

mental animal. A second instillation of the same material was made 6 hours later. At first plain broth and heated filtrate controls were carried out, but were later discontinued as inadequate. A different type of control is planned at a period when human colds are not prevalent.

Of the first 7 animals inoculated intra-nasally according to the technic described above, 3 contracted upper respiratory infections similar in their manifestations to the spontaneous infections of the simian and to the "common cold" in human beings. In 2 experiments the symptomatology was ambiguous and in 2 no symptoms developed.

The experiment charted below is a typical example of the phenomena observed. The incubation period of the experimental infection was about 36 hours. The symptomatology and alteration of the bacteriological flora of the upper respiratory tract are shown in the chart. In the experiment charted, a healthy chimpanzee kept in close contact with the experimental animal, contracted, presumably by contact, a spontaneous cold on the fourth day of the experimental infection.

The nature of the active filterable agent is as yet unknown. In one positive experiment the filtrate contained herpes virus, and from the filtrate in all positive experiments a Gram negative anaerobic bacillus of the type described by Olitsky and Gates was cultivated.

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### Oral Immunization Experiments with Disrupted, Dissolved and Avirulent Pneumococci. Time of Appearance of Immunity.\*

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Earlier experiments<sup>1</sup> have demonstrated that when dead pneumococci are fed to white rats, the animals become immune to an injection of virulent germs of the same type. The present report deals with the observations made when mechanically disrupted, bile dissolved and avirulent organisms are fed. It also includes a report of experiments in which rats were fed the pneumococcus grown in

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<sup>1</sup> Ross, Victor, *J. Lab. and Clin. Med.*, 1927, xii, 566.

whole milk.† The mechanically opened cells and the bile-dissolved ones were fed in order to learn whether the intact cell is necessary in this mode of conferring immunity, or whether the contents alone will serve as well. In one of the latter experiments some of the animals were examined a few days following the first feeding, instead of much later as was the custom in the earlier part of this work. The purpose was to observe how quickly the protection is created. Feeding with avirulent pneumococcus was carried out with a group of rats to determine whether virulence is an essential factor. Type I pneumococcus was used throughout. The resistance of the rats was tested by injecting the organism intraperitoneally in a volume of 0.20 cc.

*Effect of Feeding Mechanically Disrupted Germs.* The organisms were grown in glucose meat extract broth, centrifuged and desiccated in partial vacuum. The scaly material was then pulverized in a pebble mill for about 18 hours. Microscopic examination failed to detect any intact pneumococci. Each rat received the equivalent of 50 cc. of culture per day for 16 days. The powdered material was mixed with milk and placed in the cage. The animals were well protected by this method, surviving 1000 to 10,000 fatal doses. Doubtless, some of the germs escaped disruption in the mill, but it is believed that these were too few in number to have been responsible for the pronounced effect observed.

*Effect of Feeding Organisms Grown in Milk.* Sterile milk was inoculated with virulent broth cultures of pneumococcus and incubated for 18 hours. Several methods for killing the germs were tried. Heating at 60° C. for one hour was used. This causes separation and curdling. After shaking thoroughly, the milk was placed in the cage. The average amount consumed by each rat was 3 cc. per day. Feeding took place from 11 to 17 days. The rats were protected against 100 fatal doses, in one instance against 1000 fatal doses. The experiment was repeated using a group of rats which were older. The younger rats appear to give better results, an observation made also when bile dissolved cells were employed. This difference was not observed when the HCl killed whole cell was used.<sup>1</sup> Although it requires confirmation, it seems that better immunity was obtained when 3 cc. pneumococcus milk culture is fed than when the organisms from the same quantity of

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† It has previously been shown that pneumococci grown in broth, centrifuged and suspended in milk give excellent results. I am indebted to Dr. Krumwiede for the suggestion to grow the organisms directly in milk, thus avoiding the need for centrifuging.

broth culture are used. Heating at 60° C. for one hour does not apparently destroy the antigen. It has previously been shown that heating at 80° C. for 2 hours is destructive.<sup>2</sup>

*Effect of Feeding Avirulent Pneumococcus.* The organism was obtained by treating a glucose meat extract culture with HCl acid, and subculturing before growth was killed. The organism which was quite virulent before, became avirulent for mice, failing to kill when injected intraperitoneally in 1 cc. quantities. It was bile insoluble and showed slight and equal agglutination in the 3 fixed types of antisera. No soluble specific substance was formed. The avirulent cultures were killed by 2 hours' contact with N/15 HCl acid and the centrifuged germs mixed with cracker meal. Nine daily feedings of the pneumococci from 50 cc. culture were given each rat. No immunity follows the administration of the avirulent germ.

*Feeding of Bile Dissolved Pneumococcus.* Two experiments were done in which the pneumococcus was grown in beef heart broth, and after being centrifuged, was dissolved in sodium glycocholate. About 100 mgm. of the bile salt were used for a litre of growth. After remaining at room temperature for about 2 hours, microscopic examination generally showed the presence of an occasional intact cell. This material which was prepared fresh daily, was mixed with cracker meal and fed to the rats on 21 days in quantities equivalent to 50 cc. culture per rat per day in the first experiment, and the equivalent of 25 cc. culture per rat per day for 6 days in the second. The latter gave a better immunity, perhaps because the animals used were younger. Some of the rats were resistant to 10,000 fatal doses. When the Berkefeld (N) filtrate of sodium glycocholate dissolved pneumococci was administered to rats by mouth in quantities equivalent to 30 cc. culture per rat per day for 3 days protection was definitely present 3 days after the first feeding. Two experiments were done to determine whether the immunity appears sooner. It was found that 48 hours after a single feeding of Berkefeld filtrate of sodium glycocholate dissolved pneumococci the animals survived an injection of at least 1000 fatal doses. The experiments in which mechanically opened, bile dissolved and milk grown pneumococci were used, were also carried out on mice. As was the case following the use of HCl acid killed germs, the results obtained with these forms were comparatively poor. An effort is being made to learn why there is this difference between rats and mice.

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<sup>2</sup> Ross, Victor, *J. Immunol.*, 1926, **xii**, 237.