

As the results of several experiments were identical, those of only one are given. All guinea pigs shaved but not traumatized acquired *enteritidis* bacteriemia in 3 to 15 days. Longer incubationary periods were observed in those in which the intervals between shaving and swabbing were longer. One traumatized animal got fever on the second day. All these animals gave positive blood and stool cultures, both before and after death. Two controls showed no fever and stool cultures were always negative. They were discarded after one month's observation.

The results seem to show that bacteriemia with *S. enteritidis* may be readily produced in guinea pigs through a parenteral route with even a relatively small dose. The rapidity of infection seems to be related to the degree of trauma, and the more severe this is, the more rapidly the infection is produced. Animals with no apparent trauma may require an incubationary period of 15 days before fever appears. This brings *S. enteritidis* in line with *Br. melitensis*,^{4,5} yellow-fever virus,⁶ and *Mycobacterium tuberculosis*,⁷ in ability to pass through reasonably intact skin and to set up infection, which may explain the mode of infection in the cases of bacteriemia which have occurred in local epidemics.

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Pathogenicity of Different Types of *C. diphtheriae* for Chinese Hamsters.

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Since Anderson, *et al.*,¹ described 3 types of *C. diphtheriae*, the relative virulence of these organisms for common laboratory animals has been the subject of much study. Povitsky, Eisner, and Jackson² observed that it took 4 times smaller dose of *gravis* than *mitis* types to kill guinea pigs. Murray³ showed that in virulence for rabbits,

⁴ Hardy, A. V., Hudson, M. G., and Jordan, C. F., *J. Inf. Dis.*, 1926, **45**, 271.

⁵ Eyre *et al.*, *Report Mediterranean Fever Committee*, 1906-1907, Part VI, p. 44.

⁶ Bauer, J. H., and Hudson, N. P., *Am. J. Trop. Med.*, 1928, **8**, 371.

⁷ Ono, I., *Kekkaku Abstr. Sect.*, 1935, **13**, 20.

¹ Anderson, J. S., Happold, F. C., McLeod, J. W., and Thomson, T. G., *J. Path. and Bact.*, 1931, **34**, 667.

² Povitsky, O. R., Eisner, M., and Jackson, E., *J. Inf. Dis.*, 1933, **52**, 246.

³ Murray, J. F., *Brit. J. Exp. Path.*, 1935, **16**, 384.

the order was *gravis*, *intermediate*, *mitis*. Gundel and Ezrin⁴ found that the organisms of the *gravis* type were not only more virulent than those of *intermediate* and *mitis* types, but definitely more invasive in guinea pigs. However, Parish and his coworkers⁵ could demonstrate no such high virulence of the *gravis* type either in rabbits or in guinea pigs. In view of such conflicting reports, a further study of this problem in another species of laboratory animal seems to be desirable. As the Chinese hamsters have been found to be highly susceptible to *C. diphtheriae* infection, and since they can be easily procured in large numbers and at low cost,⁶ these animals have been chosen for the present study.

Eight strains of *gravis*, 5 of *intermediate*, and 8 of *mitis* types were employed. Two of each type were kindly supplied by Professor McLeod of the University of Leeds and the rest were isolated locally from cases of diphtheria. Eighteen-hour Loeffler's slant-cultures were emulsified in 2 cc. normal saline and shaken vigorously in order to produce an even suspension. An approximately equal concentration of organisms was obtained by diluting the different cultures to an equal opacity. These suspensions were then diluted 10, 100, and 1000 times with normal saline. Two-tenths cc. of the original suspension and of each dilution were inoculated intramuscularly into 5 hamsters each weighing from 25 to 30 gm. Animals which did not succumb to the test culture within 9 days were considered survived. The highest dilution of a culture which killed the majority of animals was taken as a measure of virulence of the strain. Recovery of organisms from various internal organs and the thoracic cavity was attempted on those that died from 20 to 72 hours after inoculation of 0.2 cc. of 1-10 or 1-100 dilution. Cultures were made on blood-agar containing 0.02% of potassium tellurite.

Results. It was found that every culture of the 3 types tested was virulent for hamsters at the dilution of 1-100 but none in that of 1-10,000. The only difference in virulence was shown at the dilution of 1-1000 at which a few strains of each type were able to kill the experimental animals.

Of various internal organs and the thoracic cavities from which the recovery of organisms was attempted, it was found that among 72 hamsters the lungs yielded the highest percentage of positive cultures, *viz.*, 22%. Thoracic cavities gave 8%, adrenals 4%, kidneys and testes 3% each and spleen 1.5%. Liver and heart's blood

⁴ Gundel, M., and Ezrin, N., *Zentralb. f. Bakt.*, 1936, **136**, 24.

⁵ Parish, H. J., Whatley, E. E., and O'Brien, R. A., *Brit. Med. J.*, 1932, **12**, 915.

⁶ Lu, K. J., and Zia, S. H., *Proc. Soc. Exp. Biol. and Med.*, 1935, **33**, 334.

TABLE I.
Virulence of different types of *C. diphtheriae* in hamsters.

Dilutions	Gravis			Intermediate			Mitis		
	Leeds Cork 16* 9*	5173 5337 4435 6441 6580 6695	Lon- don* 121* 8	Hull Park 5016 5456	Hull Leeds 136* 29*	6079 5275 8232 9317 3510 6520			
10-1	5†	5	5	5	5	5	5	5	5
10-2	3	5	5	5	5	5	5	5	5
10-3	4	4	5	0	3	2	0	1	0
10-4	0	0	0	—	0	—	—	—	0

* = strains supplied by Professor McLeod.

†Number = animals died.

0 = no death.

— = not done.

were always sterile. It is interesting to point out that in this study no appreciable difference in invasiveness was noticed in any of the 3 types.

The result of the present study seems to indicate that in Chinese hamsters, at least, the organisms classified as the *gravis* type showed neither greater pathogenicity nor enhanced invasiveness. Whatever difference that was obtained must be attributed to the difference in the individual strain rather than to the difference in types.

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Specific Carbohydrate from *Asterococcus mycoides* for Serologic Tests of Bovine Pleuropneumonia.

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It is known that a substance active in the specific precipitative reaction is present in the serum of infected animals and can also be extracted from the affected lungs in cases of bovine pleuropneumonia. These observations suggest the presence of a haptenic carbohydrate. An attempt, therefore, was made in the present experiment to isolate a specific carbohydrate from pure cultures of *Asterococcus mycoides*, the causal agent of bovine pleuropneumonia, and to determine its serologic activity with immune rabbit's serum.

The strain of the organism used here was isolated from the pleural exudate of a fatal case of pleuropneumonia in a cow. Forty flasks, each containing 200 cc. of liver-digest broth enriched by addition of 5% unheated horse serum, were inoculated with the organism and incubated for 5 days at 37°C. The growth was centrifuged for 2 hours at 3,000 r.p.m. and the sediment was collected, washed in saline, centrifuged again and finally resuspended in distilled water. For the sediment obtained from one liter of culture 20 cc. of water was employed. This suspension was left in the icechest overnight. At the end of this period, 0.5% of potassium hydroxide was added following which the suspension became transparent and markedly slimy. This was centrifuged and to the clear fluid glacial acetic acid was added. A floccular precipitate, designated here as nucleoprotein, developed. The precipitate was collected, dried in the incubator and preserved for serologic tests. The remaining clear fluid