

injections of the specific substance from *V. cholerae* adsorbed to charcoal gave, after 2 hours at 37°C., agglutinin titers of (1) 1:1600, (2) 1:100, (3) 1:50, (4) 1:100, and (5) 1:50, respectively. Precipitation reactions, made by adding 2 drops of the immune serum to 0.2 cc. of a 1:5000 dilution of the specific substance were distinctly positive in 2 cases, and weakly positive in 2 others.)

Control animals injected with the substance alone produced no precipitins, and the sera showed only traces of agglutination in a dilution of 1 to 10. A solution of the specific substance still gave a biuret reaction, and on hydrolysis with acid a precipitate was formed which contained alcohol soluble acids.

In one experiment in which an azodye made from diazotized tartranilic acid and resorcinol⁵ was adsorbed to aluminum hydroxide and injected into guinea pigs, a certain degree of sensitization seemed to be brought about, since some of the animals showed mild anaphylactic symptoms and one of them died in acute shock on the injection of a corresponding azoprotein after an appropriate interval.⁵ It was, however, not possible to reproduce this result in a second experiment.

The observations reported show in confirmation of those of Gonzalez and Armangué that the hapten of the Forssman substance can exhibit distinct antigenic activity, and one may reasonably assume that this will hold for other sorts of haptens, without the aid of antigenic proteins. On the other hand, the experiments do not disprove the idea that in the high activity of natural complex antigens the proteins play a significant rôle.

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Single Cell Dissociation of the Mycobacterium of "Rat Leprosy."*

MORTON C. KAHN AND HELEN SCHWARZKOPF.

From the Department of Public Health and Preventive Medicine, Cornell University Medical College, New York City.

An acid-fast organism isolated by Chapin¹ from a leprous rat in 1912 has been kept in the culture collection of the National Tubercu-

⁵ Landsteiner, K., and Levene, P., *J. Exp. Med.*, 1930, **52**, 347.

* This experiment is part of a group investigation being carried on in conjunction with the Medical Research Board, National Tuberculosis Association.

¹ Chapin, *U. S. Treas. Dept., Pub. Health Rep.*, 1912, **27**, 161.

losis Association for considerable time. Transfers distributed over the country from this and other sources have been designated as "bacillus of rat leprosy", mycobacterium of "rat leprosy", "rat leprosy bacillus", etc. This mycobacterium is probably not identical with acid-fast organisms in tissue from actually leprous rats.^{2, 3, 4, 5} These observers found it very difficult, if not impossible, to cultivate the organisms but stated that the disease could be transmitted from rat to rat by injecting infected tissue. The organism under discussion was received some years ago from the National Tuberculosis Association and labelled "Rat leprosy bacillus No. 368". It grows luxuriantly on Petroff's or Lowenstein's egg medium (pH 7.4) after an incubation of about 4 days. The growth membrane appears deeply orange colored. Growth also takes place on slants of nutrient agar containing no glycerol at pH 7.4, but acid-fast properties are lost after a week of incubation on this medium. Such properties may be regained after planting on the egg media which contain glycerol. The organisms measure from 0.3 to 3.0 mu. in length and about 0.3 mu. in breadth. Numerous granules have been observed on acid-fast and gram-stained preparations. The organism is acid-fast and gram-positive.

After several transfers on petri plates of Petroff's egg medium one of these cultures, initially R in character, was incubated for several weeks at 37.5°C. Upon examination, typical S colonies as well as the usual R colonies were found. The S colony is smooth, round, domed and glistening moist in appearance, and measures $\frac{1}{2}$ to 2 mm. in diameter, while the R colony is flat, dry, dull and rough and wrinkled in configuration, measuring some 2 to 4 mm. in diameter. The S colony makes a homogeneous suspension in normal sodium chloride solution or distilled water; the R colonies, on the contrary, flocculate and precipitate even after prolonged shaking. These 2 colony types are distinct and clear-cut.

Several representative R and S colonies were then selected and by applying Petroff's⁶ filtering technic a number of plates were obtained from each, having widely separate colonies. These colonies for the most part bred true to type but on some plates of the S colonies, R types were found to have grown, while on one of the plates seeded with an R colony suspension 3 distinct S colonies were noted. These

² Stefansky, *Centralbl. f. Bakteriol.*, 1903, **33**, 481.

³ Dean, *Centralbl. f. Bakteriol.*, 1903, **33**, 222; *J. Hyg.*, 1905, **5**, 99.

⁴ Wherry, *J. Am. Med. Assn.*, 1908, **1**, 1903.

⁵ Zinnser and Carey, *J. Am. Med. Assn.*, 1912, **58**, 507.

⁶ Petroff, *PROC. SOC. EXP. BIOL. AND MED.*, 1927, **24**, 632.

S colonies from an initial R form, and R forms from an initial S colony again, developed after a subsequent reculturing of the second dissociants. Obviously, either the homologous colony was not homogeneous for type or spontaneous dissociation was taking place.

To answer this question several single bacteria were isolated from both the R and S types of colonies. The single bacterial cells were allowed to form micro-colonies in the micro-culture after which the resultant growth was drawn into a micro-pipette and expelled upon a slant of Petroff's egg medium. By allowing a micro-colony to form in the microscopic drop containing a single bacterial cell before any attempt is made at transplantation, a much higher percentage of successful cultures is obtained from single cells than if the freshly isolated single organism is planted directly. The details of this technic will be published later.

The cultures obtained from single organisms of both the R and S types of colonies bred true for 3 or more generations, giving rise only to their homologous colony forms. Sooner or later, however, in both instances dissociation unquestionably occurred. Typical R colonies grew on plates receiving inoculation with an actual S colony from a single cell S strain and S colonies developed on plates which were seeded with an actual R colony from an R single cell strain. The mutation is somewhat more prolific from S to R, but the R to S change may not be termed at all infrequent, as is the case among many other species of bacteria. The dissociation of these single cell strains took place spontaneously, no special effort being made to induce it. This confirms observations on single cell cultures of avian *Mycobacterium tuberculosis*.[†]

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Relative Immutability of Hydrogen Ion Concentration of the Bile. Its Buffering Effect in Bactericidal Experiments.

REUBEN OTTENBERG AND JOSEPH KAHN.

From the Laboratories of Mount Sinai Hospital, New York.

In 1926 we noticed that solutions of phenol-tetra-chlor-phthalein and phenol-tetra-iodo-phthalein are decidedly bactericidal for typhoid bacilli. These dyes, however, when concentrated in the gall-bladder of animals after intravenous injection (Abel and Rown-

[†] Kahn, Proc. 33rd Ann. Meeting Soc. Am. Bact., 1931, 10.