

occur after administration of large doses of insulin, have any causal relationship to the phenomenon observed.

Conclusion. The rise in blood pressure occurring during the inhalation of 6.2% oxygen for 3 minutes increases in proportion to the fall of blood sugar obtained after intravenous injection of large doses of insulin in dogs. Intravenous injection of glucose which offsets to a certain extent the hypoglycemia induced by insulin diminishes the blood pressure response to oxygen deficiency.

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Production of Small-Colony Variants of *Staphylococcus aureus*.

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We have previously reported the production of small-colony variants, or G forms, after prolonged serial transfer of *S. aureus* in lithium chloride broth or plain broth media.^{1, 2} Swingle³ subsequently confirmed these results and also obtained these forms after extended growth in nutrient broth or casein-digest medium. Under these conditions the variants occur only rarely and in small numbers, seldom exceeding 1.0% of the colonies on a plate. Chinn⁴ in a study of the metabolic activity of the small colony variants of *S. paratyphoeae* Sonne and *S. aureus* found them to be metabolically far less active than the normal forms, having a reduced generation-time, reduced fermentative powers, lessened ability to reduce methylene blue and a slower cataphoretic velocity. This lowered metabolic activity and the ability to revert to forms identical with the parent culture furnishes some evidence that small-colony variants may be forms whose metabolism has been greatly reduced by some unknown factor.

Quastel and Woolridge⁵ studying the enzymes of *B. coli* found that among many inorganic salts barium chloride was the most effective in inhibiting the metabolic activity of washed suspensions of these organisms. These results, and the known lowered metabolic

¹ Hoffstadt, R. E., and Youmans, G. P., *J. Infect. Dis.*, 1932, **51**, 216.

² Hoffstadt, R. E., and Youmans, G. P., *J. Bact.*, 1934, **27**, 551.

³ Swingle, E. L., *J. Bact.*, 1935, **29**, 467.

⁴ Chinn, B. D., *J. Infect. Dis.*, 1936, **59**, 137.

⁵ Quastel, J. H., and Woolridge, W. R., *Biochem. J.*, 1927, **21**, 1224.

activity of the small-colony variants, indicated that barium chloride might be effective as an incitant of their production.

Concentrations of barium chloride of 0.5 to 5.0% were made up accurately in 1.0% peptone solution. Since *S. aureus* produces only slight growth in plain peptone medium the acetone-soluble portion of meat extract containing Hughes'⁶ growth factor was added. This was prepared by extracting 50 gm. of meat extract dissolved in 100 cc. distilled water with 6 to 8 volumes of acetone. The acetone was decanted and removed *in vacuo* at 50 to 60°C. The residue was taken up in 50 cc. of distilled water and re-extracted with 6 to 8 volumes of acetone which was decanted from the precipitate and removed as before. The final residue was dissolved in 50 cc. distilled water and after the pH had been adjusted to 7.0 was sterilized by filtration through a Berkefeld N filter. Four percent of this activating solution was added to the barium chloride peptone medium after the latter had been adjusted to pH 7.0 and sterilized either by Berkefeld filtration or by autoclaving. This final medium was tubed aseptically in 5 cc. amounts. All media were tested for sterility before use.

Fifteen strains of *S. aureus* were employed. After a preliminary 24-hours' growth in peptone-activator medium a constant amount was inoculated with a capillary pipette into media containing varying concentrations of barium chloride and into the same medium which did not contain barium chloride. These cultures were incubated at 37°C. and subcultures upon meat infusion agar plates made every 2 days. The plates were examined for the presence of small-colony variants after 24 to 48 hours' incubation.

The ability of the different strains of *S. aureus* to grow upon the barium chloride medium varied considerably. The completely inhibiting concentrations of barium chloride varied from 1.0 to 5.0% with different strains, though the ability of any one strain to grow in certain concentrations was relatively constant. Growth, as judged by both turbidity and plating, was very slow, sometimes requiring from 2 to 4 days to become evident. This was especially true in the higher concentrations of barium chloride.

The small-colony variants appeared very rapidly under the influence of barium chloride, all strains showing their presence within 6 days and most of them within 2 days. (Table I.) Four strains were completely changed to the small-colony form after 4 days' incubation and 7 of the strains after 6 days. These results are in marked contrast to those obtained by older methods which ordinarily

⁶ Hughes, T. P., *J. Bact.*, 1936, **23**, 437.

TABLE I.
Approximate percentage of small colony variants found upon plates made from
Barium Chloride Broth Cultures of *Staphylococcus aureus*.

Strains of <i>Staphylococcus aureus</i>															
Time in days	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
2	10	100	10	90	50	NG*	NG	90	50	75	NG	NG	NG	NG	NG
4	10	100	10	95	25	75	75	90	95	99	100	NG	NG	100	100
6	50	100	25	100	25	10	75	100	95	100	50	100	50	100	100

* No growth.

require weeks or months to produce less than 1.0% of small-colony variants. By the use of barium chloride complete conversion into the variant form may take place within 4 days. The number of small-colony variants produced was roughly proportional to the concentration of barium chloride in which growth was obtained, the higher concentrations producing a relatively higher percentage of variants. Control cultures of the broth containing no barium chloride did not produce small-colony variants even after 30 days of incubation. The chlorides of sodium, magnesium and calcium were without effect.

The variants obtained in the above manner differed in no way from those obtained by other methods. The colonies on agar were extremely small and cultures exhibited the same lowered metabolism, the same manner of reversion and the same ability, if properly handled, to maintain their variant-characteristics over periods of weeks or months without reversion.

When barium chloride was added to plain meat infusion or meat extract broth the appearance of the small-colony variants was very irregular. Upon the barium chloride peptone-activator medium, however, consistent results were always obtained.

The rapidity of appearance and the great number of small-colony variants produced in the presence of barium chloride tends to support the theory that these variants of *S. aureus* are merely forms possessing a lowered rate of metabolism which have evolved under the influence of an unfavorable environment. Furthermore, the work of Quastel and Woolridge⁵ showing the effectiveness of barium chloride as an inhibitor of the enzymatic functions of *B. coli* offers a possible explanation of the mechanism by which barium chloride brings about the production of these small-colony variants.