

TABLE II.
Agglutinin Titer in Serum, Hepatic Bile, and Gall-Bladder Bile to Bacterial Antigen in Dogs.

Dog No.		<i>Staphylococcus</i>	<i>Streptococcus</i>	<i>B. coli</i>	<i>B. typhosum</i> flagellate	<i>B. typhosum</i> aflagellate
19	Serum	1:800	1:1600	1:800	1:400	1:1600
	Gall-bladder bile	1:120	1:120	1:240	1:240	1:120
	Hepatic bile	1:60	1:60	1:120	1:120	1:60
20	Serum	1:400	1:800	1:3200	1:1600	1:3200
	Gall-bladder bile	1:120	1:120	1:240	1:240	1:60
	Hepatic bile	1:60	1:30	1:60	1:120	1:30
42	Serum	1:400	1:400	1:800	1:800	1:1600
	Gall-bladder bile	1:160	1:80	1:160	1:80	1:160
	Hepatic bile	1:20	1:10	1:10	1:40	1:10

Conclusions. Immunized dogs which have developed serum agglutinins to certain strains of the staphylococcus, streptococcus, colon and typhoid bacteria, have those agglutinins present in the hepatic and the gall-bladder bile. The data indicate that antibody is concentrated in the gall bladder.

8330 P

Lysis of Tubercle Bacilli in Vitro.

WILLIAM STEENKEN, JR. (Introduced by L. U. Gardner.)

From the Research and Clinical Laboratory, Trudeau Sanatorium, Trudeau.

The results of studies on the dissociation of the H₃₇ strain of human tubercle bacilli have been reported.¹ The variants found were designated "R" indicating resistant to environment and "S" indicating sensitive to environment. This communication records an interesting lytic phenomenon observed during the study of the effect of ageing H₃₇ "R" and "S" variants on gentian violet glycerol egg and plain glycerol egg media of different pH. For clarity it seems advisable to discontinue this usage of the symbols "R" and "S" and to employ them in the usual sense as indicative of a rough or smooth colony structure. To indicate virulence or avirulence the letters (v) or (a) are appended thus "Rv" and "Ra". Since a smooth variant of H₃₇ which manifests a typical morphology and virulence has not been obtained this terminology has been adopted to cover

¹ Steenken, W., Jr., Oatway, W. H., Jr., and Petroff, S. A., *J. Exp. Med.*, 1934, **60**, 515

the virulent and avirulent strains of variants having an "R" morphology.

From a dissociated H₃₇ culture¹ extreme variants "Ra" and "Rv" were obtained. Each of these variants was seeded in flasks of gentian violet egg and plain glycerol egg media of the following pH 5.0, 5.5, 6.0, 6.2, 6.5, 6.8, 7.0, 7.3, 7.5, and incubated at 37.5°C. in order to follow single colonies. Structural and growth differences of the variants were observed at the end of 6 weeks' incubation. At this time there were no visible colonies on flasks below pH 5.5. The flasks were then returned to the incubator and were examined every 2 weeks over a period of 5 months to observe further changes.

During the first 3 months the cultures of the "Rv" variant showed typical wrinkled or stippled, colonies that were slightly raised and had spreading veil-like peripheries. After a period of 3 or 4 months' incubation at 37.5°C. such colonies on media of pH 6.0, 6.2, and 6.5 became slightly moist and their central portions semifluid with loss of recognizable structure. Extension of the liquefaction toward the periphery resulted in destruction of the original characteristics of the colonies and some of them completely disappeared. The pH of the media at this time was 4.2 in contrast to the original value of pH 6.1.

At the same time small secondary resistant colonies developed which were raised and had large convolutions with clear-cut peripheries. When such colonies were seeded on gentian violet glycerol egg media of pH 5.5 they showed a good growth within 3 weeks with colonies closely resembling the original ones but with no evidence of surface liquefaction. But when such colonies were removed from the media and their under surface examined with a binocular "colony microscope" deep excavations filled with mucoid fluid were discovered.

In smears stained by the Ziehl-Neelsen method the bacillary bodies from these colonies were very pleomorphic ranging from small acid-fast and non-acid-fast dustlike granules to large round or ovoid acid-fast bodies. There were also many acid-fast and non-acid-fast bacilli and a few branching forms. As the colonies grew older the number of granules and round acid-fast bodies progressively increased.

When inoculated into guinea pigs these "Ra" variants produced no spreading macroscopic disease or local tissue damage, but they did create skin hypersensitiveness.

The original "Ra" variant over the same period of time remained practically unchanged except for the development of a slightly

moister surface but there was no evidence of lysis. When transferred to new media the "Ra" colonies grew freely and continued to manifest the same type of colony structure and virulence for the guinea pig.

The same phenomena have been observed with several other "Ra" cultures that were obtained from biopsy and autopsy materials and from sputa. These results will be reported in a later and more detailed publication together with complete data regarding virulence, hypersensitivity and bacillary morphology.

The above observations on the tubercle bacillus parallel those of d'Herelle and Bordet on other bacteria. They demonstrated that the sensitive or "S" type gives rise to a resistive or "R" type which is refractory to the lytic principle.

8331 P

Motility and Fertilizing Capacities of Fowl Sperm in the Excretory Ducts.

S. STERLING MUNRO. (Introduced by Carl R. Moore.)

From the Poultry Division, Dominion Experimental Farm, Ottawa, Canada.

Young¹ showed clearly that spermatozoa of mammals require and undergo a "ripening" process after formation, while being transported through the seminiferous tubules and epididymis during which they acquire not only the power of movement but also the ability to fertilize eggs of their own species.

Certain fundamental differences exist between the anatomy and biology of the avian and mammalian testis which make pertinent the question of just how analogous are the processes underlying sperm physiology in the two classes.

Spermatozoa removed from the testes, epididymides and vas deferens of 30 males of the domestic fowl have been examined for motility when suspended in either Ringer's solution or the diluent recommended by Baker.² Using the motility classifications of Moore³ the activity of sperm from the testis, epididymis and vas deferens was found to be x, xx or xxx and xxxx respectively. There was

¹ Young, W. C., *Brit. J. Exp. Biol.*, 1931, **8**, 151.

² Baker, J. R., *Quart. J. Exp. Phys.*, 1931, **21**, 139.

³ Moore, C. R., *J. Exp. Zool.*, 1928, **50**, 455.