THE EPIDEMIOLOGY OF A RABBIT RESPIRATORY INFECTION.

V. EXPERIMENTAL SNUFFLES.

BY LESLIE T. WEBSTER, M. D.

(From the Laboratories of The Rockefeller Institute for Medical Research.)

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A number of facts have been assembled to indicate that \textit{Bacterium lepisepticum} is the incitant of snuffles as it occurs at The Rockefeller Institute.\textsuperscript{1-3} We wish now to report in detail\textsuperscript{4} the experimental induction of snuffles by the intranasal injection of this organism.

The experiments may be divided into two series; first, those in which regular stock rabbits were employed, which led to confusing results, and second, those in which carefully bred rabbits were used, which led to definite results.

For the first series of experiments, the rabbits were obtained from outside dealers. They were then placed in intimate contact with advanced cases of snuffles, or were inoculated intranasally, by various methods, with different strains of \textit{Bacterium lepisepticum}, or were subjected to extremes of temperature or to operation prior to exposure to snuffles or inoculation with the organism just mentioned. As a whole, the results were difficult to interpret because the incidence of fresh infection among the experimental animals was small, while among the control uninoculated animals, spontaneous snuffles\textsuperscript{3} was relatively frequent.

For example, four rabbits with clinical snuffles of 9 months duration were caged each one with a normal rabbit observed during the same period of time. Six similar normal rabbits were grouped two per cage as controls. To lower their

\textsuperscript{1} Webster, L. T., \textit{J. Exp. Med.}, 1924, xxxix, 843.

\textsuperscript{2} Webster, L. T., \textit{J. Exp. Med.}, 1924, xxxix, 857.

\textsuperscript{3} Webster, L. T., \textit{J. Exp. Med.}, 1924, xl, 109.

resistance, some were then subjected to a temperature of 98.2°F. and some to 43.0°F. for 2 weeks. In the ensuing 3 months period of observation, two of the four normal rabbits in contact with the infected animals developed snuffles, but since four of the six controls came down with spontaneous snuffles, the significance of the experimental result remained in doubt.

In another similar experiment, the result was more favorable. Of four young rabbits, each placed in contact with an old snuffles rabbit and later subjected to bleeding or environmental change, three came down with snuffles while the controls remained normal throughout the 3 months period of observation.

Another experiment had to do with a group of eight rabbits in which an attempt was made to induce snuffles by introducing a culture of *Bacterium lepisepticum* into the nares on a cotton plug. In each case, removal of the plug after 24 hours was followed by a nasal discharge. In the controls it was serous in character and of short duration, while in the animals which had received the plugs containing *Bacterium lepisepticum*, a mucopurulent discharge developed which persisted from 4 days to 3 weeks.

Experiments such as these were of value only as they indicated the possibility that snuffles might be communicable, that environmental changes might influence the result, and that the appearance of snuffles is associated with the presence of *Bacterium lepisepticum* in the nasal passages. They indicated further that variation in the susceptibility of the stock rabbits might be responsible for the confused results.

It was thought that part of this confusion might be avoided if the susceptibility-resistance factor was analyzed and controlled. Consequently it was decided to eliminate any specific acquired immunity of the rabbits to snuffles by establishing and maintaining a breeding room free of infection and free of *Bacterium lepisepticum*, the suspected etiological agent. This was done by choosing for breeding purposes rabbits which were clinically normal and with nasal passages free from the bacterium on at least three examinations. These animals received careful attention in a large, airy room, the ample cages being cleaned every day. At short intervals, the nasal passages of each animal were cultured and any carriers of *Bacterium lepisepticum* were removed. In this way we have maintained for 9 months a rabbit stock free from snuffles.

Further evidence of a similar nature is reported in the preceding paper.

It may be of interest to note that a few of these rabbits carry *B. bronchisepticus* in their nasal passages.
Rabbits from this breeding room react in a characteristic and predictable manner to an intranasal inoculation of *Bacterium lepisepicum*. The technique of some experiments and the results follow.

*Experiment 1.*—A culture of *Bacterium lepisepicum* of low virulence was obtained from a rabbit showing snuffles and grown for 17 hours in broth, pH 7.4, containing \( \frac{1}{2} \) cc. of defibrinated rabbit blood. Five 800 gm. rabbits from the special breeding room received \( \frac{1}{4} \) cc. of this culture into each nasal cavity by means of a blunt 2 cc. Luer syringe used as a medicine dropper. They were then placed in individual cages and observed for 3 months. Five uninoculated rabbits from the breeding room were kept under similar conditions as controls. Autopsies and bacteriological studies were employed to check the clinical findings.

Table I shows that three of the rabbits remained well but carried the bacilli in their nasal passages for more than 1 month and that two developed snuffles on the 1st and 2nd days after inoculation, the disease lasting 5 weeks and 3 months respectively and terminating in a fatal pleuropneumonia. The five uninoculated controls remained well and free of *Bacterium lepisepicum* throughout the period of observation.

*Experiment 2.*—A virulent culture of *Bacterium lepisepicum*, obtained from a rabbit by Dr. T. M. Rivers and grown for 18 hours in rabbit blood broth, was inoculated, as in Experiment 1, into the nares of six specially bred 800 gm. rabbits. These animals, together with five similar uninoculated rabbits, were then placed in separate cages and observed for 4 months. Autopsies and bacteriological studies were performed.

Table II shows that of the inoculated rabbits one remained well and carried the bacilli less than 1 month, another on the 1st day after inoculation developed snuffles which lasted less than 1 month, a third came down on the 1st day after inoculation with snuffles which lasted 6 weeks, while three developed pneumonia and died 3 to 4 days after inoculation.

*Experiment 3.*—Twelve 800 gm. rabbits from The Rockefeller Institute farm in New Jersey, free from snuffles and *Bacterium lepisepicum* as proven by four examinations covering a period of 2 weeks, received intranasally cultures of the same virulent Rivers strain as had been employed in the previous experiment. Each rabbit received \( \frac{1}{4} \) cc. into each naris by means of a blunt 2 cc. Luer syringe. They were then caged separately and observed for 5 months. At autopsy the gross findings and bacteriology confirmed the clinical observations.
### TABLE I.

**Intranasal Inoculation of Bacterium leptosicmicum (R 82) into Specially Bred Rabbits (Experiment 1).**

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<tr>
<td></td>
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<td>Immediate result</td>
<td>Onset</td>
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<tr>
<td>101 107</td>
<td><em>Bacterium leptosicmicum</em> intranasally</td>
<td>Well.</td>
<td>—</td>
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<tr>
<td></td>
<td></td>
<td>Snuffles.</td>
<td>1st day</td>
</tr>
<tr>
<td>110 113</td>
<td></td>
<td>Well.</td>
<td>—</td>
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<tr>
<td>1, 2, 3, 4, 5</td>
<td></td>
<td>Well.</td>
<td>—</td>
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<tr>
<td>Rabbit No.</td>
<td>Experimental procedure</td>
<td>Clinical course</td>
<td>Persistence of Bacterium lepisepticum in nasal passages</td>
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<tr>
<td>100</td>
<td>Bacterium lepisepticum intranasally</td>
<td>Septicemia meningitis. 3rd day. 1 day. Death 4th day. Septicemia. Meningitis. Nares infection.</td>
<td>4 days.</td>
</tr>
<tr>
<td>103</td>
<td>“</td>
<td>Snuffles. 1st “ 1 wk. Well.</td>
<td>2 wks.</td>
</tr>
<tr>
<td>106</td>
<td>“</td>
<td>Well. 2nd day. 1 day. Death 3rd day. Pneumonia. Nares infection.</td>
<td>1 wk.</td>
</tr>
<tr>
<td>112</td>
<td>“</td>
<td>“ 2nd “ 1 “ “</td>
<td>3 “</td>
</tr>
<tr>
<td>115</td>
<td>“</td>
<td>“ 1st “ 6 wks. Well.</td>
<td>7 wks.</td>
</tr>
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<td>Controls</td>
<td>“</td>
<td>Well. “ “</td>
<td>“</td>
</tr>
<tr>
<td>1, 2, 3, 4, 5</td>
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TABLE III.

Intranasal Inoculation of Bacterium leptisepticum (Rivers Strain) into Specially Bred Rabbits (Experiment 3).

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<tbody>
<tr>
<td>206</td>
<td>Bacterium leptisepticum intranasally.</td>
<td>Snuffles.</td>
<td>1st day.</td>
<td>5 mos. +</td>
<td>Snuffles.</td>
</tr>
<tr>
<td>208</td>
<td>&quot; &quot;</td>
<td>Well.</td>
<td>6 days.</td>
<td></td>
<td>Well.</td>
</tr>
<tr>
<td>212</td>
<td>&quot; &quot;</td>
<td>Snuffles.</td>
<td></td>
<td></td>
<td>Death. Pneumonia. Nares infection.</td>
</tr>
<tr>
<td>213</td>
<td>&quot; &quot;</td>
<td>1st &quot;</td>
<td>1 day.</td>
<td></td>
<td>Well.</td>
</tr>
<tr>
<td>214</td>
<td>&quot; &quot;</td>
<td>4th &quot;</td>
<td>5 mos. +</td>
<td></td>
<td>Snuffles.</td>
</tr>
<tr>
<td>215</td>
<td>&quot; &quot;</td>
<td>1st &quot;</td>
<td>1 mo.</td>
<td></td>
<td>Well.</td>
</tr>
<tr>
<td>216</td>
<td>&quot; &quot;</td>
<td>1st &quot;</td>
<td>4 days.</td>
<td></td>
<td>Recurrence lasting 4 mos. +.</td>
</tr>
<tr>
<td>217</td>
<td>&quot; &quot;</td>
<td>1st &quot;</td>
<td>4 &quot;</td>
<td></td>
<td>Death. Pneumonia. Nares infection.</td>
</tr>
<tr>
<td>219</td>
<td>&quot; &quot;</td>
<td>3rd &quot;</td>
<td>5 mos. +</td>
<td></td>
<td>Snuffles.</td>
</tr>
<tr>
<td>220</td>
<td>&quot; &quot;</td>
<td>1st &quot;</td>
<td>5 &quot; +</td>
<td></td>
<td>&quot;</td>
</tr>
<tr>
<td>221</td>
<td>&quot; &quot;</td>
<td>1st &quot;</td>
<td>5 &quot; +</td>
<td></td>
<td>&quot;</td>
</tr>
<tr>
<td>222</td>
<td>&quot; &quot;</td>
<td>1st &quot;</td>
<td>5 days.</td>
<td></td>
<td>Death. Pneumonia. Nares infection.</td>
</tr>
</tbody>
</table>
Table III shows that one rabbit remained well but carried the bacilli for over 5 months, that one showed 24 hours after inoculation a nasal discharge and matted paws lasting 1 day, seven developed snuffles which persisted 1 to 5 months, and three developed a nasal discharge and died with pneumonia 4 to 6 days after inoculation.

**Experiment 4.**—Seven 800 gm. rabbits from The Rockefeller Institute farm in New Jersey and twelve 700 gm. rabbits specially bred at the Institute in New York were used for this experiment. The New Jersey farm rabbits were free from snuffles and had shown no *Bacterium lepisepticum* in their nasal passages on three observations covering a period of 2 weeks. The specially bred rabbits had been examined several times while in the breeding room and were regularly free from snuffles and the bacterium.

Each rabbit received in each naris $\frac{1}{4}$ cc. of an 18 hour blood broth culture of *Bacterium lepisepticum* (Rivers strain) and was then placed in a separate cage for observation. Gross pathology and bacteriological studies at autopsy confirmed the clinical observations.

Of the specially bred rabbits, two remained well and carried the bacilli for less than 1 month, two developed snuffles on the 1st or 2nd day and recovered in less than 1 month, while eight rabbits showed a seropurulent nasal discharge and died of pneumonia 2 to 12 days after inoculation.

Of the New Jersey farm rabbits, one remained well and carried the bacilli less than 1 month, two developed snuffles on the 1st day after inoculation and recovered within 2 weeks, one on the 1st day developed snuffles which persisted more than 1 month, while three rabbits showed a slight nasal discharge, but developed pneumonia, and died 2 to 3 days after inoculation.

**Experiment 5.**—Seven 800 gm. rabbits from The Rockefeller Institute breeding room, with no history of snuffles and regularly free from *Bacterium lepisepticum* in the nasal passages, were inoculated into each naris with $\frac{1}{4}$ cc. of a 17 hour blood broth culture of the original Rivers strain and were placed in separate cages for observation.

One rabbit remained well and carried *Bacterium lepisepticum* less than 1 month, four remained well and carried the bacilli more than 1 month, one rabbit developed snuffles on the day following inoculation, which persisted more than 1 month, and one rabbit showed a slight nasal discharge and died of pneumonia 12 days after inoculation.
GENERAL RESULTS.

When the results of these five experiments are considered together, a number of interesting facts appear (Text-fig. 1). Thus, it is apparent from each experiment that normal rabbits react in different ways to an intranasal injection of *Bacterium lepisepticum* and that this reaction indicates varying degrees of susceptibility. Therefore we have divided the rabbits into six categories according to their response, progressing from the most resistant to the most susceptible. (1) In the most resistant animals, the bacterium remains in the nasal passages for a few days without producing any noticeable disturbance; (2) in a more susceptible group, the bacterium remains in...
the nasal passages for a period of months, in their case also without producing clinical symptoms; (3) in still more susceptible animals, clinical snuffles results, followed by recovery in a few days; (4) in yet others, chronic snuffles results and persists over a period of months; while the highly susceptible rabbits either (5) die of pneumonia and septicemia after a period of weeks or (6) die of pneumonia within a few days of the inoculation.

It is furthermore apparent that different strains of *Bacterium lepisepticum* and, indeed, different cultures of a given strain, vary in pathogenicity or virulence. Inoculations with Strain R 82 (Experiment 1) and the unpassed Rivers strain (Experiment 5) yielded a relatively great number of healthy carriers and snuffles cases with few pneumonia fatalities, while the virulent Rivers strain had resulted in few carriers and cases of snuffles, but a large number of fatalities from pneumonia.

**DISCUSSION.**

In the preceding pages it has been shown that the mere instillation of a virulent culture of *Bacterium lepisepticum* into the external nares of rabbits bred under circumstances of protection from infection with snuffles usually suffices, first, to set up an endemic carrier state, and second, to incite in certain animals a clinical condition identical with snuffles. It appears, therefore, that in the rabbit an active culture of *Bacterium lepisepticum* is not eliminated by the normal upper respiratory mucous membrane in the manner described by Bloomfield for certain pathogenic and non-pathogenic species in man. 7

There is still confusion and difference of opinion in regard to the identity of the inciting microorganism of snuffles. 8 Recently Ferry and Hoskins 9 have concluded that the chronic form of the disease is induced by *Bacillus bronchisepticus* and the acute form by *Bacterium lepisepticum*. McCartney and Olitsky 10, 11 who studied the

11 Strain R 63 used by McCartney and Olitsky in their experiments had received eleven direct intrapleural passages but had not increased in virulence.
disease in stock rabbits state that *Bacterium lepisepticum* cannot be regarded as the sole inciting organism.

It is now obvious, we believe, that there are two sources for the lack of harmony in bacteriological experiments and findings: first, preexisting disease in the rabbits employed for testing, and second, the grade of virulence of the culture of *Bacterium lepisepticum* used for purposes of inoculation. Our experiments, carried out with rabbits bred with a view to protection from exposure to snuffles in any of its stages, and with strains of *Bacterium lepisepticum* of various degrees of virulence have led us to the conclusion that several degrees of natural resistance may be detected among groups of rabbits. According to these degrees of resistance a series of clinical and pathological effects is produced, either in course of ordinary exposure or experimentally, which may be classified as follows: (1) The carriers of *Bacterium lepisepticum* are rabbits of relatively high resistance which have been exposed to active cultures. (2) Moderately resistant rabbits tend on infection to come down with the local, nasal infection, snuffles. (3) Rabbits of maximum susceptibility on infection with *Bacterium lepisepticum* develop pneumonia associated with or without other manifestations of local or general infection.

In any given large group or community of rabbits, the quantitative ratio of the incidence of these several types of infection forms an index, if perhaps only a rough one, to the resistance of the rabbits and the virulence of the microbe.

**CONCLUSIONS.**

Rabbits were bred at The Rockefeller Institute with special relation to protection from exposure to *Bacterium lepisepticum*. These healthy rabbits were then submitted to intranasal instillation of a given dose of cultures of *Bacterium lepisepticum*. The results of the inoculation were various according to the natural degree of resistance of the individual animals.

The following graded effects could be distinguished: Some of the animals became (1) short time carriers of *Bacterium lepisepticum*; others (2) long time carriers of the same organism; some developed (3) local nasal infection (snuffles) of short duration; others (4) local
nasal infection (snuffles) of long duration; while yet others had (5) snuffles, followed by pneumonia and signs of general infection after a few weeks; and finally there were instances of (6) local nasal discharge attended by general infection and pneumonia after a few days.

The effects produced in any given healthy stock depend also upon the degree of virulence of *Bacterium lepisepticum* of which great differences exist among the same and different strains.