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The Transformation of Pneumococcal Types in Vitro.

MARTIN H. DAWSON AND RICHARD H. P. SIA.*

From the Department of Medicine of the College of Physicians and Surgeons, and the Presbyterian Hospital, New York.

Griffith[1] was the first to show that type-specific, S, pneumococci may be transformed from one specific S type into other specific S types through the intermediate stage of the R form. He showed that the transformation of R forms, derived from one specific S type, into S forms of heterologous types may be effected *in vivo* by the following procedure: The subcutaneous injection, in white mice, of small amounts of living R forms together with suspensions of heterologous S cultures, killed by heating. Griffith further reported that all attempts to secure transformation of type by *in vitro* methods were unsuccessful.

Griffith's observations were confirmed and extended in recent publications by one of the authors.[2] In these communications it was also reported that all *in vitro* attempts to effect transformation of type were unsuccessful.

Recently we have renewed the *in vitro* studies and have succeeded in evolving a relatively simple technique for inducing transformation of pneumococcal types in the test tube.

For the purposes of convenience we have confined our present studies to the transformation of a 2R culture into Type III S organisms. However, since it has been shown in previous work that an R culture may be transformed *in vivo* into S organisms of *any*

* On leave of absence from the Peiping Union Medical College, Peiping, China.

1 Griffith, F., *J. Hygiene*, 1928, xxvii, 113.

2 Dawson M. H., *J. Exp. Med.*, 1930, li, 99, 123.

heterologous type it is probable that similar transformations may also be effected *in vitro*.

The procedure consists in seeding minimal amounts of an R culture into a suspension of S organisms of heterologous type, killed by heating. Certain conditions, while not absolutely necessary, apparently facilitate the transformation process. Some of these conditions are: (1) The amount of the R inoculum introduced, (2) the incubation of the cultures for a longer time than the conventional period, (3) the addition of a small amount (10%) of anti-R serum, (4) the addition of a small amount of blood-broth. The degree of heat to which the organisms have been exposed also materially affects the results. Suspensions of organisms heated for periods as long as four hours at 60°C. have been effective. Likewise suspensions of organisms heated for 15 minutes at 80°C. have been effective. However, organisms heated for 15 minutes at 100°C. have lost the capacity for inducing the transformation.

By this technique, transformation of type may be induced with very small quantities of heat-killed suspensions—quantities as small as the equivalent of 0.1 cc. of culture. Filtrates of actively growing cultures have not proven effective nor have filtrates of heat-killed suspensions of S organisms. Suspensions of S organisms, broken up by freezing and thawing and subsequently subjected to a temperature of 60°C. for 15 minutes have likewise proven ineffective. However, suspensions of S organisms first killed by heating for 15 minutes at 60°C. and subsequently frozen and thawed have proven highly effective. In this process the heat-killed cells, for the most part, maintain their integrity. In previous *in vivo* experiments autolysates of S cultures, heated for 15 minutes at 60°C., failed to induce the transformation. It would therefore appear that, under the conditions employed, the property of the heat-killed S organism responsible for bringing about change of type is destroyed or altered by the disruption of the S cell.

Experiments carried out under anaerobic conditions are now being undertaken.

In all experiments the vaccines were prepared in the same manner as described in previous publications. Similar controls were adopted to eliminate the possibility of the persistence of viable forms in the heat-killed suspensions.