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Reversion of Avirulent "Rough" Forms of Pneumococcus to Virulent "Smooth" Types.

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The fact that virulent, type-specific, capsulated, "S" forms of pneumococcus can be degraded to avirulent, non-type-specific, unencapsulated, "R" forms is well established. (Friel,[1] Stryker,[2] Griffith,[3] Amoss,[4] and Reimann.[5]) The reverse process, however, the conversion of avirulent, "R" forms into virulent, "S" forms, has been a matter of some controversy.

Felton and Dougherty,[6] by means of an automatic transferring device in a milk-containing medium, were able to restore virulence to a strain of pneumococcus which had become completely avirulent. They employed pure line strains derived from single cells, and report that some cultures showed a maximal increase in virulence while others remained avirulent. Their investigation preceded the recognition of "R" and "S" forms of pneumococcus. Griffith[3] states that "R" strains may revert in all respects to the "S" type or remain unchanged after many generations in subculture or plain blood broth. Single cell cultures, however, were not employed. Reimann[5] reports that he was unable to enhance the virulence of an "R" strain derived from Type I even after 105 mouse passages. Levinthal[7] was able to effect the transformation by growing "R" forms in serum broth at 25° C. and subsequently passing the cultures through mice.

For bacteria other than pneumococcus the same differences of opinion appear to exist. (De Kruif,[8] Schütze,[9] Savage and White.[10]) Recently, however, Jordan[11] has demonstrated the interconvertibility of "R" and "S" forms of *B. paratyphosus B*, and Soule[12] has shown the reversibility of the dissociated form of *B. subtilis*.

In the present investigation pure line strains derived from single cell cultures of pneumococcus were employed. The single cells were isolated by the method of Avery and Leland.[13]

I. Reversion by Animal Passage. With eight pure line strains of "R" pneumococci, derived from Types I, II, and III "S", it has been found possible in all cases, with one exception, to effect reversion to the "S" form by means of mouse passage. The single exception was that of an "R" form derived from Type I, which, under prolonged cultivation, appears to have lost permanently its ability to revert. Upon reversion the organisms acquire all the

properties of the "S" form, including virulence, so that 10^{-6} cc. of the reverted culture regularly kills white mice, while 0.5 cc. of the original "R" culture fails to do so.

II. Reversion by Growth in Anti "R" Sera. Soule,[12] investigating the microbic dissociation of *B. subtilis,* states that, "by the incorporation of "S" or "R" immune sera in fluid media "R" forms can be obtained from "S" forms and "S" forms from "R" respectively.

Since it is known that "S" types of pneumococcus when grown in homologous immune (anti "S") sera change to the degraded "R" forms, it was thought of interest to determine whether the reverse process would occur if "R" organisms were grown in media containing anti "R" sera.

Accordingly, a series of pure line "R" cultures were grown in broth to which the serum of rabbits immunized to "R" forms was added. By this method six "R" strains derived from Types II and III have been caused to revert to type-specific "S" forms. So far, however, it has not been found possible to effect this change with those Type I R strains which were selected. As in the method of animal passage, reversion by cultural methods is accompanied by the acquisition of maximal virulence, the ability to form capsules, and to elaborate the specific soluble substance upon type specificity depends.[14]

In all cases in which the transformation has been effected, reversion has invariably been toward the specific type from which the "R" form was originally derived. Thus far the reversion from "R" to "S" pneumococcus by *in vitro* methods has only succeeded when the serum employed contained anti "R" antibodies. The nature of the stimulus to reversion is at present being further investigated.

This method, then, in certain instances at least, represents a simple *in vitro* means of enhancing virulence and restoring type-specificity to avirulent "R" forms of pneumococci.

This is a preliminary report.

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[1] Friel, A. R., *Pub. S. A. Inst. Med. Res.*, 1915, No. 5.

[2] Stryker, L. M., *J. Exp. Med.*, 1916, xxiv, 49.

[3] Griffith, F., *Rep. Pub. Health, Ministry of Health*, 1923, No. 18, (1).

[4] Amoss, H. L., *J. Exp. Med.*, 1925, xli, 649.

[5] Reimann, H. A., *J. Exp. Med.*, 1925, xli, 587.

[6] Felton, L. S., and Dougherty, K. M., *J. Exp. Med.*, 1924, xxxix, 137.

[7] Levinthal, W., *Klin Wochschr.*, 1926, ii, 2020.

[8] De Kruif, P. H., *J. Exp. Med.*, 1922, xxv, 561.

[9] Schütze, H., *J. Hyg.*, 1921, xx, 330.

[10] Savage, W. G., and White, P. B., *Spec. Rep. 91, Med. Res. Council*, 1925.

[11] Jordan, E. O., *J. Am. Med. Assn.*, 1926, lxxxvi, 177.

[12] Soule, M. H., *J. Bact.*, 1927, xiii, 41.

[13] Avery, Roy C., and Leland, Stanley J., *J. Exp. Med.*, 1927, xlv, 1003.

[14] Dochez, A. R., and Avery, O. T., *J. Exp. Med.*, 1917, xxvi, 477. Heidelberger, M., and Avery, O. T., *ibid.*, 1923, xxxviii, 73; 1924, xl, 301.

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Further Experiments on the Transplantation of Neural Crest (Mesectoderm) in Amphibians.

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In previous experiments[1] the author has shown that when mesectoderm was excised in the cranial regions of early stages of embryos of *Amblystoma punctatum* deficiencies were produced in the branchial skeleton, Meckel's cartilage, palatoquadrate bar, anterior trabeculae and loose mesenchyme in the head. It was further found[2] that when groups of mesectoderm cells were transplanted to new positions on the side of the body they differentiated into cartilages in the presence of the strange mesentoderm (mesoderm) of the somites and in the absence of mesentoderm of the visceral arches.

A similar study has been extended to the Anurans. In *Rana palustris* the author reported[3] a migration of bands of mesectoderm over the mesentoderm of the visceral arches from the dorsal part of the cranial portion of the neural folds similar to the condition found in Amblystoma. The migration begins before the closure of the neural folds—an earlier stage than in Amblystoma. It was found in those studies[3] in *Rana* that when the mesectoderm was removed deficiencies were produced in the visceral skeleton on the operated side.

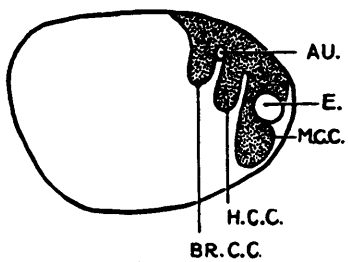

FIG. 1.

Camera-lucida drawing of *Rana palustris* embryo just before closure of neural folds showing extent of migration of mandibular (M. C. C.), hyoid (H. C. C.), and branchial (BR. C. C.) mesectoderm (stippled) at time of operation. AU., auditory placode. E., optic vesicle. X 15.