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Rapid Production of Highly Potent Scarlatinal Toxin.

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In producing mass cultures of streptococci and pneumococci, Mueller and Klise¹ found it distinctly advantageous to maintain approximate neutrality of cultures growing in 2% glucose broth; this was accomplished by frequent additions of sterile NaOH. The yield of cocci and/or of soluble specific substance was considerably enlarged. We have applied their method in a study of the production of other bacterial products—the erythrogenic toxins and the lysin of the N.Y.5 strain of hemolytic streptococcus.

Comparisons of the activities of this strain in 1 L. quantities of tryptic digest broth, pH 7.9, 3.82 mg. N₂/ml, were made under 3 conditions: I, 2% glucose and phenol red were added to basic medium and, after growth began, the color was kept faintly pink by repeated small additions of 10% NaOH—84 ml were used in 24 hours; II, basic medium containing indicator and 2% glucose; III, basic medium and indicator. Each 2 L. flask was seeded with 5 ml of a 6 hour broth culture; at successive 4 hour intervals, samples were withdrawn for tests. Observations are recorded in the figure and table.

Hemolytic activity of centrifugates was at once measured at 37°C. in the presence of 0.5 ml of 5% rabbit cells; total volume, 1 ml; incubation, 1 hr.

TABLE I.
Observations on pH, N₂, and glucose contents, viability, and suspension stability of cultures.

| | I | II | III |
|-----------------------------|-----------|------------|------------|
| pH, 24 hr. | 7.5 | 5.47 | 5.92 |
| Glucose 12 hr., mg/ml | 2.2 | 15.3 | |
| 24 hr., mg/ml | 1.9 | 11.7 | |
| N ₂ 24 hr. mg/ml | | | |
| Whole culture | 3.77 | 3.71 | 3.59 |
| Filtrate | 3.23 | 3.23 | 3.16 |
| Viable cocci, 24 hr. | | | |
| 0.00001 ml | Overgrown | 0 | 1 |
| 0.0001 ml | | 0 | 20 |
| 0.001 ml. | | 6 | 168 |
| Suspension | Smooth | Flocculent | Flocculent |

¹ Mueller, J. H., and Klise, K. S., *PROC. SOC. EXP. BIOL. AND MED.*, 1932, **29**, 454.

The bacterial bulks were measured by uniform centrifugation and are charted as volume percent. The cocci in lot 1 were notably small and usually gram-negative; used as a bacterial vaccine this

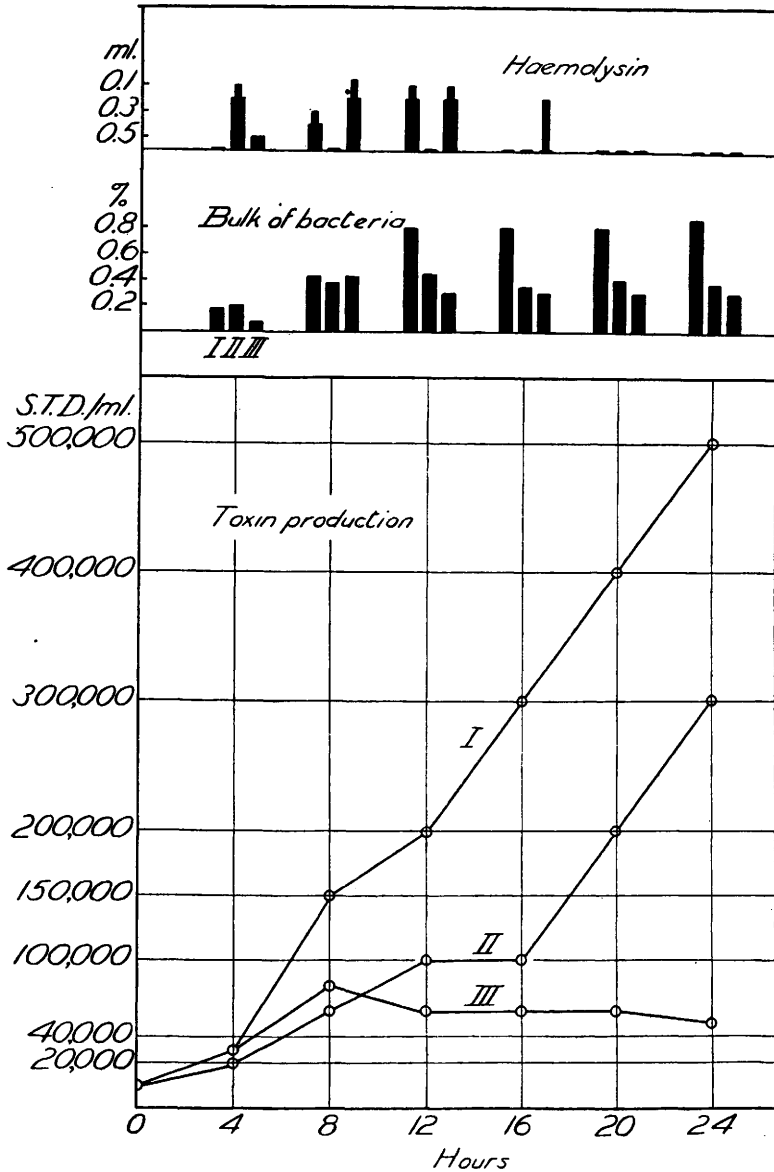


FIG. 1.
 Observations on growth, toxin and lysin formation.
 (The symbols II and III, relating to toxin production, should be interchanged.)

suspension has evoked larger local reactions than ordinary suspensions of apparently the same density.

Toxins were titrated on subjects who responded with bright, 20 mm. reactions. Estimates of the skin-test dose (STD) are considered accurate only within 50%. The potency of toxin "B",² also produced by this strain, was measured and found to parallel that of the Dick toxin ("A") except that in lot II after 8 hours it rapidly declined and at 20 hours there were less than 100 STD/ml of "B" toxin remaining. This behavior affords an additional criterion of differentiation between "A" and "B" toxins. The stability of toxins prepared as in lot I does not appear to differ markedly from that of toxins prepared in the ordinary way; after 3 months there was about a 50% loss in potency.

Among other things the observations indicate a connection between rapidity of growth and toxigenesis. They do not suggest identity of hemotoxin with erythrotoxic toxin.

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Attempts to Produce Hyperplasia of Thyroid Gland by Infection and by Injury to the Adrenal Gland.

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The etiology of exophthalmic goiter is still unknown. However, we do know one change that occurs regularly in typical exophthalmic goiter—hyperplasia of the thyroid gland. Various theories have been proposed as to the basic factor underlying this hyperplasia. In these experiments I have been interested in two: (1) Infection; (2) Disordered action of the adrenals.

Halsted¹ found hyperplasia of the thyroid gland after producing in dogs a low grade peritonitis by the intraperitoneal injection of staphylococci. Later he thought he could attribute certain hyperplastic changes in the thyroid to infection of the wound. Cole and Womack² described hyperplasia of the thyroid occurring after the implantation of infected foreign bodies into the neck muscles of dogs.

² Hooker, S. B., and Follensby, E. M., *J. Immunol.*, 1928, **15**, 601.

¹ Halsted, *Am. J. Med. Sc.*, 1914, **147**, 56.

² Cole, and Womack, *J. Am. Med. Assn.*, 1929, **92**, 453.