THE PRODUCTION OF ANTIBODIES IN RABBITS BY A SIMPLIFIED INTRATRACHEAL METHOD.

By F. S. JONES, V.M.D.

(From the Department of Animal Pathology of The Rockefeller Institute for Medical Research, Princeton, N. J.)

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During the last few years interest has been revived in antibody production by means of injections into the trachea. Besredka[1] called attention to the laryngo-tracheal method of administering sera to guinea pigs. Among other experiments two guinea pigs were vaccinated with killed cultures of the diphtheria bacillus. One was treated intratracheally, the other inoculated into the peritoneal cavity. 11 days after the last injection each received an intratracheal injection of living culture. The animal that had been vaccinated by the intratracheal route survived; the other died. Both animals had developed agglutinins for the diphtheria organism.

Pfenninger[2] undertook a more detailed study of antibody production after the administration of antigens through the trachea. He incised the skin overlying the trachea and injected through a needle inserted between the cartilaginous rings. The injections were made 7 days apart. Comparisons were made of the intravenous, the intraperitoneal, and the intratracheal methods. Pfenninger pointed out that the intratracheal method for antibody production is a little better on the whole than the intravenous route. He considered both superior to the peritoneal route. Many of his rabbits died after several intravascular inoculations, but all those treated by the tracheal and peritoneal methods survived.

D'Aunoy[3] used much the same procedure. He concludes that the intratracheal injection is comparable with intravenous inoculation and superior to intraperitoneal injection for the production of agglutinins, bacteriolysins, bactericidins, and precipitins. Hemolysins were formed more slowly by intratracheal administration but ultimately the serum obtained was equal to that produced in the intravenous series. Attention is directed to the relative safety of the intratracheal method, since none of the rabbits died during the experiments. Several deaths occurred in the intravenous series.

A simple method for the administration of liquids into the trachea seemed desirable, preferably one which would produce the minimum amount of injury and

[1] Besredka, A., *Ann. Inst. Pasteur*, 1920, xxxiv, 361.

[2] Pfenninger, W., *Ann. Inst. Pasteur*, 1921, xxxv, 237.

[3] D'Aunoy, R., *J. Infect. Dis.*, 1922, xxx, 347.

enable the operator to inject animals rapidly. Factors other than the introduction of antigen into the respiratory tract have not been taken into account when injections through the walls of the trachea have been made. Winternitz, Smith, and Robinson[4] point out that injection of material through the walls of the trachea affords an opportunity for infection of the submucosa and the peritracheal tissue. They observed histological evidences of infection and were able to trace the injected organisms through the submucosa of the trachea and larger bronchi to the hilus of the lung by way of the peribronchial and perivascular structures.

Snel[5] had previously shown by experiments with guinea pigs that the anthrax bacillus failed to attack the intact lung. When the vegetative forms or spores were introduced by means of a catheter passed through the larynx, infection failed to take place. In guinea pigs injected by means of a needle inserted through the walls of the trachea a locus of infection was produced and the animals developed a septicemia.

Kitt[6] also cites similar observations by Arloing, Cornevin, and Thomas in immunization against blackleg. They showed that following the introduction of virulent material into the veins or the tracheæ, calves resisted subsequent subcutaneous or intramuscular injections. Great care was employed to avoid leakage into the perivascular and peritracheal tissues since the virus would multiply in these structures with fatal results.

An apparatus by which one could introduce fluids into the lower respiratory tract without appreciable injury to the mucosa and underlying structures would imitate more nearly the natural condition. In addition, a method which would enable one to space the doses at frequent intervals would be of considerable advantage.

Woven or rubber catheters introduced through the glottis have been employed by many for intratracheal inoculation, but considerable skill is required in their use.

Method of Intratracheal Injection.

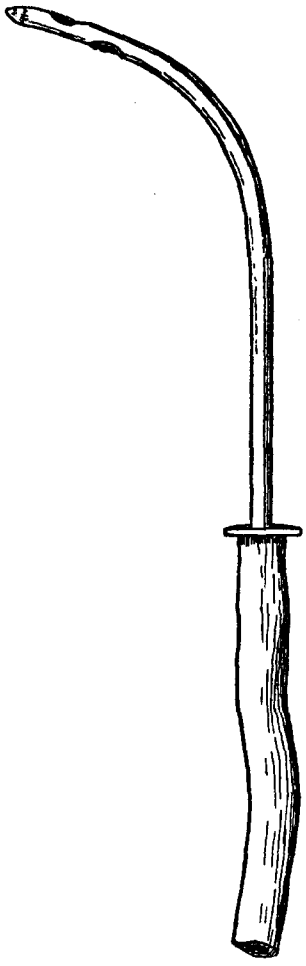
It has been stated that as simple a device as possible seemed advisable. It was found that a metal tube with a rounded end could be easily introduced into the trachea through the glottis. A milk or teat tube 9 cm. long, with an external diameter of 3 mm., was bent to a final angle of about 70° (Text-fig. 1). Such a tube has two open-

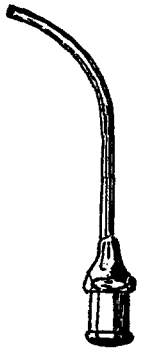
[4] Winternitz, M. C., Smith, G. H., and Robinson, E. S., *Proc. Soc. Exp. Biol. and Med.*, 1919–20, xvii, 195.

[5] Snel, J. J., *Z. Hyg. u. Infectionskrankh.*, 1902, xl, 103.

[6] Kitt, T., in Kolle, W., and von Wassermann, A., Handbuch der pathogenen Mikroorganismen, Jena, 2nd edition, 1912, iv, 819.

ings on opposite sides situated a short distance from the end so that very little force is required to inject fluids. Rabbits are given sufficient ether to insure complete relaxation. The mouth is opened and the tongue grasped gently by means of rubber forceps and drawn





TEXT-FIG. 1. TEXT-FIG. 2.

TEXT-FIG. 1. Tube for the administration of liquids into the trachea of rabbits. Actual size.

TEXT-FIG. 2. Tube for the administration of liquids into the trachea of guinea pigs. Actual size.

forward. The tube, previously immersed in paraffin oil, is inserted into the mouth and carried backward over the tongue and downward through the larynx. The tube is then connected to a syringe and the fluid slowly forced into the trachea. The tube is retained for a few seconds to allow drainage and then slowly withdrawn. After a little practise the whole operation may be done with considerable rapidity —usually from $1\frac{1}{2}$ to 3 minutes elapse between the beginning of anesthesia and the completion of the injection. The anesthesia may be so controlled that the animal has almost recovered when the tube is withdrawn.

A similar tube made from a $2\frac{1}{2}$ inch 14 gauge hypodermic needle will answer for guinea pigs (Text-fig. 2). Obviously the operation is more difficult in the guinea pig on account of the small mouth and the great development of the tongue at its base. A properly directed light is of considerable value when guinea pigs are used.

Comparison of the Production of Antibodies in Rabbits by Means of the Intratracheal and Intraperitoneal Routes.

Throughout the experiments the rabbits were immunized in pairs. The dose of antigen was always the same; the volume never exceeded 1.5 cc. In the case of bacterial and red cell suspensions the amount of material was increased from one series to another by concentration. Small volumes were indicated, since it was felt that the likelihood of all the material being retained in the respiratory tract was greater. All injections were made in series. Injections were made daily for 3 days, and 5 days after the last injection the animals were bled and the next series begun. The rabbits subjected to the peritoneal treatment were anesthetized for the same length of time as those injected into the trachea. Inasmuch as the protocols add little of interest, an outline of one is given in Table I.

In these experiments a heavy suspension in salt solution from a 24 hour agar culture of the hog-cholera bacillus was killed by heating to 60°C. for $\frac{1}{2}$ hour. This suspension was stored in the refrigerator as the stock vaccine. From it the antigens for the various series were prepared from week to week.

The results obtained in the case of these two rabbits have been recorded in Table II.

TABLE I.

Protocols of Rabbits Treated Intratracheally and Intraperitoneally with Killed Cultures of the Hog-Cholera Bacillus.

Rabbit No.	Method of administration.*	Series No.	Turbidity of antigen by Gates apparatus.	Time elapsing between beginning of anesthesia and completion of injection.	Thermic reaction.
				min.	
1	Intratracheal.	1	1.0	1½	None.
				2	Severe.
				1¼	"
2	Intraperitoneal.	1	1.0	1½	Slight.
				2	None.
				1½	"
1	Intratracheal.	2	0.8	2	Moderate.
				1½	"
				2	"
2	Intraperitoneal.	2	0.8	2	Slight.
				1½	"
				0	None.
1	Intratracheal.	3	0.5	2	Moderate.
				2	Severe.
				2	Moderate.
2	Intraperitoneal.	3	0.5	2	None.
				2	Slight.
				2	None.
1	Intratracheal.	4	0.4	2	Severe.
				2	Moderate.
				2	"
2	Intraperitoneal.	4	0.4	2	None.
				2	"
				2	"
1	Intratracheal.	5	0.3	2	Moderate.
				2	Severe.
				2	"
2	Intraperitoneal.	5	0.3	2	Slight.
				2	None.
				2	"

* The dose was always 1 cc.

TABLE II.

Agglutinin Production in Rabbits Immunized by the Intratracheal and Intraperitoneal Routes.

Rabbit No.	Method of administration.	Time.	Serum dilutions.												
			1:5	1:10	1:20	1:50	1:100	1:200	1:500	1:1,000	1:2,000	1:5,000	1:10,000	1:20,000	1:50,000	1:100,000
		wks.														
1	Intratracheal.	1	C.*	C.	C.	++	+	±	±	−	−					
2	Intraperitoneal.	1	"	"	+++	+	+	+	±	−	−					
1	Intratracheal.	2	"	"	C.	C.	C.	C.	++++	++	+	±	−			
2	Intraperitoneal.	2	"	"	"	"	"	"	++++	+++	+	±	−			
1	Intratracheal.	3			"	"	"	"	C.	C.	++++	+++	++	+	−	−
2	Intraperitoneal.	3			"	"	"	"	"	"	+++	+++	++	+	±	−
1	Intratracheal.	4				"	"	"	"	"	++++	+++	++	++	+	±
2	Intraperitoneal.	4				"	"	"	"	"	C.	++++	+++	++	±	−
1	Intratracheal.	5					"	"	"	"	++++	+++	++	+	±	−
2	Intraperitoneal.	5					"	"	"	"	C.	++++	+++	++	+	±

* The readings were recorded as follows: C., complete clearing of the fluid; ++++, heavy deposit with slight turbidity of the liquid; +++, less marked agglutination; ++, moderate clumping; +, distinct deposit; and ±, slight deposit.

From the tables it will be noted that the temperature reactions following the intratracheal inoculation are on the whole more severe. The temperature of animals that have been vaccinated through the tracheal route usually falls 1°C. within an hour or two. This is followed by a rather sharp rise for several hours. The initial drop in temperature after the intraperitoneal administration is much less and the secondary increase is not so pronounced. After 3 or 4 weeks of treatment those animals injected intratracheally with bacterial vaccines usually develop a severe dyspnea immediately after the treatment. In two rabbits treated for considerable periods consolidation associated with *Bacillus lepisepticus* has been observed.

It will be noted that agglutinins developed to about the same degree in both animals. This has held true in experiments with other organisms.

Similar results have been obtained in the production of hemolysins and precipitins. For the production of a hemolytic serum rabbits were immunized with a suspension of the washed red cells of the sheep. As in the preceding experiments the amount of material injected at one time was limited to 1 cc. The animals received three series of weekly injections. The first series contained a 10 per cent suspension of red cells, the second a 20 per cent suspension, and in the third the cells were increased to 40 per cent. In this way constantly increasing doses of antigen were possible. The total number given was equivalent to 42 cc. of a 5 per cent suspension of sheep red cells. The results are given in Table III.

The rabbit treated by the intratracheal route formed hemolysin to a greater degree during the first series of inoculations than the other which received intraperitoneal injections. After the second series the animal injected intraperitoneally possessed the better serum. At the end of the third series the titer of both sera was about the same.

It was possible to produce a good precipitating serum in the same manner. In these experiments cow serum was administered by both routes. The maximum dose given at one time was 1.5 cc. A total of 12 cc. was administered over a period of 3 weeks. The results are recorded in Table IV. In more recent work it was found that 6 to 8 cc. of a foreign serum may be introduced into the trachea without causing noticeable ill effects.

TABLE III.

Hemolysin Production in Rabbits Immunized by the Intratracheal and Intraperitoneal Routes.

Rabbit No.	Method of administration.	Time.	Serum dilutions.										
			1:5	1:10	1:20	1:50	1:100	1:200	1:500	1:1,000	1:2,000	1:5,000	1:10,000
		wks.											
3	Intratracheal.	1	C.*	C.	++++	+++	++	+	±	±			
4	Intraperitoneal.	1	"	++++	++	+	±	−	−	−			
3	Intratracheal.	2	"	C.	C.	C.	C.	C.	C.	C.	+++	+	−
4	Intraperitoneal.	2	"	"	"	"	"	"	++++	+++	±	±	−
3	Intratracheal.	3			"	"	"	"	C.	C.	++++	+	−
4	Intraperitoneal.	3			"	"	"	"	"	"	++++	++	±

* The usual method of recording hemolysis has been employed. The serum was stored in the refrigerator from 2 to 4 weeks before it was tested. Fresh guinea pig serum was used for complement.

During the first 2 weeks there was considerable difference in the antibody content of the two sera. At the end of the 1st week the serum of the rabbit (No. 5) immunized by intratracheal inoculation detected 1/1,000 cc. of cow serum; that of the other (No. 6) failed to react in the presence of 1/100 cc. By the end of the 2nd week the serum of Rabbit 5 had almost reached its maximum titer and reacted slightly with 1/20,000 cc. of cow serum. After 2 weeks 1/2,000 cc. of antigen was required to produce precipitation with the serum of Rabbit 6. At the end of the 3rd week both sera were equally efficient.

TABLE IV.

Precipitin Production in Rabbits Immunized with Beef Serum by Injection into the Tracheal and Peritoneal Cavities.

Rabbit No.	Method of administration.	Time.	Amount of antigen (in cc.) producing precipitation with 0.1 cc. of serum.*									
			1/100	1/200	1/500	1/1,000	1/2,000	1/5,000	1/10,000	1/20,000	1/50,000	1/100,000
		wks.										
5	Intratracheal.	1	+	+	+	+	±	−	−	−		
6	Intraperitoneal.	1	±	±	−	−	−	−	−	−		
5	Intratracheal.	2	+	+	+	+	+	+	+	±		
6	Intraperitoneal.	2	+	+	+	+	+	−	−	−		
5	Intratracheal.	3	+	+	+	+	+	+	+	+	±	−
6	Intraperitoneal.	3	+	+	+	+	+	+	+	+	−	−

* Definite precipitation has been recorded as +; ± indicates a slight turbidity and a trace of sediment.

DISCUSSION AND SUMMARY.

The method described of producing antibodies by the administration of antigens through the larynx is simple. The results obtained, however, conform closely to those obtained through intraperitoneal injection. The procedure is relatively a safe one and may well be employed in experimental inoculations. The advantages of rapidity and painlessness are obvious. In addition, gross injury has not been observed. Injections may be repeated at frequent intervals without danger to the life of the animal. The tube illustrated in Text-fig. 1

extends a little over 1 cm. into the trachea. The tube designed for the guinea pig reaches 2 or 3 mm. below the glottis. While the doses indicated in the protocols are small, 7 to 10 cc. of liquid have been given to rabbits by means of the tube without ill effects. It has been shown by the injection of India ink that the material is well distributed throughout the lungs in both the rabbit and guinea pig.

Under certain conditions it may be advisable to inject more deeply into the trachea, especially in the rabbit. The tube illustrated in Text-fig. 1 is not adaptable for this purpose. A cannula of larger diameter has proved of distinct advantage as a shield for introducing flexible catheters well down the trachea. A cannula 9 cm. long and 4 mm. in diameter when bent at an angle of 45° may be passed through the larynx of a rabbit without difficulty. A No. 8 (French) woven or No. 10 soft rubber catheter may be inserted into the cannula beyond the bend before the tube is passed through the glottis. After the tube has entered the glottis the catheter then may be introduced as deeply as desired into the teachea. The metal cannula enables the operator to "feel" the glottis.

From the experiments one seems justified in concluding that the results obtained by administering antigens by way of the trachea are about the same as those obtained by the intraperitoneal route. With the tubes described injury has been largely eliminated. The procedure is relatively a safe one.