It will be observed that the range of C_{H+} at which the smallest amount of beef infusion is required is for each type precisely the zone of the acid agglutination recorded in the preceding paper. This experiment indicates that the beef infusion, *per se*, does not cause the agglutination. It merely widens the acid agglutination zone. This would seem to throw light upon the mechanism of the granular growth character of type G in plain broth.

Suspensions of types D and G were similarly tested against decreasing concentrations of peptone at varying C_{H+}. In these experiments the results were of a different nature, as might have been expected from the failure of peptone to agglutinate type G at $P_H = 7.5$ to $P_H = 6.8$. In the case of peptone, the optimum for type G lies at a range between $P_H = 3.0$ and $P_H = 2.5$. That for D, at $P_H = 2.5$. Peptone, therefore, seems to shift the optimum zone in the direction of a higher C_{H+}, an effect analogous to that observed in the glycocol-HCl buffer mixtures. In the case of microbe D, strong concentrations of peptone (1–2 and 1–4) actually suppress flocculation completely at $P_H = 3.0$. This effect is analogous to the pre-zone phenomenon in immune reactions, since for the higher dilutions of peptone at this C_{H+}, complete agglutination readily occurs.

It would appear from the foregoing that while the flocculation in all cases under consideration is due to H-ions, at the same time other factors, such as glycocol, peptone or beef infusion, either shift or broaden the acid agglutination optimum.

20 (1767)

Dissociation of microbic species.

V. Further considerations in regard to the virulence of microbes D and G.

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The wide variations in virulence between microbes D and G, bacillus of rabbit septicemia, has been demonstrated in the first paper of this series.[1] Microbe D, the type found in natural in-

[1] *Jour. Exper. Med.*, 1921, xxxiii, 773.

fections, is possessed of powerful invasive properties, while its mutant form G is characterized by very low virulence.

The fixity of the character of high virulence for type D is demonstrated by the following experiment. Strain R-19, type D, was tested a few days after its isolation from a rabbit dead of broncho-pneumonia. The test was carried out by injecting high dilutions of a six-hour serum broth culture intrapleurally into young rabbits of 600 grams weight. The strain proved itself fatal in 10^{-8} c.c. of the serum broth culture. This culture was transplanted every seven days on serum agar. Tests made one and three months after the first experiment indicated its virulence to be still of the same titer.

The individuals of a given strain of type D appear to differ very little in the characteristic of virulence. Six pure-line strains, isolated by the Barber method from stock strain R-15, were tested for virulence by the method just described. All were fatal in dose of 10^{-6} c.c.

The virulence of type D not only remains constant during passage on serum agar, but persists under conditions that may be considered as distinctly unfavorable. For example, a pure-line strain of type D was planted in plain broth. It was allowed to remain at 37° C. for 9 days and 12 hours without further transplantation. At the end of this time a culture was streaked on a serum agar plate. Marked D → G mutation had occurred, counts showing D = 40, G = 60. A colony of each type was fished into serum broth tubes. These were incubated for the usual time, diluted appropriately, and injected into two series of rabbits of 600 grams weight. The D culture, injected over a range from 10^{-7} to 10^{-1} c.c., proved fatal in every case. The G culture, on the other hand, failed to provoke a noticeable effect, even when 0.5 c.c. of whole culture was injected.

It has been remarked in the second paper of this series that pure-line strains of microbe D may mutate during daily passage in plain broth. A pure culture of type D, virulence 10^{-6}, was transplanted daily in plain broth for 25 passages. Its virulence remained constant during this time. At the 30th passage a few G colonies were observed on the serum agar sub-plates. In two months, sub-cultures on serum showed a large preponderance of G

colonies. The virulence had fallen to 10^{-4} c.c. Twenty-five days later, no D colonies could be demonstrated. The mutation D → G was complete, or type G had completely outgrown type D. 0.1 c.c. of culture failed to produce a fatal effect. The attenuation of this culture is to be referred to the gradual replacement of the primordial D by the mutant G form. It is possible to predict the virulence from the relative preponderance of the two types, as evidenced by colonies on serum agar plates. It is possible to procure sub-cultures of very high or of very low virulence by selection of one type or the other, so long as any of the D type remain.

While the virulence of microbe G is very low, 1.0 to 2.0 c.c. of whole culture may occasionally produce fatal infections, especially in young rabbits. The organisms recovered from such animals at necropsy retain their granular growth character, but may gain perceptibly in virulence. After three animal passages, a type G culture has been observed to reach a virulence of 10^{-4} c.c. But despite this increase in invasive power, the non-fluorescence of its colonies persisted, its granular growth character intensified, and its acid agglutination optimum rose to $> P_H = 5.6$. It is apparent from this experiment that it is unsafe to state that low virulence goes invariably hand in hand with the other characters gained in mutation. Experiments are under way to determine whether this artificially produced virulence of type G is permanent or evanescent.

The route of infection is important in determining the ability of the low-virulent type G to gain a foothold in the animal body. A culture of microbe G which produced no perceptible effect when injected into rabbits *intrapleurally* in dose of 1.0 c.c. gave rise to abscesses when injected *subcutaneously* in 0.1 c.c. These lesions remained sharply circumscribed, but the type G organism could be recovered from them for several weeks after the appearance of the abscess.

The phenomenon of vicariously greater susceptibility to subcutaneous injection would seem to be due to the rapidity with which phagocytes are mobilized against type G when this organism is injected into the pleural cavity. Within 6 hours after intrapleural injection of 1.0 c.c. of serum broth culture of type G, no

free organisms could be demonstrated in the aspirated fluid. Polymorphonuclear cells were present in large numbers and phagocytosis was intense. The virulent type D, on the other hand, gains its foothold primarily by reason of the late appearance of phagocytes following its intrapleural injection.

The occurrence of the low-virulent type G would seem to afford an excellent opportunity for the investigation of the properties or products of secretion which give the parent D type its characteristic of high virulence.

21 (1768)

II. The prevention of the development of rickets in rats by sunlight.

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In June, 1919, Huldschinsky[1] reported that the ultraviolet ray exerted a curative action in rickets. The criterion on which he relied was the evidences furnished by the X-ray of calcium deposition at the ends of the long bones. He found that there were definite signs of calcium deposition after four weeks of treatment and that at the end of eight weeks healing was almost complete. In May, 1920, Huldschinsky[2] again reported the curative effects of treatment with the ultraviolet ray in rickets in a series of thirty children, aged between one and one half and six and one half years, who exhibited all clinical manifestations of the disease. In all, healing was accomplished after twenty-two to twenty-six treatments covering a period of two months. In April, 1920, Putzig[3] corroborated the findings of Huldschinsky. He obtained

[1] Huldschinsky, K., *Deutsch. Wchnschr.*, 1919, xlv, 712.

[2] Huldschinsky, K., *Ztschr. f. orthop. Chir.*, 1920, lxxxix, 426.

[3] Putzig, H., *Therap. Holbmonatschr.*, 1920, viii, 234.