In normal guinea pigs, the areas of skin injected with old tuberculin were not affected by a subsequent intravascular injection of a typhoid filtrate. Hence it is probable that the specific inflammation produced by tuberculin in the hypersensitive tissue, rather than products of the tubercle bacillus *per se,* prepared the skin for the action of the typhoid filtrate introduced into the blood stream.

Conclusion. The intravascular injection of a filtrate from the culture of typhoid bacilli produces hemorrhagic necrosis in tuberculous guinea pigs at the site of positive tuberculin reactions.

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Independent Variation of Biological Characters of Bacteria as a Result of Dissociation.

G. M. MACKENZIE, HELEN FITZGERALD AND VERNAL IRONS.

From the Biological Laboratory, Mary Imogene Bassett Hospital, Cooperstown.

Numerous observations on bacterial dissociation indicate that the changes occurring as a result of dissociation may involve many of the cultural characteristics. Although alterations in colonial morphology are the most obvious, many other characteristics may also undergo modification. De Kruif demonstrated changes in virulence[1] and acid agglutination optimum.[2] Profound serological alterations have been studied by Arkwright,[3] Schütze,[4] Savage and White,[5] Goyle.[6] The modifying effects of dissociation on many biochemical reactions, including fermentative capacities, have also been reported.[7] In a recent study of the fermentations of variants of *Salmonella schottmülleri,* Nungester and Jung[8] obtained R-variants which formed gas and others that did not; they observed that an S-culture may lose its capacity to form gas without change in colonial morphology. These observations, they concluded, were

[1] De Kruif, P., *J. Exp. Med.*, 1922, **35**, 631.

[2] De Kruif, P., *J. Gen. Physiol.*, 1922, **4**, 387.

[3] Arkwright, J. A., *J. Path. and Bact.*, 1921, **24**, 36.

[4] Schütze, H., *J. Hygiene*, 1921, **20**, 330.

[5] Savage, W. G., and White, P. B., *Spec. Rep. Series, Med. Research Council,* London, 1923, **25**, 90.

[6] Goyle, A. N., *J. Path. and Bact.*, 1926, **29**, 149.

[7] Hadley, P., *J. Inf. Dis.*, 1927, **40**, 1.

[8] Nungester, W. J., and Jung, R. W., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 681.

in accord with the concept of independent variation of bacterial properties.

Our studies on S- and R-variants of *Salmonella schottmülleri, Salmonella morgani* and *Shigella paradysenteriae (Flexner)* appear to demonstrate that many of the biological characters—serological, morphological and physiological—of these cultures are capable of independent variation during the dissociative and revertive processes.

Well-stabilized R-variants were obtained from the 3 normal S-cultures by growth in formalin (1:8000) beef infusion broth. Each of the R-cultures regularly produced 100% rough colonies on agar plates. The R-variants were then subjected to environmental conditions favoring reversion: (1) growth in 2% homologous immune serum broth; (2) growth in broth containing 2% vaccine prepared by heating an 18-hour growth of the original smooth culture for 4 hours at 56°C.; (3) rapid transfers in plain beef infusion broth. A variety of "reversion" cultures, derived from the 3 R-variants, was obtained. Serological, morphological and physiological characteristics of the 3 original S-cultures, the 3 R-variants, and the 11 "reversion" cultures were studied. Observations were made on colonial morphology, cellular morphology, motility, acid and gas production in carbohydrate media, formation of indol, saline stability, acid agglutination optimum, type of growth in broth, and antigenic composition determined by cross agglutinations and reciprocal absorptions. Alterations of antigenic behavior were such that it has been possible to demonstrate independent variation of the agglutinative, absorptive, and agglutinogenic properties of the antigens. A variant culture may show impaired agglutinative capacity with little or no alteration of absorptive and agglutinogenic properties. Other variants may show unimpaired agglutinative capacity and partial suppression of absorptive capacity, or the changes may affect the agglutinogenic capacity more than the other two. In brief, the agglutinative, agglutinogenic and absorptive properties vary independently of each other. Similarly, the other characters of these cultures varied independently of each other and of the antigenic characters. A motile S-variant of *Salmonella schottmülleri* formed acid from the same carbohydrates as the original culture, but on repeated tests failed to form any gas, and in serological behavior the only alteration was a decreased absorptive capacity. In this instance, therefore, gas formation and absorptive capacity had varied independently of the other characters. One R-variant of *Salmonella schottmülleri* was motile, another non-motile. An S-variant of *Shigella paradysenteriae* was unstable in 0.85% NaCl; another,

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.

A. HAMBLETON. (Introduced by H. Wasteneys.)

*From the Mara Laboratories, Queen Alexandra Sanatorium, London, Canada.**

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:

* Aided by the National Research Council of Canada.

[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.