

In 7 of 10 domestic rabbits kept for 7 months or more, cancers arose in pigmented Shope papillomas.⁴ Some of the cancers were papillomatous, but none of their cells reacted with dopa.

From these observations it is plain that the pigmentation of Shope papillomas is not determined by the strain of virus inducing the growths but by the epidermis upon which it acts. Where melanoblasts are present pigmented growths arise because these cells become involved in the pathological process though not themselves affected by the virus.

8084 P

Colony Morphology of Tubercle Bacilli. II. Influence of pH of Culture Medium on Colony Morphology and Virulence.

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Extensive studies have been made with reference to the range of pH in which tubercle bacilli will grow and the changes in pH which take place during growth on fluid medium. These studies have been reviewed by Wells and Long.¹ Recently Birkhaug² has studied the changes in pH induced in fluid medium during growth of R and S forms of avian and mammalian tubercle bacilli. However, the influence of pH on colony morphology of Mycobacteria has been less extensively investigated. Steenken, Oatway, and Petroff³ found that R colonies of degraded virulence were characteristic of the growth at pH 6.1 on either Petroff's or Calmette's medium; whereas Petroff's medium at pH 7.2 supported the growth of S variants of greater virulence. Recently it was observed in this laboratory⁴ that by adjusting the pH of Corper's medium to various values, three principal types of colonies could be obtained: rough, smooth, and intermediate. The present report is an extension of the latter work.

⁴ Rous, Peyton, and Beard, J. W., *Proc. Soc. Exp. Biol. and Med.*, 1935, **32**, 578.

¹ Wells, H. G., and Long, E. R., *The Chemistry of Tuberculosis*, 1932, The Williams and Wilkins Company.

² Birkhaug, K. E., *Ann. Inst. Pasteur*, 1935, **54**, 19.

³ Steenken, W., Jr., Oatway, W. H., Jr., and Petroff, S. A., *J. Exp. Med.*, 1934, **60**, 515.

⁴ Smithburn, K. C., *J. Exp. Med.*, 1935, **61**, 395.

Twenty-five strains of tubercle bacilli were used in the experiments. Included in these were: R and S variants of an avian strain, a freshly isolated avian strain, 11 bovine, and 11 human strains. Ten of the human strains were isolated within the last 16 months. Corper's glycerinated egg-yolk medium with Congo Red was used throughout. 450 cc. lots of the latter were adjusted to various pH values between 6.0 and 7.2 by intervals of 0.2 pH, the pH determinations being done by the glass electrode method through the courtesy of Dr. D. A. MacInnes. Phosphate buffer (M/1.5 KH_2PO_4 or M/1.5 Na_2HPO_4) was added in the quantity just sufficient to bring to the desired pH value. After adjusting the pH, the medium was placed in tubes and inspissated in the usual manner. In the second and third generations of cultures the medium was adjusted only to pH 6.0, 6.4, 6.8, and 7.2.

Stock cultures of each of the 25 strains were suspended in saline and 3 tubes of medium of each pH were seeded from each suspension. Therefore, in the first generation each of the 24 tubes of each strain of organisms was seeded from a single suspension. In the second and third generations the transfers were made in the same manner except that organisms grown at any one pH value were seeded on medium of the same pH value. Colony selection was not done.

All cultures were examined with the binocular dissecting microscope after 3 and 4 or 5 weeks' incubation at 37°C. The relative amount of growth and colony characteristics of each culture were recorded.

Sharp differences in colony morphology at various pH values were noted in each of 3 avian, 11 bovine, and 4 human strains in the first generation. In the second generation the remaining 7 human strains showed the same differences as were noticed in the 4 human strains of the first generation. These differences may be summarized as follows:

1. Avian Strains. Rough colonies only at pH 6.0 and 6.2. All other cultures showed smooth, creamy colonies. This was true of the avian R as well as the avian S and freshly isolated avian strains. The avian S and fresh avian strains also showed smooth colonies at pH 6.0 in the third generation.

2. Bovine Strains. Rough colonies at pH 6.0 and 6.2. Smooth colonies from pH 6.4-6.8. These were rounded, shiny and non-granular. Intermediate, flat, spreading, finely granular, semi-transparent, "stippled" colonies from pH 7.0-7.4.

3. Human Strains. Rough colonies at pH 6.0 and 6.2. Smooth colonies at pH 6.4 and in a few instances at pH 6.6. These were also rounded, shiny and non-granular. Intermediate colonies from pH 6.8-7.4. These were similar in appearance to the bovine intermediate forms.

The data above indicate the dominant colony forms at the various pH values. When other colony forms appeared than those indicated, they were in the minority.

Total growth varied remarkably at the different pH values but showed no appreciable variation in the human, bovine or avian types. Growth was most vigorous at pH 6.0 and 6.2 and in general less vigorous the more alkaline the medium.

Animals have been inoculated with these colony variants derived from a freshly isolated human strain, a highly virulent bovine strain, and a bovine strain of very low virulence. From the latter experiments it has been found that the rough colonies are of least virulence, the smooth of greatest virulence, and the intermediate colonies are of intermediate virulence. Smooth colonies derived from the bovine strain of low virulence are apparently not more virulent than the undissociated culture from which they were derived.

Since all other variables were avoided, save that of hydrogen ion concentration of the standard medium, these experiments demonstrate the rôle which one factor may play in determining the colony morphology and virulence of tubercle bacilli.

8085 P

Histological Observations on Resistance to Transplantable Leukemia in Immunized Mice.

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Recently it has been demonstrated that immunization to transplantable lymphatic leukemia in mice can be actively induced by suitable injection of dilute doses of leukemic cells,¹ by injection of nor-

¹ MacDowell, E. C., Taylor, M. J., and Potter, J. S., *Proc. Soc. Exp. Biol. and Med.*, 1934, **82**, 84.