

THE PERMEABILITY OF THE LINING OF THE LOWER RESPIRATORY TRACT FOR ANTIBODIES.

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In a previous communication¹ it has been shown that the membranes of the lower respiratory tract permit the absorption of proteins to a limited degree. In the course of experiments to ascertain to what extent the mucous membrane of the trachea, air passages, and alveoli would permit the passage of serum, foreign sera rich in antibodies were introduced into the trachea. Since the laryngotracheal administration of antisera as a practical procedure has been suggested by Besredka,² it seemed desirable to record the results of some observations by the writer. Besredka found that rabbits and guinea pigs can be protected against tetanus and diphtheria toxin by first administering concentrated antitoxin intratracheally. Levaditi³ points out that an intratracheal injection of serum from a convalescent may stop short an attack of poliomyelitis.

Inasmuch as the writer's experience has been that foreign serum is absorbed slowly and in relatively small quantities, it seemed reasonable to infer that the antibodies contained in the serum would not penetrate the membranes to a greater degree. That this is the case is shown in the following series of experiments.

EXPERIMENTAL.

Inasmuch as the absorption of agglutinin is easily measured, in most of the experiments serum rich in agglutinin was introduced into the trachea by the method previously described.⁴ The control ani-

¹ Jones, F. S., *J. Exp. Med.*, 1924, xl, 63.

² Besredka, A., *Ann. Inst. Pasteur*, 1920, xxxiv, 51.

³ Levaditi, C., *Ectodermoses neurotropes*, Paris, 1922.

⁴ Jones, F. S., *J. Exp. Med.*, 1923, xxxvii, 789.

mals received a comparable amount of the same serum intraperitoneally. In the observations dealing with the passage of agglutinin, the animals were bled at regular intervals and the collected serum tested with the original antigen.

In the first series of experiments cow serum containing agglutinins for the non-hemolytic mastitis streptococcus was employed. It agglutinated suspensions of the streptococcus at a dilution of 1:10,000.

TABLE I.

The Absorption of Agglutinin in Cow Serum Administered into the Trachea and Peritoneal Cavity.

Time serum was obtained.	Rabbit 1. Weight 2,618 gm. 6 cc. of bovine streptococcus serum injected intratracheally.				Rabbit 2. Weight 1,960 gm. 5 cc. of bovine streptococcus serum injected intratracheally.				Rabbit 3. Weight 2,530 gm. 5.8 cc. of bovine streptococcus serum injected intraperitoneally.					
	Results of tests of rabbit sera for the presence of agglutinins specific for a bovine streptococcus.													
	Serum dilutions.				Serum dilutions.				Serum dilutions.					
	1:20	1:50	1:100	1:200	1:20	1:50	1:100	1:200	1:20	1:50	1:100	1:200	1:500	1:1,000
Before injection.	-	-	-	-	++	-	-	-	++	+	-	-	-	-
After injection.														
hrs.														
1	++	+	-	-	C.	C.	#	-	C.	++++	++++	+++	+	+
2	++++	#	-	-	++++	-	-	-	"	C.	++++	++++	+	-
3	C.	-	-	-	+	-	-	-	"	++++	++++	+++	+	+
4	++	-	-	-	++++	+	-	-	"	C.	++++	++++	+	+
5	+	-	-	-	++++	+	-	-	"	++++	++++	+++	+	+
6	+	-	-	-	++++	+	-	-	"	++++	++++	+++	+	+

The results following injections of this serum into the trachea and peritoneal cavity are given in Table I.

The results of the various tests indicate that the antibody injected into the trachea is absorbed into the circulation in relatively small amounts. Assuming that all the serum injected into the rabbit is absorbed during the course of the experiment, a strong agglutination should result when the serum of the animal is diluted 200 times. Such is not the case in the rabbits (Nos. 1 and 2) treated intratracheally.

The titer of the serum of the control rabbit (No. 3) injected intraperitoneally approaches the theoretical figure and indicates that the maximum absorption has taken place within 2 hours following the injection. In a similar experiment in which bovine serum rich in agglutinins for *Bacillus abortus* was injected intratracheally, the agglutinins for *Bacillus abortus* were not appreciably increased in the blood stream.

Confirmatory results were obtained when serum hemolytic for rabbit red cells was introduced into the trachea and peritoneal cavity. The hemolytic serum was obtained by injecting suspensions of washed red blood cells of the rabbit into the peritoneal cavities of guinea pigs. After sera sufficiently high in hemolysin had been obtained the guinea pigs were bled. The sera of all the guinea pigs were mixed and injected into the trachea or the peritoneal cavity of rabbits. The effect of the serum was measured by hemoglobin estimations and red cell counts at intervals following the injections. The details of the methods of administration and the effects upon the red cells *in vivo* are given in Table II.

The rabbits injected intratracheally with the hemolytic serum failed to react to a measurable degree, for one can scarcely admit as evidence the transient diminution in the number of red cells of Rabbit 5. Both rabbits survived and suffered no appreciable ill effects. In sharp contrast to these results are the marked effects following intraperitoneal injection. The red cells of Rabbit 4, which was injected intraperitoneally with 6.5 cc. of serum, began to decrease $3\frac{1}{2}$ hours after injection. The hemoglobin remained stationary during the first $6\frac{1}{2}$ hours. By the 23rd hour the anemia was severe. Rabbit 7, which received 3.5 cc. of serum intraperitoneally, ultimately reacted in a similar manner. It is of interest to note that in this instance there was an apparent increase in the red cells and hemoglobin during the first 6 hours following injection. At this time the capillaries of the ears were dilated and the blood was almost black in color.

It becomes evident that antibodies contained in a heterologous serum are not appreciably absorbed through the walls of the lower respiratory tract. The possibility suggested itself that antibodies contained in a homologous serum might be more readily absorbed.

TABLE II.

No. of rabbit.	Time blood was examined.	Hemoglobin.	Red cells.	Remarks.
4 (Weight 2,400 gm.)	Before injection.	90	6,000,000	6.5 cc. of hemolytic serum injected intraperitoneally. Died 56 hrs. after injection.
	After " "			
	<i>hrs.</i>			
	$\frac{1}{2}$	92		
	1	90		
	$1\frac{1}{2}$	89	6,048,000	
	$2\frac{1}{2}$	87		
	$3\frac{1}{2}$	90	5,240,000	
	$5\frac{1}{2}$		4,600,000	
	$6\frac{1}{2}$	90	4,528,000	
	23	35	1,304,000	
	31	25	1,248,000	
	48	25	752,000	
5 (Weight 2,400 gm.)	Before injection.	68	4,400,000	6.5 cc. of hemolytic serum injected intratracheally. Rabbit failed to show symptoms.
	After " "			
	<i>hrs.</i>			
	$\frac{1}{2}$	67		
	1	68		
	$1\frac{1}{2}$	68		
	$2\frac{1}{2}$	68		
	4		3,928,000	
	5	67	4,330,000	
	6	67	4,172,000	
	$23\frac{1}{2}$	68	4,172,000	
	31	68	3,976,000	
	48	68	4,000,000	
	72	67	4,200,000	
6 (Weight 2,195 gm.)	Before injection.	80	4,172,000	7.0 cc. of hemolytic serum injected intratracheally. Rabbit failed to show symptoms.
	After " "			
	<i>hrs.</i>			
	$1\frac{1}{2}$	80	4,528,000	
	3	90	5,132,000	
	$4\frac{1}{2}$	85	5,128,000	
	6	83	5,088,000	
	24	80	4,784,000	
	31	90	5,004,000	

TABLE II—*Concluded.*

No. of rabbit.	Time blood was examined.	Hemo-globin.	Red cells.	Remarks.
7 (Weight 2,210 gm.)	Before injection.	80	4,880,000	3.5 cc. of hemolytic serum injected intraperitoneally. Died 60 hrs. after injection.
	After " "			
	hrs.			
	1½	90	5,432,000	
	3	90	5,632,000	
	4½	107	6,304,000	
	6	108	6,208,000	
24	52	2,768,000		
31	46	2,008,000		

TABLE III.

The Absorption of Agglutinin Contained in Homologous Serum Administered into the Trachea and Peritoneal Cavity.

Time blood was obtained.	Rabbit 8. Weight 2,350 gm. 6 cc. of serum from a rabbit immunized with the hog-cholera bacillus injected intratracheally.						Rabbit 9. Weight 2,670 gm. 6 cc. of serum from a rabbit immunized with the hog-cholera bacillus injected intraperitoneally.					
	Results of tests of rabbit serum for agglutinins specific for the hog-cholera bacillus.											
	Serum dilutions.						Serum dilutions.					
	1:10	1:20	1:50	1:100	1:200	1:500	1:10	1:20	1:50	1:100	1:200	1:500
Before injection.	-	-	-	-	-	-	++	+	#	-	-	-
After " "												
hrs.												
½	-	-	-	-	-	-	C.	C.	++	+	#	-
1	-	-	-	-	-	-	"	"	++++	+	#	-
2	-	-	-	-	-	-	"	"	C.	++++	++	#
3	-	-	-	-	-	-	"	"	"	++++	++	+
4	+	-	-	-	-	-	"	"	"	++++	++	+
5	+	-	-	-	-	-	"	"	"	++++	++	+
6	+	#	-	-	-	-	"	"	"	++++	++	+
7	+	+	#	-	-	-	"	"	"	++++	++	+
24	++	+	+	-	-	-	"	"	"	++++	++	+

Little and Orcutt⁵ have shown that the new-born calf for a short period after birth readily absorbs agglutinins for *Bacillus abortus* from the colostrum of the mother through the walls of the gastrointestinal canal. Smith and Little⁶ fed young calves cow serum of high titer for *Bacillus abortus* and showed that the agglutinins were absorbed through the intestinal wall into the blood stream within a few hours. To test whether the epithelium of the alveoli and air tubes is more readily permeable to antibody in a homologous serum, the serum from a rabbit immunized with a strain of the hog-cholera bacillus was injected into the trachea of one rabbit and into the peritoneal cavity of another. This serum in a dilution of 1:10,000 would cause marked agglutination of the hog-cholera bacillus. As in the previous experiment, the rabbits were bled before and at regular intervals after the injection. The various samples of serum were tested for the presence of agglutinins with suspensions of the hog-cholera bacillus. The details are given in Table III.

The results of this experiment were similar to those obtained when foreign serum containing antibodies was injected into the trachea. The respiratory epithelium appears to be impermeable for antibodies contained in a homologous serum as it is for those contained in the serum of other species.

DISCUSSION AND SUMMARY.

The experimental results indicate that the walls of the lower respiratory tract are relatively as impermeable for antibodies as they are for serum proteins. The kind of serum employed does not influence this fact. Antibodies contained in homologous (rabbit) serum failed to enter the blood stream when injected into the trachea in greater concentration than those contained in heterologous (guinea pig and cow) sera.

That a little serum and its contained substances are absorbed after intratracheal injection has been shown both by the experiments dealing with sensitization and anaphylaxis previously reported and by the experiments recorded in this paper.

⁵ Little, R. B., and Orcutt, M. L., *J. Exp. Med.*, 1922, xxxv, 161.

⁶ Smith, T., and Little, R. B., *J. Exp. Med.*, 1923, xxxvii, 671.

Besredka's assertion that the laryngotracheal method is an efficient one for the administration of serum in the treatment of disease has not been borne out by the experimental evidence here presented. From the results one would conclude that it is one of the least efficient routes for administration.

In contrast to the relative impermeability for proteins of the mucous membranes of the lower respiratory tract is the relative permeability of the endothelium of the peritoneal cavity. Antibody is there absorbed readily, whether it be in heterologous or in homologous serum. The indications are that a considerable portion of the antibody injected into the peritoneal cavity reaches the blood stream in 2 or 3 hours.