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.

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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

* Aided by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago. Acknowledgment is also due for a grant from the Ella Sachs Plotz Foundation.

1 Vennesland, B., and Hanke, M. E., *J. Bact.*, 1940, **39**, 139.

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

vessel is carefully noted. Previous reports[1,2] should be consulted for details of sterilizing and inserting glass electrodes, establishing sterile salt bridge connections, stirring, use of poising agents, criteria for growth, and tests for contamination.

Cultures of all 3 organisms were obtained from the Department of Bacteriology, University of Chicago. In addition, a culture of *Cl. welchii* was obtained from Dr. Hac,[3] of the Department of Obstetrics and Gynecology. The limiting potentials of these 2 strains of *Cl. welchii* were found to be the same. Best growth was obtained from an 18-hour subculture in the case of *Cl. welchii*, and 24 hours in the cases of *sporogenes* and *histolyticum*. In addition to the peptone medium previously used (10 g glucose, 5 g NaCl, M/15 phosphate, 5 g peptone, 3 g meat extract, in 1 liter), a gelatin yeast medium was also employed (10 g glucose, 5 g NaCl, 0.1 g $MgSO_4$, M/15 phosphate, 10 g gelatin, 5 g yeast extract, in 1 liter). The limiting potentials found with these two media were identical.

In any experiment, at least 3 (sometimes as many as 8) vessels were inoculated from the same culture, potentials were controlled, and observations were made on growth or lack of growth, simultaneously in all 3 (or more) vessels. In one of these vessels, strict anaerobic conditions were maintained, so that growth occurred in a minimum time, which was about 5 hours in the case of *Cl. welchii*, 6 to 10 hours in the case of *sporogenes*, and 8 to 12 hours in the case of *histolyticum*. In the other vessels the Eh values were controlled and maintained at 2 or more different levels, so that, in one vessel at a given pH growth would occur, and in another vessel at the same pH at a more positive Eh growth would not occur. In other pairs of vessels, the Eh value was kept the same in both, while the pH was varied so that growth occurred in one, but not in the other of a pair. In some experiments, after it was established that growth did not occur under a given set of conditions, the Eh value was adjusted to a more negative (a more favorable) value, and then growth was observed. In this way the limiting pH and Eh values for growth could be established with confidence. In the controlled vessels the time required for growth was usually greater than in the strictly anaerobic vessels, although experiments were usually not carried on for times longer than twice the time required for growth in the anaerobic controls. It should perhaps be emphasized that the success of these experiments depends upon constant and continuous potentiometric observation and control of pH and Eh for 12 to 30 hours. This was accomplished by having a crew of 3 or 4 people working 3- or 4-hour shifts.

In most experiments the Eh values were controlled by bubbling through the medium controlled proportions of air and nitrogen, as described by Vennesland and Hanke;[1] in a few cases the electrolytic method of Hanke and Katz[2] was used. The former is more satisfactory for determination of the limiting potential chiefly because it avoids the changes in pH which follow the electrolytic formation of acid or base. Also the time required for growth at a given favorable Eh and pH is greater, when electrolytic control is used. This may be due not only to the unfavorable pH effects of electrolysis; but the local liberation of oxidizing agents at the surface of the electrode, may exert some inhibitory action on the microörganisms. When suitably carried out, so that prolonged and intensive electrolysis is avoided especially in the early part of the experiment when the inoculum is recently introduced, both methods show the same result. However, for precise determination of limiting Eh values for growth the air-nitrogen method of Eh control is preferred.

In calculating the results, the 12 ten-minute observations of the Eh in any 2-hour period are averaged. If the average deviation of these exceeds 8 millivolts the experiment is discarded as unsatisfactory. Usually the average deviation was 3 to 6 millivolts. These successive two-hour averages for a given vessel are then averaged to give the grand average for the entire period. In order to be considered satisfactory, the average deviation of these 2-hour values for the grand average must not have exceeded 8 millivolts.

Results. Table I gives the result of 60 observations on the growth or lack of growth

3 Hac, L. R., and Hubert, A. C., PROC. SOC. EXP. BIOL. AND MED., 1943, **53**, 58; *idem.*, 1943, **53**, 61.

TABLE I.
Observations on Growth or Lack of Growth in Cultures of *Cl. welchii* at Controlled Eh and pH Values.

Eh in millivolts			Eh in millivolts			Eh in millivolts		
pH	Growth	No growth	pH	Growth	No growth	pH	Growth	No growth
5.78		111	6.45		166	6.86		150
5.92		155	6.45		166	6.88	114	
5.93		153	6.47	146		6.92		128
5.98		117	6.47	145		6.95	91	
5.98	92		6.49		181	6.95		107
6.03	106		6.50	141		6.96		129
6.06		114	6.55	134		6.98	84	
6.07	98		6.55		147	6.99	91	
6.07	111		6.55	141		7.06		87
6.18	131		6.57	149		7.07	67	
6.20		144	6.58		145	7.10		150
6.30	143		6.58		150	7.20		116
6.32		157	6.59		164	7.21	79	
6.35	157		6.60		191	7.26		103
6.36	146		6.60		167	7.32		57
6.38		163	6.60		166	7.35	40	
6.40	143		6.67		148	7.42		74
6.40	156		6.71	129		7.74		36
6.42		172	6.73		131	7.74		31
6.44	162		6.75		123	7.88	—12	

TABLE II.
Observations on Growth or Lack of Growth of *Cl. sporogenes* at Controlled Eh and pH Values.

Eh in millivolts			Eh in millivolts			Eh in millivolts		
pH	Growth	No growth	pH	Growth	No growth	pH	Growth	No growth
6.08		97	6.30	136*		6.60	136*	
6.08		106	6.40	144		7.08		115
6.13	118†		6.45		152	7.09	114	
6.14		147	6.50		163	7.02	124†	
6.15	121		6.60	116		6.97		140†
6.19		145†	6.60	36		6.97	117†	
6.27	133†		6.60		155*			

* These data are taken from Hanke and Katz.[2]
† These data are taken from Katz.[4]

in cultures of *Cl welchii* at a variety of Eh levels between pH 5.8 and 7.9. Tables II and III give similar data for *Cl. sporogenes* and *Cl. histolyticum*. In Table IV these data are summarized to give the most positive Eh value at which growth will just occur (the limiting Eh value for growth) at a few rounded pH values.

From the data, the following conclusions may be drawn. (1) There is a marked difference between the oxidation-reduction potential requirements of the three organisms; at pH 6.4 *Cl. welchii* will grow at a slightly more positive Eh level (+ 160 mv) than *Cl. sporogenes* (145 to 150 mv), while *Cl. histolyticum* requires much more reducing conditions (85 to 90 mv). (2) pH has a marked effect on the limiting Eh value for growth. In the case of *Cl. welchii,* the maximum limiting Eh value of 160 mv is reached at 6.4 and falls off rapidly at lower or higher pH value. For *Cl. sporogenes* the maximum limiting Eh value is at pH 6.4 to 6.6, and falls off more slowly with increasing pH than that of *Cl. welchii,* so that at pH 7.0, *Cl. sporogenes* will grow at

[4] Katz, Y. J., Ph.D. Dissertation in Biochemistry, University of Chicago, 1941.

TABLE III.
Observations on Growth or Lack of Growth of *Cl. histolyticum* at Controlled Eh and pH Values.

Eh in millivolts			Eh in millivolts			Eh in millivolts		
pH	Growth	No growth	pH	Growth	No growth	pH	Growth	No growth
6.38	63		6.54		119	6.73		139
6.42		105	6.58	94		6.98	60	
6.43		94	6.58	76		7.00		76
6.43		96	6.61		95	7.00		76
6.45	85		6.63	85		7.00		118
6.52		84	6.68	72		7.05	60	

TABLE IV.
Summary of Limiting Eh Values for Growth of three Clostridia at a Few Rounded pH Values.

Cl. welchii		*Cl. sporogenes*		*Cl. histolyticum*	
pH	Eh	pH	Eh	pH	Eh
6.0	106	6.0	<100		
6.2	131	6.2	130		
6.4	160	6.4	144	6.4	85
6.6	150	6.6	136-152	6.6	90
6.8	114				
7.0	90	7.0	114	7.0	60-76
7.2	80				
7.4	35-70				
7.8	0-30				

a more positive Eh (114 mv) than will *Cl. welchii* (90 mv). *Cl. histolyticum* also requires a more negative Eh value at pH 7.0 than at pH 6.4 or 6.6.

Certain minor inconsistencies in the data do not detract from the above conclusions. They do indicate the limit of accuracy of this type of experimentation. In Table I at pH 6.57 growth occurred at Eh 149 millivolts, but not at pH 6.58 Eh 145; at pH 6.71 growth occurred at Eh 129, but not at pH 6.75 Eh 123. In Table III growth fails at pH 6.52 Eh 84 mv, while it does occur at pH 6.58 Eh 94 mv. No inconsistencies greater than 10 millivolts have ever been observed, and this, it is felt, represents the maximum error of the conclusions.

Similar experiments were carried out with *Cl. tetani* but satisfactory quantitative conclusions could not be drawn because of the long time required for growth. In the anaerobic controls growth occurred at Eh-50 to 150 millivolts, in 8 to 12 hours, but when small amounts of air were admitted to maintain the Eh value at a more positive level, the time required for growth was increased to 40 or 50 hours. In 4 successful experiments it was observed that at pH 6.5, *Cl. tetani* will grow at Eh 85 millivolts but not at Eh 120; at pH 7.2 it will grow at Eh 55 millivolts but not at Eh 120. From this it seems that *Cl. tetani* requires more reducing conditions than *Cl. welchii* or *Cl. sporogenes*, and is more like *Cl. histolyticum* in its Eh requirements.

Summary. The oxidation-reduction potential requirements of three clostridia have been determined at a variety of pH values. The observations reveal striking and characteristic differences between the three kinds of organisms and a marked effect of pH on the limiting Eh for growth.

Acknowledgment is hereby made to the following students whose collaboration made this study possible: Richard Boyagian, Charles Gabelman, Eugene Goldwasser, Ruth Hanke, Alan Robertson, and Louis Thomas.