indistinguishable in colonial morphology, formed homogeneous suspensions. A large number of such changes has been observed without evidence of linkage of any 2 characters.

There has been no uniform pattern for the altered characters of the R-variants, nor for those of the reversion cultures derived from them. We have found no evidence in studying these cultures that during either the dissociative or the revertive process the characters of a culture change hand-in-hand. The alterations occur in various groupings, and each character appears to be capable of varying independently of the others. In no instance did we observe complete reversion of all the characteristics. Only one of the 11 reversion cultures has reverted to complete antigenic identity with the original culture, but this culture showed for many weeks a difference in cellular morphology, growing in long filamentous forms.

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On the Mechanism of True and of Non-Specific Complement Fixation Reactions.

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The complement fixation test for tuberculosis gives a non-specific or falsely positive reaction if a balanced physiological saline, akin to Tyrode's solution, replaces the plain 0.9% NaCl used in the test. The balanced saline which gives the strongest non-specific reaction contains 0.888% NaCl, 0.009% $CaCl_2$, and 0.003% $MgCl_2$; both Ca and Mg salts are required, and it is only over a very limited range of concentration of these salts that the non-specific reaction occurs. Petroff's[1] whole bacillus antigen is more effective than any other examined in producing this non-specific reaction, while "fat-free" tuberculo-antigens do not give this non-specific effect. In the absence of antigen, *i. e.*, in the anticomplementary control tubes, balanced saline assists hemolysis, so that one-third of the normal unit of complement may produce complete hemolysis.

The mechanism of this falsely positive reaction is as follows:

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[1] Petroff, S. A., *Z. f. Tuberk.*, 1923, **39**, 100; quoted by Willis, H. S., Laboratory Diagnosis and Experimental Methods in Tuberculosis, Springfield, 1928, 166.

1. The surface properties of the (Petroff) antigen particles are modified when incubated with complement and plain saline, presumably due to irreversible adsorption of protein from the diluted guinea pig serum used as complement; the isoelectric point of the washed antigen is changed from pH 3.2 to pH 3.7 by this treatment.

2. In the presence of balanced saline, the surface properties of the antigen undergo a further change, in that the particles agglutinate readily, and fix complement; *i. e.*, the antigen now acts as does an antigen sensitized by immune serum. The tendency of the antigen to agglutinate is a reversible one, which is present while the antigen is in balanced saline, and absent if the antigen is transferred to plain saline.

Further details of the factors involved in this reaction will be given elsewhere.[2] The critical Na to Ca ratio which will produce this non-specific reaction is also approximately the ratio of these ions present in the blood and in those balanced physiological salines which are essential to many forms of cell life; it is also the Na to Ca ratio, which, due to antagonism of the Na and Ca ions, exerts zero effect upon the stability of oil-water emulsions (Clowes[3]).

This non-specific reaction is an example of an immune reaction being duplicated by means of non-specific reagents, and the following experiments give some information upon the mechanism of complement fixation. A non-specific complement fixation reaction (complement + antigen + balanced saline), and a true positive reaction (immune serum + complement + antigen in plain saline), were carried out simultaneously, the same amount of antigen being used in each reaction. By preliminary titration, the amount of immune serum used in the latter reaction was so adjusted that the antigens in the 2 reactions possessed equal complement fixing power. Direct comparison of the 2 antigens then indicated that their particles had the same degree of cohesion, and when shaken with oil, they exhibited the same percentage partition between the oil and water phases. The only physical difference observed was in the electrical potential on the washed antigen particles.

Complement fixation thus appears to be produced by an antigen which has acquired appropriate surface properties, regardless of whether these properties were produced by immune or by non-specific reactions; *i. e.*, complement fixation in itself is not a specific immune reaction, but only a secondary effect due to the surface properties of suitably modified antigens.

[2] Hambleton, A., *Can. J. Res.*, 1932, **7**, 583, 596.

[3] Clowes, G. H. A., *J. Phys. Chem.*, 1916, **20**, 407.