

EPIDEMIOLOGICAL STUDIES ON RESPIRATORY INFECTIONS OF THE RABBIT.

VI. ETIOLOGY OF OTITIS MEDIA.

By DAVID T. SMITH, M.D., AND LESLIE T. WEBSTER, M.D.

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

(Received for publication, November 10, 1924.)

During the course of a number of autopsies on stock and experimental animals at The Rockefeller Institute, it was frequently noted that the middle ears contained pus. These observations led us to investigate otitis media as it occurs in rabbits with the purpose of determining its etiology and relation to other native infections and of gaining information which might possibly be useful in explaining similar conditions of the human ear.¹

Occurrence.—In a series of consecutive autopsies on 68 experimental and 34 stock animals, 33 (32 per cent) were found to have middle ear infection. Of these, 31 had been used for experimental purposes and 2 came from supposedly normal stock (Table I).

Symptoms.—There are no outstanding characteristic symptoms of otitis media in the rabbit. Except for the relatively few instances in which pus is found in the external canal or the occurrence of torticollis and rotating movements, indicating an involvement of the vestibular apparatus, the diagnosis cannot be made before autopsy. Very frequently, however, animals with otitis have snuffles in either the acute or the chronic state and may lose weight and strength and finally die.

Gross Pathology.—Autopsies at various stages have shown that the progress of the infection is much like that exhibited by the human ear disease. In the first stage the tympanic membrane and the lining of the tympanic cavity are red. The cavity contains a sero-sanguineous fluid. Soon it becomes filled with thick, white, creamy

¹ Smith, D. T., *Am. J. Dis. Child.*, 1924, xxviii, 1.

pus. This may remain for weeks or months until it is gradually absorbed or until the animal is killed or dies of some intercurrent infection. At times, but less frequently than in the case of human otitis, the ear drum ruptures and pus is discharged into the external canal. This is generally followed by absorption of the remaining material and a healing of the ear drum with scar formation. When absorption has taken place one finds the membrane lining the cavity somewhat thickened and of a dirty grayish color.

Bacteriology.—Material from the middle ear, free from contamination, was obtained by the following technique.

The brain was first carefully removed with sterile instruments. Then, the inside of the skull was seared with a hot soldering iron and the middle ear opened

TABLE I.

	No. of rabbits.	Sinusitis.	Per cent.	Otitis.	Per cent.	
Animals previously used for the study of	experimental syphilis.....	27	16	59	15	55
	“ meningitis.....	16	7	44	7	44
	“ tumor.....	14	3	21	5	35
	“ thyroidectomy....	10	3	30	3	30
	“ dysentery.....	1	0	0	1	0
Total.....	68	29	42.6	31	45.5	
Normal.....	34	3	8.4	2	5.0	
Grand total.....	102	32	31.0	33	32.0	

with a pair of sterile bone forceps. The tympanic cavity of the rabbit is quite large and it was relatively easy to get a loopful of pus from the center of the cavity without touching the walls or the broken surface of the petrous bone.

Smears and cultures were made from the pus found in the ears of twenty-five rabbits. The films were stained by Gram's method and generally showed large masses of pus cells, mainly of the mononuclear type, in various stages of degeneration. Bacteria were relatively few in number and were both extra- and intracellular.

Cultures were made by streaking a loopful of pus over the surface of a freshly prepared rabbit blood agar plate. When both ears were involved, each was cultured separately. Table II shows that *Bacterium leprosepticum* was isolated from twenty of the twenty-five animals. It was found in pure culture in thirteen and mixed with a diphtheroid organism or *Staphylococcus albus* in seven other cases.

Relation of Otitis Media to Infection of the Nasal Passages.—It was noted early that rabbits with snuffles very frequently showed otitis media also. Among the thirty-two animals with nasal infection, mentioned in Table I, twenty-five (78 per cent) of them showed evidence of middle ear involvement, and although an occasional rabbit with otitis showed no macroscopic lesion in the nasal passages, when a considerable number were examined the percentage incidence of both diseases was found to be practically the same.

TABLE II.
Bacteriology of the Otitis Media.

<i>Bacterium lepi-septicum</i>	13
“ “ and <i>Staphylococcus albus</i>	4
“ “ “ diphtheroid.....	3
Diphtheroid.....	2
“ and colon-like organism.....	1
Gram-negative mucoid organism.....	2
Total.....	25

TABLE III.
Results of Cultures on the Nares and Middle Ear Cavities.

No. of rabbits.	<i>Bacterium lepi-septicum</i> cultured.	Miscellaneous bacteria cultured.
9	Nares and ears.	0
3	Ears.	0
3	Nares and ears.	Diphtheroids from ears.
2	0	“ “ “
4	Nares and ears.	Staphylococcus “ “
1	Ears.	<i>B. bronchisepticus</i> “ “

These findings suggested the advisability of determining the bacteriological relationship between the infections. This was accomplished by culturing separately the nares and middle ear cavities of twenty-two rabbits which had both otitis and nasal passage involvement. Table III shows the results of these cultures. *Bacterium lepi-septicum* was recovered from the nares and ears of fourteen animals, in pure culture from Nos. 1 to 9 inclusive, and in combination with diphtheroids or staphylococci from Nos. 13, 15, 18, and 19.

In seven cases (Nos. 10, 11, 12, 14, 20, 21, and 22) it was obtained from the ears but not from the nasal passages.² Two rabbits (Nos. 16 and 17) showed no *Bacterium lepi-septicum* in either nares or ears.

Experimental Otitis Media with Cultures of Bacterium lepi-septicum.—

It was evident from the studies just described that *Bacterium lepi-septicum* is the organism most frequently found in both otitis and nasal infections. Consequently, if an etiological relationship exists between these two conditions, cultures of this organism when introduced into the nasal passages of normal non-immune rabbits might be expected to induce both nasal and middle ear infections. An experiment was devised to test the supposition.

A group of young rabbits was available which had been reared under special conditions that precluded contact with *Bacterium lepi-septicum* at any time previously. Into the nasal passages of eleven of these animals 1/4 cc. of a 16 hour culture of the organism was introduced. Clinical observations and subsequent autopsies and bacteriological cultures were made. Table IV summarizes the results. Three of the animals died of pneumonia; of the eight survivors, six developed snuffles and otitis and became carriers of the organism and one developed otitis media alone. Only one animal escaped infection.

Another group of nine animals of the same age and from the same source was treated in a similar way except that a preliminary attempt had been made to vaccinate them with killed and living cultures. The results are shown in Table V. Five came down with snuffles, these and two others became carriers, and all seven developed otitis media. From one case of otitis *Staphylococcus albus* alone was recovered, from the other six cases *Bacterium lepi-septicum*. Two of the nine escaped infection with the organism used in the experiments. Although none of this series died of pneumonia, yet the percentage of middle ear infection was about as great as among the rabbits which had not been vaccinated.

Intranasal Inoculations with Bacillus bronchisepticus.—Because *Bacillus bronchisepticus* is found frequently in the nasal passages of stock rabbits and because it has been regarded by some as the cause

² This discrepancy may be due to an error in technique. Webster, L. T., *J. Exp. Med.*, 1924, xxxix, 837, 843.

TABLE IV.
*Outcome of Experimental Inoculation with Bacterium lepi-
 septicum.*
First Experiment.

Rabbit No.	Treatment.	Snuffles.	<i>Bacterium lepi- septicum</i> carrier.	Pus in ears.		Culture of ears.
				Left.	Right.	
23	1/4 cc. <i>Bacterium lepi- septicum</i> intranasally.	+	+	+	+	<i>Bacterium lepi- septicum</i> both ears.
24	"	+	+	+	0	" left ear.
25	"	+	+	+	+	" both ears.
26	"	+	+	0	+	" right ear.
27	"	+	+	+	+	" both ears.
28	"	+	+	+	+	" "
29	"	0	0	+	+	" "
30	"	0	0	+	0	Ears sterile.
31	"	Died of pneumonia.				
32	"	"	"			
33	"	"	"			
Total.....	11	6	6	7	7	

TABLE V.
Outcome of Experimental Inoculation with Bacterium lepirosepticum.
Second Experiment.

Rabbit No.	Treatment.	Snuffles.	<i>Bacterium lepirosepticum</i> carrier.	Pus in ears.		Culture of ears.
				Left.	Right.	
34	1/4 cc. <i>Bacterium lepirosepticum</i> intranasally.	+	+	+	+	<i>Bacterium lepirosepticum</i> both ears.
35	"	+	+	0	0	" " left ear.
36	"	+	+	+	+	" " both ears.
37	"	+	+	+	0	" " left ear.
38	"	+	+	0	0	Ears sterile.
39	"	0	+	+	+	<i>Bacterium lepirosepticum</i> both ears.
40	"	0	+	+	+	" " " "
41	"	0	0	0	Serum.	<i>Staphylococcus albus</i> both ears.
42	"	0	0	0	0	Ears sterile.
Total.....	9	5	7	7	7	<i>Bacterium lepirosepticum</i> 6. <i>Staphylococcus albus</i> 1. Sterile 2.

TABLE VI.
Outcome of Experimental Infection with *B. bronchisepticus*.

Rabbit No.	Treatment.	Snuffles.	<i>B. bronchisepticus</i> carrier.	Pus in ears.	Cultures of ears.
43	1 cc. <i>B. bronchisepticus</i> intranasally.	0	+	0	Ears sterile.
44	"	0	+	0	"
45	"	0	+	0	"
46	"	0	+	0	"
47	"	0	+	0	"
48	"	0	+	0	"
49	"	0	+	0	"
50	"	0	+	0	"
51	"	0	+	0	<i>B. bronchisepticus</i> right ear.
52	"	0	+	Serum.	<i>Staphylococcus albus</i> both ears.
53	"	0	+	Red drum.	" " "
54	"	0	+	Serum.	<i>B. bronchisepticus</i> right ear.
					" and <i>Staphylococcus albus</i> right ear.
55	"	0	+	Viscous pus.	<i>B. bronchisepticus</i> both ears.
56	"				

Died of pneumonia.

of rabbit snuffles, it was thought advisable to determine whether intranasal instillation of this organism was followed by otitis media.

A group of fourteen rabbits from the same source as those used in the previous experiments was selected. Into the nasal passages of the animals 1 cc. of a 16 hour culture of four strains of *Bacillus bronchisepticus* was introduced. Table VI summarizes the results. One animal died of a localized abscess-like pneumonia. All of the survivors became carriers of the organism, although no symptoms of snuffles developed. At autopsy the nasal passages of all were clean and the middle ears appeared normal in all except four. In one of these the ear drum and the lining of the tympanic cavity appeared bright red, while the cavity itself contained a slight amount

TABLE VII.

Summary of Results of Experimental Otitis Media.

Experiment.	No. of rabbits.	Snuffles.		Per cent.	Carriers.	Per cent.	Otitis.	Per cent.	Cultures.
		Died.							
1/4 cc. <i>Bacterium lepi-</i> <i>pticum</i> intranasally.....	20	3	11	65	13	76	14	82	13 <i>Bacterium lepi-</i> <i>septicum</i> .
1 cc. <i>B. bronchisepticus</i> intrasally.....	16	3	0	0	13	100	4	30	4 <i>B. bronchisep-</i> <i>ticus</i> .
Controls.....	11	0	0	0	0	0	0	0	1 diphtheroid.

of serous fluid. In two other cases a small amount of serum was noted. Only one showed a thick viscous material, mucoid in nature, not like the pus encountered in the above experiments. *Bacillus bronchisepticus* was isolated from the nasal passages of all the rabbits and from the ears of the four mentioned above. It was obtained in pure culture from three of these cases, but in the fourth was associated with *Staphylococcus albus*.

Controls.—This entire series of experiments was controlled by selecting a group of eleven rabbits from the same stock and keeping them under the same living conditions as the experimental animals. At the end of the period of observation they were killed and autopsied. None of them had macroscopic or microscopic evidence of nasal

infection, and none showed signs of otitis media. Cultures from the nares showed no *Bacterium lepi-septicum*. Those from the middle ears were found to be sterile save in the case of two animals. From these, a few diphtheroid organisms were recovered.

This experiment shows, therefore, that rabbits raised under conditions which preclude exposure to *Bacterium lepi-septicum*, as to certain other infective agents, are free of middle ear infection but that this organism dropped through the external nares into the upper nasal passages of such animals is capable of inducing a purulent otitis media similar in every respect to that which occurs spontaneously in stock and experimental animals.

SUMMARY.

A common form of otitis media occurring in rabbits is described. The condition in the great majority of instances is associated with the inflammation of the upper respiratory tract known as snuffles and with the presence of *Bacterium lepi-septicum*. This organism, when introduced into the external nares of rabbits hitherto protected from infection with it, is capable of inducing a purulent otitis media indistinguishable from the natural one.

In the rabbit the connection between the nasal passages and the middle ear is such that a pathogenic agent introduced into the nose may readily invade the entire group of cavities connected with the upper respiratory tract.