based on the findings that strong alcohol does not dissolve certain interfering substances such as ergothioneine, proteose, the barium salt of oxyproteic acid, or barium urate but does dissolve barium thiocyanate a somewhat improved procedure has been employed as follows: An aliquot of urine generally 50 cc. is treated with a saturated aqueous solution of barium hydroxide as long as a precipitate forms. The mixture is filtered. The filtrate is brought to dryness on the water bath and is extracted with warm absolute alcohol and filtered. The procedure of extraction with alcohol and filtering is repeated. Then the alcohol is evaporated, the residue dissolved in 25 cc. of water and precipitated with silver nitrate. Then the Rupp-Schied-Thiel iodine procedure is applied.

Such a procedure has given a return of 97.2% for potassium thiocyanate in pure solution, a return of 95% for thiocyanate added to the urine and shows in urine amounts of thiocyanate very much smaller than the original Rupp-Schied-Thiel procedure applied to urine directly. Four normal urines tested for thiocyanate by this procedure gave values of 14.2 mg., 5.31 mg., 17.6 mg., and 25.5 mg. of HCNS in 24-hour samples as compared respectively with 101.3 mg., 66.2 mg., 116.1 mg., and 140.6 mg. HCNS in the original Rupp-Schied-Thiel procedure.

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Dissociation of Pneumococcus: A New Colony Variant.

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I. *A New Colony Variant of Pneumococcus.* R colonies of pneumococcus, suitably spaced on blood agar, are allowed to age for 6 to 7 days at 37°C. Under these conditions many of the colonies give rise to a variety of secondary growths including daughter colonies, peripheral excrescences and a peculiar form of localized, marginal outgrowth with a rough, fungoid appearance. These various forms of secondary growth have been observed in some detail and the rough, localized, marginal outgrowth represents an unusual phase of development. When transfers are made from such areas to fresh blood agar plates a new type of colony is produced. Its ap-

pearance varies considerably with age, but at all periods of growth is characteristically different from the parent colony. Young colonies (4-8 hours) present an extremely rough and irregular surface with delicate, filamentous edges. With age the surface is coarsely rough with an irregular fimbriated border. At 12-16 hours the surface is slightly convex, but after 24 hours the colonies become flattened. They continue to show an irregular margin and are approximately twice the diameter of the parent R colonies grown under similar conditions. With further ageing the colonies develop an extraordinary, spreading, filamentous type of marginal growth.

Stained smears of such colonies show an unusual morphological picture, varying with age, but quite distinctive at all periods of growth. The bacteria consist mostly of plump cocci and cocco-bacilli arranged in long chains. In younger colonies the organisms are almost exclusively Gram positive, but in the older ones there is considerable variation in staining properties. In addition to the plump coccal and cocco-bacillary forms, large numbers of granular elements of various sizes are present.

The stages in the development of the marginal outgrowth which produces the new type of colony have been carefully studied. The available evidence suggests that the transition to the new form is a gradual one. At first the organisms tend to grow in clusters suggestive of a staphylococcal arrangement. The next become pleomorphic and frequently elongated in an extreme, lanceolate manner. In the next stage they appear as pointed, diphtheroid-like elements arranged in a fashion suggesting broken twigs. Long, bizarre, rod forms may appear, which are partly Gram positive and partly Gram negative. Finally, the plump cocco-bacillary forms appear. Each of these morphological stages is represented by a characteristic colony type showing a gradual transition to the new form. The intermediate stages are moderately stable in both liquid and solid media; but, after a time, there appears to be a tendency toward reversion to the original parent form or a progression toward the new colony type.

When transferred to liquid media, colonies of the new type produce a sedimented type of growth resembling a tuft of cotton in the bottom of the tube. The supernatant is usually clear but there may be present loose, flocculent clumps in an otherwise limpid medium. Stained smears of such growths reveal a tangled mass of enormous chains and skeins of plump coccal forms. On solid media the new colony form appears quite constant and has continued to reproduce

its own form for many transfers. In liquid media the new variant reproduces its own form for a number of transfers, but usually between the fourth and sixth subculture a change occurs. Diplococci of the usual morphology appear in the cultures, and colonies of 2 distinct types develop on plates streaked from such cultures: (1) those above described and (2) the original parent type. Further transfers in liquid media result in the appearance of an increasing number of cells of the original parent type until the entire culture appears to revert to its original form.

The morphological evidence suggests that the development of the new colony type is an indirect and not a direct product of the parent colony. On the other hand, reversion of the new colony type to the parent form appears to be sudden and abrupt. The organisms so far studied in the new variant form are bile-soluble, autolyze readily and possess relatively little virulence for white mice. Bacteria-producing colonies similar to the extremely rough variant above described and showing similar growth characteristics are frequently found in normal throats.

In a recent communication, Hadley[1] postulated the existence of this new variant form of pneumococcus and predicted its salient characteristics in a remarkable manner. The basis for Hadley's forecast and the place of the new colony variant in the phenomenon of bacterial dissociation will be considered in a subsequent communication.

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Effect of Sodium Chloride in Treatment of a Patient with Addison's Disease.

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It has been shown recently that the sodium content of the blood of patients suffering from Addison's disease is decreased[1] as it is in adrenalectomized cats,[2, 3] and that adrenalectomy in dogs results in a

[1] Hadley, P., personal communication.

[1] Loeb, R. F., *Science*, 1932, **76**, 420.

[2] Marine, D., and Baumann, E. J., *Am. J. Physiol.*, 1927, **81**, 86.

[3] Zwemer, R. L., personal communication.