different amounts of amylase will be required to hydrolyze the digest to the end-point in a given time.

From the evidence adduced in this and a previous paper (Chesley[1]), it seems probable that the disparity in "digestibility" (as given by the various methods) is caused by varying proportionate quantities of amylopectin, amylose and dextrins in the different soluble starch samples.

Summary. The achromic iodine method, with several variations, has been investigated for its validity in determining amylase.

The iodine method is not a valid quantitative procedure for the estimation of amylase. There are considerable differences in the quantities of enzyme required to digest different samples of soluble starch to the same end-point in the same time, under identical experimental conditions.

The copper reduction method is a valid quantitative method for measuring the saccharogenic power of an amylase. Dextrin and 3 samples of soluble starch were found to be saccharified at the same rate.

Apparently the disparities in digestibility which exist among different soluble starch samples are caused by varying relative amounts of amylopectin, amylose and dextrins.

7454 P

A Method for Obtaining Stable S Colonies of Human Tubercle Bacilli.

ELEANOR G. ALEXANDER. (Introduced by William H. Park.)

From New York University.

The so-called "S" colonies of the human tubercle bacillus obtained by Petroff,[1] Reed and Rice,[2] and others, as described or shown on photographic plates, although more easily emulsified in saline and of greater virulence than R colonies, appear flat, wrinkled, dull, and irregular rather than convex, glistening, round, and smooth-edged as do S colonies of bovine and avian tubercle bacilli, and the S colonies of most other bacteria which have been dissociated. The flat colonies seem to be intermediates and not true S. This paper reports a successful attempt to dissociate human tubercle

[1] Petroff, S. A., and Steenken, W., Jr., *J. Exp. Med.*, 1930, **51**, 831.

[2] Reed, G. B., and Rice, C. E., *Canad. J. Res.*, 1931, **4**, 389; **5**, 111.

bacilli completely into S colonies resembling those of the bovine and avian types, and, moreover, to develop or modify a medium favoring the continued formation of such S colonies in pure culture by means of some substance other than anti-R serum.

The Bordet-Gengou[3] medium proved not only an excellent medium for growth, but lent itself well to experimentation. Unmodified Bordet-Gengou medium was used to maintain undissociated control strains. The human strains grown on Bordet-Gengou medium all exhibited morphology characteristic of human type colonies.[3] These appeared to be much nearer the S than the R. It was found that a small optimal amount of ferric chloride, 0.0004%, representing 2.5×10^{-4} mols of iron per liter, added to Bordet-Gengou medium stimulated the dissociation of 2 human strains studied into completely smooth, convex, glistening colonies. These S colonies were produced in pure culture by one strain in the first generation, and by the other strain in the third generation on this modified medium. The fourth and fifth generations continued to produce S colonies.

Avian R bacilli were dissociated to S on modified Bordet-Gengou medium. The optimal amount of ferric chloride in this case was 0.004%, representing 2.5×10^{-3} mols of iron per liter.

The lesions produced by the convex glistening S colonies of both avian and human strains differed radically from those produced by R bacilli. They were diffuse, inflammatory, and small or microscopic, whereas those produced by R bacilli were much fewer, limited, calcified, and larger. Virulence tests indicate that the S colonies of these strains possess somewhat greater virulence than the R. Details of experimental methods used in dissociation and virulence tests will be included in a subsequent publication.

[3] Mishulow, L., *J. Inf. Dis.*, 1932, **51**, 416.