equal parts of acetone (Baker's C. P.), while the monkeys injected with the supernatant fluid did not develop poliomyelitis. The ninhydrin tests for protein were either negative or only very slightly positive with these precipitates.

In one experiment the virus was recovered both in the supernatant fluid and in the washed precipitate after treating the filtrate with acetic acid to pH 5.0 and heating to 55°C., while in another the virus was found only in the supernatant fluid after acidifying to pH 4.4 and heating to 58°C. The washed precipitate from the latter was negative. Heating to 71°C., to 75°C., and to 78°C., destroyed the virus.

After a number of unsuccessful attempts it was most interesting to find the virus in the final precipitate after treating the filtrate with lead acetate and sodium bicarbonate and then leaving it on ice over night following the addition of equal parts of acetone. Monkeys injected both with the supernatant fluid after the removal of the major part of the proteins and with the acetone precipitate developed poliomyelitis. The latter gave a negative ninhydrin test.

In the same experimental series a potent virus was obtained in the precipitate which formed following acidification of the filtrate to pH 4.4, heating to 58°C. and holding it on ice over night with equal parts of acetone. The washed acetone precipitate was ninhydrin negative and produced a typical flaccid paralysis in a monkey.

Several attempts were made to extract a potent virus from the acetone precipitate with 95% alcohol and with ether. Both the evaporated extractive material and the residue failed to infect monkeys. Negative results were also obtained after several attempts both to precipitate the virus with different proportions of absolute alcohol and to recover it in the supernatant fluid after adding varying amounts of phosphotungstic acid.

5207

Metabolism Studies on the Brucella Group. II. The Fermentation of Monosaccharides.

C. E. ZOBELL AND K. F. MEYER.

From the George Williams Hooper Foundation for Medical Research, University of California, San Francisco.

It was generally believed that members of the Brucella group lack the power to ferment the common sugars until McAlpine and

Slanetz[1] showed that all the varieties except the bovine *abortus* strains are able partly to decompose and utilize the available glucose in the medium. Meyer and Eddie[2] failed to find any definite correlation between the type of Brucella and glucose utilization. More recently Coleman and coworkers,[3] Mallardo,[4] and Duncan and Whitby[5] found slight acid changes in arabinose, xylose, galactose and levulose. However, the degree of acid production varied with the different strains and could not be correlated with the origin of the cultures.

Since preliminary tests indicated that the chlorphenol red indicator used by Coleman and coworkers is reduced by certain Brucella strains it appeared desirable to test the fermentative properties of a large series of strains by the following procedure: A 1% filtered beef infusion rendered sugar-free by the action of *B. saccharolyte*, 0.5% NaCl, 0.2% agar adjusted to a pH 6.9 was mixed with 1% filtered goat serum and 1% carbohydrate (filtered solutions), tubed in 3.5 cc. amounts, tested for sterility and inoculated with a uniform sized loopful of a slant culture suspended in saline. The tubes were held at 37° C. and the pH of the cultures determined with brom thymol blue according to the method of Clark,[6] on the 3rd, 7th, 10th, 14th, and 21st day of incubation. Changes in the reaction became perceptible in 3 to 7 days, the maximum intensity being reached in 10 to 21 days. Duplicate determinations were made on 194 strains from host origins in 15 countries of the world.

The semi-solid consistency of the medium favored the growth of the bacteria and the low buffer content permitted a rapid change in the H-ion concentration. In the control medium containing no carbohydrate the final reaction was pH 7.6 or above, with all strains. The tests for ammonia were invariably positive. Rhamnose and the alcohol, mannitol, were never fermented as indicated by the pH 7.6. A simultaneous attack of carbohydrate and the nitrogenous compounds was recorded in media containing arabinose, xylose, galactose, glucose and levulose, indicated by a terminal reaction less than pH 7.6 with these sugars. They were attacked with intensity in the order named. The growth energy was not influenced although many strains increased the H-ion concentration to pH 6.0 or below. Regardless of the final pH the production of ammonia could

[1] McAlpine and Slanetz, *J. Infect. Dis.*, 1928, **42**, 73.

[2] Meyer and Eddie, *J. Lab. and Clin. Med.*, 1930, **15**, 447.

[3] Coleman, *J. Lab. and Clin. Med.*, 1930, **15**, 641.

[4] Mallardo, *J. Trop. Med.*, 1930, **33**, 125.

[5] Duncan and Whitby, "A System of Bacteriology," 1930, **5**, 396.

[6] Clark, *J. Bacteriol.*, 1917, **2**, 61.

always be demonstrated by making the medium alkaline with NaOH. There is no evidence of a protein sparing action by the carbohydrates.

All strains produced acid in excess of alkali in arabinose (final average pH 6.2), xylose (pH 6.6), and galactose (pH 6.8). Greater variations in the power to ferment were noted in the culture series containing glucose. Of 80 bovine strains tested 13 or 16% gave a final pH 6.2 or below, 28 or 33% had a final reaction of pH 7.3 or above. Practically every bovine strain fermented glucose to some degree. Five caprine strains recently isolated on the Island of Malta showed very little glucose utilization, while 3 strains isolated from Arizona goats produced sufficient acid to reduce the pH to 6.7. The average final reaction of 86 strains of human origin was pH 7.07 with 13% giving readings of pH 6.2 or less in glucose media. Porcine strains failed to utilize glucose in appreciable amounts, the final reaction averaging pH 7.5.

Levulose utilization almost paralleled that of glucose in the different groups. In general it was fermented slightly less than was glucose.

The fermentation of the monosaccharides can not be correlated in any way with the type of Brucella grouped either according to host origin, serologic tests, hydrogen sulfide production or genesistatic behavior.

5208

Lactate Production in Muscle Stimulated Briefly.

E. G. MARTIN, J. FIELD, II AND V. E. HALL.

From the Laboratory of Physiology, Stanford University.

In dogs anesthetized with amytal we have observed the lactate concentration in gracilis muscles immediately after brief periods of activity induced by short tetanizations repeated rhythmically. The muscles were stimulated *in situ* through the obturator nerve. The circulation was intact and the arterial pressure satisfactory. As soon as possible after stimulation ceased they were frozen *in situ* with carbon dioxide snow, excised and ground to powder in liquid air. Lactate determinations were made by the method of Friedmann, Cotonio and Shaffer.[1]

[1] Friedmann, T. E., Cotonio, M., and Shaffer, P. A., *J. Biol. Chem.*, 1927, **73**, 335.