the response of positively established conditioned reactions.

It is concluded that insulin coma and electroshock may act specifically on inhibited conditioned reactions without causing dedifferentiation.

I am much indebted to Miss Kathryn Seese for her help in this work.

15016

Postmortem Studies on Hypertensive Rats Chronically Intoxicated with Lead Acetate.

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Detailed descriptions of the clinical picture of chronic lead poisoning are found in almost all acceptable textbooks of general medicine. The accompanying hypertension in some cases of the disease has been the subject of many publications, but this problem is still unsolved. Exhaustive studies on the pathologic changes that may occur in chronic plumbism, especially among humans, have served to a certain extent to complicate the problem. Vigdortchik[1] states that "nephrosclerosis is not necessarily a companion of lead hypertonia; in most cases hypertonia is accompanied by no kidney modifications although work with lead undoubtedly predisposes to some kidney trouble and to nephrosclerosis in particular. Such kidney modifications in lead cases occur about 3 times as often as in non-lead cases." Belknap[2] denies the presence of hypertension among human beings chronically intoxicated with lead and in contact with the poison for a period of 5 to 20 years. Other investigators[3,4,5] claim that lead is perhaps the only vascular poison, and intoxication in man leads to hypertension not only during the acute episodes, but also in chronic plumbism. They agree that prolonged exposure to the poison results in an arteriosclerotic nephritis.

Fouts and Page[6] failed to produce hypertension in dogs chronically intoxicated with lead. They suggest further studies of this phenomenon on human beings since the evidence on which it is based is very slim. Dominguez failed to produce hypertension in rabbits with either lead acetate or lead carbonate.[7] Nevertheless, Griffith and Lindauer[8] were successful in producing a hypertonia in rats within 30 to 80 days after daily administration of 70 mg of lead by stomach tube.

The object of the experiment reported herein was to study the pathologic changes that occur in the tissues of rats which have developed hypertension following chronic lead poisoning.

Method. Thirty young and healthy albino rats were employed in this experiment. Lead acetate in doses of 90 mg (70 mg of lead) was administered daily for 50 days with omission of every 7th day. The poison was dissolved in 2 cc of water and was given by stomach tube.

Before the administration of the first dose of lead the systolic blood pressure of all rats was measured by the indirect method devised by Griffith.[9] These readings varied between 90 and 130 mm Hg. Subsequent blood pres-

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2 Belknap, E. L., *J. Indust. Hyg. and Toxicol.*, 1936, **18**, 380.

3 Fishberg, A. M., *Hypertension and Nephritis*, Ed. 4, 1939, Lea and Febiger Co., Philadelphia.

4 Zadek, E., *Z. f. Klin. Med.*, 1931, **116**, 241.

5 Teleky, cited by Zadek.[4]

6 Fouts, P. J., and Page, I. H., *Am. Heart J.*, 1942, **24**, 329.

7 Dominguez, R., *Arch. Path.*, 1928, **5**, 577.

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9 Griffith, J. Q., Jr., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 394.

TABLE I.
Effect of Chronic Lead Poisoning on Systolic Blood Pressure of 11 Rats. (Normal systolic pressure 90 to 130 mm of Hg.)

	Day of experiment	
	45th day B.P.	60th day B.P.
Rat No. 1	186	202
2	180	194
3	150	190
4	144	210
5	170	200
6	110	176
7	130	170
8	150	174
9	140	188
10	136	170
11	110	130

sure determinations were made in each of the animals that survived the intoxication on the 45th and 60th days. All animals were anesthetized with ether during the procedure.

At the conclusion of the experiment the organs were removed and immediately fixed in Zenker-formol solution.

Results and Discussion. Ten of the 11 animals that survived 60 days developed hypertension (Table I).

At autopsy it was our impression that the hearts were enlarged, apparently as the result of minor degrees of both hypertrophy and dilatation. However, they were not weighed. This finding was equivocal in the rat which failed to develop hypertension. The kidneys also appeared larger than normal and their external surfaces were mottled and pale, but the normal markings were distinct on the cut surfaces. Two rats had a unilateral hydronephrosis, the cause of which was not determined. Gross pathologic changes were not observed in any of the other viscera.

The heart, kidney, and liver were studied microscopically in all cases, the aorta in 10, the renal arteries and veins in 4, the adrenal gland in 7, and the lungs in 5. The hearts of the hypertensive rats showed histologic changes consistent with a slight degree of hypertrophy. Except for this, significant histologic findings were limited to the kidneys and liver.

The most striking finding was the presence of solid and rounded, or irregular and granular, basophilic deposits in the epithelium of the renal tubules. These deposits, which were blue-black in hematoxylin and eosin preparations, usually occurred in the cytoplasm of the cells lining the convoluted tubules in the region of the junction of the cortex with the pyramid, frequently surrounding or partially obscuring the nuclei. A few were also present in the capillary endothelial cells. The bodies were stained blue-gray with fresh, unripened hematoxylin and were deeply basophilic in methylene blue preparations. These tests are considered indicative of the presence of lead by Mallory and Parker[10] although they are not entirely specific. These authors recommend fixation in 95% or absolute alcohol whereas our material was fixed in Zenker-formol. Precipitation with H_2S was not attempted. The inclusions were not stained by von Kossa's method for calcium salts. It seems likely that these inclusions represent lead or lead salt deposits. Rauh[11] has described lead deposits (demonstrated by H_2S precipitation) in the epithelium of the loops of Henle in the kidneys of guinea pigs given lead orally. He observed similar deposits in the medial coats of blood vessels and in capillary endothelium only after subcutaneous administration.

Acidophilic intranuclear inclusions, such as described by Blackman[12] as occurring in the renal tubular epithelial cells and hepatic cells, were noted in the kidneys of 3 animals of this series. These inclusions were all small and only a few were found after prolonged search.

Similarly, morphologic changes indicative of destruction or regeneration of the tubular lining cells, as described also by Blackman, were very scanty. In the kidney of only one animal were irregular, elongated or hypertrophied cells and mitotic figures at all conspicuous. Blackman's studies were made on poisoned children, but similar observations in rats have been recorded by Finner and Calvery.[13] Fairhall and Miller,[14] however, have

[10] Mallory, F. B., and Parker, F., Jr., *Am. J. Path.*, 1939, **15**, 517.

[11] Rauh, F., *Arch. f. Exp. Path u. Pharmakol.*, 1934, **174**, 352.

[12] Blackman, S. S., Jr., *Bull. Johns Hopkins Hosp.*, 1936, **58**, 384.

[13] Finner, L. L., and Calvery, H. O., *Arch. Path.*,

demonstrated that such cellular alterations in the kidneys of rats disappear rapidly after return to a normal diet. Our rats had received no lead in the 10 days prior to sacrifice.

Basophilic deposits similar to those in the kidney were also seen in the liver, more commonly, however, in Kupffer cells than in hepatic cells. No intranuclear inclusions were seen in this organ.

These basophilic cytoplasmic bodies were observed in the kidneys and liver of the nonhypertensive rat as well as in the other animals.

The arterioles and arteries of the kidneys, the aorta, and the blood vessels of the other organs studied showed no histologic abnormalities.

Summary. An experiment is reported which confirms the successful production of hypertension in rats by the administration of lead for prolonged periods.

Post-mortem studies on 10 hypertensive rats and one similarly treated rat, which failed to develop hypertension, revealed no significant blood vessel alterations.

The most striking and consistent finding was the presence of basophilic cytoplasmic inclusions, which may represent lead or lead salts, most commonly in the tubular epithelial cells of the kidney and the Kupffer cells of the liver.

Renal changes similar to those described by others in chronic lead intoxication in both humans and experimental animals were also observed but were irregular and inconspicuous features.

1939, **27**, 433.

14 Fairhall, L. T., and Miller, J. W., *Pub. Health Rep.*, 1941, **56**, 1641.

15017

Oxidation-Reduction Potential Requirements of *Cl. welchii* and Other Clostridia.*

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Previous work[1,2] has shown that the limiting oxidation-reduction potential for growth of *Bacteroides vulgatus* and of *Cl. sporogenes* is at Eh + 0.150 volt at pH 6.6; at potentials more positive than this limiting value the organisms do not grow, while at more negative potentials they grow consistently on a suitable nutrient medium at this pH. This study is concerned primarily with the effect of pH on the limiting potentials for growth of *Cl. welchii*, *Cl. sporogenes*, and *Cl. histolyticum*.

Experimental Methods. In general the procedure is to maintain constant the Eh and pH of an inoculated medium for 10 to 30 hours, and to distinguish those Eh and pH values at which growth occurs, from those at which growth does not occur. The apparatus and procedure have been previously described.[1,2] The electrode culture vessels consist of 100 ml wide mouth bottles, each of which contains 75 ml of medium, and is fitted with a rubber stopper which supports gas inlet and outlet tubes, glass electrode, platinum electrodes, and salt bridge connection to a calomel cell. After autoclaving, the pH of the medium is adjusted to the desired level by addition of HCl or NaOH, 0.2 ml of a brain broth culture inoculum is added, glass electrode potentials are observed every half hour, platinum electrode potentials every 10 minutes, and the time of the first appearance of growth in any

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2 Hanke, M. E., and Katz, Y. J., *Arch. Biochem.*, 1943, **2**, 183.