	75 degrees	13	75 degrees

While walking and while becoming narcotized with urethane, it showed signs of distinct extensor weakness in the right forepaw.

Because the electrodes could not be placed on corresponding parts of the muscles, direct muscle stimulation proved unsatisfactory. The animal was in too poor condition to give good action currents. Constant stimulation fatigued the extensors of both sides easily.

Cat 119, Control.—Except that this animal received no lead, it was treated like cat 107.

The ease with which the muscles were fatigued in the "leaded" animal and the rapid rise of threshold on both sides are evidences that the circulation of this animal was poor. Nevertheless, the threshold of the right side was somewhat higher than that of the left except in two instances, when fatigue was setting in rapidly. The constant threshold in the control is noteworthy.

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EXPERIMENT 5.

Cat 131, "Leaded."—Lead was given intratracheally. The weight placed on the right forepaw was 80 Gm. The animal was exercised for a period of fifty-five days (16 miles). The body weight fell from 3 to 1.8 Kg. This animal used the forelimbs to a limited extent in the drum. It sprang across the drum, exerting almost its entire effort with the hind limbs. As far as could be determined from threshold and action currents, there was no diminution in the threshold of the weighted side.

This animal showed good extension of the weighted limb before the operation, and therefore the absence of any increase in threshold is not surprising. In fact, it confirms a certain relationship between the threshold of the muscle and its clinical appearance.

COMMENT

The original purpose of these mammalian experiments was not accomplished. Our object in this study was to localize the lesion of palsy by producing paralysis and then to obtain action currents from muscle and nerve. Only toward the end of the investigation, however, was technic developed by which such action currents could be obtained without artefacts. At this time the "leaded" animals were in a poor condition, and none were really fit subjects for such an experiment. It is clear from these experiments, however, that extensor weakness follows muscular work. Both limbs performed approximately the same movements, but the muscles of one had to do more work because of the attached weights. Consequently, this suggests that lead injury is caused by muscular activity and fatigue.

Proper appreciation of these experiments requires a brief consideration of the chemical relationship between lead in the body and the products of muscular activity. In this laboratory, both Fairhall [19] and Minot [20] have shown that lead is deposited in the bones as insoluble lead phosphate, but when mobilized it is transported by the blood stream as the triple phosphate. Any bodily change which tends to disturb the acid-base equilibrium—for instance, acidosis—liberates lead from the bones and greatly increases the quantity in circulation.[21] This was illustrated by the increased excretion of lead by both patients and animals when they received a diet low in calcium and were treated with either ammonium chloride or acid. Acute respiratory infection seemed to produce the same results in animals. Lead probably acts in this way because the phosphate is very soluble in acids, especially in lactic acid

19. Fairhall, L. T., and Shaw, Charlotte P.: Lead Studies: X. The Deposition of Lead Salts, J. Indust. Hyg. 6:159 (Aug.) 1924.

20. Minot, A. S.: Lead Studies: V. A. B. C. Lead Distribution in the Organism, J. Indust. Hyg. 6:125, 137 and 140 (Aug.) 1924.

21. Minot, A. S., and Aub, J. C.: Lead Studies: XII. The Mechanisms for Storage and Excretion of Lead; to be published.

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which is liberated in large amounts during muscular activity, particularly during fatiguing exercise.[22] Therefore, when lead phosphate circulates through muscle undergoing vigorous exercise, it must be dissolved to a considerable extent by the lactic acid and thus be converted to lead lactate.

If such a soluble lead salt as the lactate comes into contact with a cell, it can unite with the inorganic phosphate present at the surface[23] and form insoluble lead phosphate and free lactic acid. In work already reported,[9] it was suggested that the precipitation of this insoluble lead salt, which is accompanied by the liberation of free acid and the removal of buffer phosphate, changes the colloidal state and the properties of the red cell surface. There is considerable evidence that this also occurs in muscle. Clinical observation has established the fact that the muscles paralyzed are those which are most used. As far as is known, only muscle and not nerve becomes fatigued, and experiments in vitro have demonstrated that lead does not interfere with the function of isolated nerve, whereas it does greatly affect muscle. This change in muscle function is preceded by an alteration of the surface permeability, and it is therefore probable that lead acts on muscle just as on red blood cells by changing the permeability of the surface.

Thus these considerations afford a possible explanation of the development of lead palsy: Lead is transported by the blood as an insoluble phosphate in colloidal state. In regions of muscular activity this is dissolved by the excess lactic acid which diffuses from fatigued muscle cells and is converted into lead lactate. As the soluble lactate comes in contact with inorganic phosphate at the surface of muscle cells, the lead is reprecipitated as insoluble phosphate—a reaction dependent on the relative concentration of lactate and phosphate.

CONCLUSIONS

1. Experiments with isolated nerve-muscle preparations from frogs have shown that the onset of fatigue in muscles which have been exposed to lead is much more rapid and complete than normal.

2. The contractility of "leaded" muscles is often completely lost, and recovery from fatigue is always impaired.

3. When muscles are immersed in Ringer's solution containing lead, the acidity of the solution increases markedly.

4. A change in the permeability of the surface of muscle cell after exposure to lead is evidenced by an increased diffusion of inorganic phosphate from the muscle into the surrounding Ringer's solution.

22. Ritchie, A. J.: The Reaction of Resting and Active Muscle, J. Physiol. 56:53, 1922. Barr, D. P.; Hunwich, H. E., and Green, R. P.: Studies in the Physiology of Muscular Exercise, J. Biol. Chem. 55:495 (March) 1923.

23. Embden and Laquer (footnote 1, tenth reference).

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5. As far as can be seen from the response of muscle to nerve stimulation, lead salts seem to have no deleterious action on the conductivity of the nerve of an isolated nerve-muscle preparation from a frog.

6. In one experiment, records of action currents taken from both nerve and muscle also indicate that lead acts on muscle and not on nerve.

7. Marked weakness or even palsy develops in fatigued extensor muscles of "leaded" rabbits and cats. This was manifested by great difficulty in extending the forepaw.

8. The threshold of these weak muscles to stimulation is much higher than that of unfatigued muscles or of the corresponding muscles in control animals.

9. Neither lead nor fatigue alone causes palsy, but both are necessary to establish the condition. This is indicated by experiments in vitro with isolated nerve-muscle preparations and by the similarity between the threshold values of unfatigued muscles of "leaded" animals and the muscles of the controls.

10. Such experiments indicate that the physiologic lesion of lead palsy is in the muscle itself and that the muscles which are fatigued are most susceptible to lead paralysis.

11. An attempt has been made to explain susceptibility to lead palsy on the basis of the chemical and physiologic reactions between the metabolic products formed during muscular activity and lead as it occurs in the human organism.

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