THE ABSORPTION OF PROTEIN SOLUTIONS FROM THE PULMONARY ALVEOLI

By CECIL K. DRINKER, M.D., MADELEINE FIELD WARREN, PH.D., AND MARGARET MACLANAHAN

(From the Department of Physiology, The Harvard School of Public Health, and the Department of Bacteriology, The Harvard Medical School, Boston)

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In a recent paper on the permeability of the lungs to antibodies, Fox (1936) has summarized the literature upon the subject and makes it clear both from his own experiments and from those of others that antibodies of "different types contained in foreign or homologous serum appear in the blood stream but slowly after intratracheal injection" and that sterile inflammation of the lungs did not affect this result. Fox made no effort to identify the possible routes of absorption whether directly into the blood or *via* the lymphatics, though he assumes the blood route was the one selected and our experiments show this to be correct. His procedure consisted in intratracheal injections of immune sera in rabbits with sampling of the blood beginning shortly after the injection and continuing for as long as 5 days.

The experiments described below had as their objectives the determination of the route, whether lymphatic or vascular, by which passage of various species of proteins from the pulmonary alveoli was effected, and the rapidity with which these substances which varied in molecular size emigrated from the pulmonary site to the blood and lymph systems. As experimental animals dogs were selected since in them it is readily possible to tie off the lymphatic entrances into the right subclavian vein. If then the thoracic duct is cannulated the lymph drainage of the lungs has been excluded from entrance into the blood, and if samples of thoracic duct lymph and blood are titrated for the presence of foreign protein one can discover the pathway of absorption, whether it be across the alveolar wall and into the blood capillaries or by the lymphatic route to the root of the lung and the thoracic duct.

Materials and Methods

Dogs anesthetized by intravenous or intraperitoneal injections of barbital-sodium, 0.35 gm. per kilo, were used in all experiments. Animals so anesthetized will often live for several days if given fluid and kept warm, and in most of them blood pressure and general conditions are excellent for 30 hours, a period quite long enough to satisfy the needs of the experiments to be described. In preparation for the experiment the thoracic duct was isolated and tied, care being taken to search for branches making separate entrance into the subclavian vein, since, if lymphatic absorption occurred, such branches would cause contamination of the blood. Through a second incision all lymphatics entering the right subclavian vein were ligatured. The situation so produced probably results in diverting the lymph drainage of the right lung to the thoracic duct, since Baum (1918) has shown that in the dog the lymphatics from the two sides communicate quite freely as they reach the tracheobronchial lymph nodes. In any event the arrangement will provide all the lymph leaving the left lung, and in all experiments effort was made to deposit the solutions used upon that side. While in most cases the left side was shown at autopsy to contain far the larger part of the injected material, some of the solution invariably reached the right lung.

Following the two operations upon the lymphatics the trachea was cannulated and in some instances preparations made to take blood pressure, and in one case to perform plasmapheresis. To introduce the solutions into the lungs, the board was elevated at the head end and a long glass catheter with a slight bend at the end to assist entrance into the left bronchus was inserted slowly and without disturbance to the animal. The amounts of solution injected varied in different animals from 20 to 50 cc. Usually they were run in at a single injection, the rate being slowed if respiration became active, and the elevated position maintained for about an hour. The animals were killed by bleeding and an autopsy performed at once. Sections of the parts of the lungs containing solutions as well as from the tracheobronchial lymph nodes were made for microscopic examination.

Precipitating sera were obtained from rabbits injected intravenously with normal horse serum, crystallized egg white, and crystallized horse hemoglobin. Horse serum, obtained from a single bleeding of one animal, was used for preparation of the antiserum and for the intratracheal injections. This anti-horse-rabbit serum exhibited a titre of 1:10,000 to 1:14,000. The egg white and hemoglobin were crystallized, the first by the method of Sørensen (1917) and the second by the method of Green (1931). The egg albumin antiserum had a titre of 1:30,000 and the crystallized hemoglobin of 1:500. Serum and lymph titrations were begun at one-half dilution and continued through various dilutions. The precipitin tests were carried out by means of the ring technique using undiluted antiserum. Readings were taken at the end of 1 hour's incubation at room temperature. Physiological salt solution was used to dilute blood serum and lymph under examination for egg white and hemoglobin antibodies, but 1.5 per

cent salt solution proved more satisfactory in the case of these fluids derived from the dogs receiving intrabronchial administration of horse serum. Controls included the titration of the dog blood and lymph before injection as well as the usual saline control.

EXPERIMENTS

Experiment 1. Intratracheal Horse Serum.—Jan. 28, 1937. Dog weighing 7.7 kilos. 10:15 a.m. 25 cc. of 10 per cent barbital-sodium solution intravenously. 11:45. Thoracic duct cannulated. Lymphatic entrances on right ligatured. Trachea cannulated.

11:50. Lymph specimen 1 (control).

12:00. Blood specimen 1 (control). 200 cc. physiological saline intravenously. 12:10 p.m. *15 cc. of horse serum plus 2.5 cc. graphite suspension injected intratracheally.* 12:15. Rectal temperature 100.1°F. 12:25. *15 cc. of horse serum plus 2.5 cc. graphite suspension injected intratracheally.*

TABLE I

Titration of Blood Samples with Anti-Horse-Rabbit Serum. Dog 1

Time	Specimen	Antiserum dilutions			Saline control	Time after intratracheal injection of horse serum
		1:3	1:6	1:12		
12:00 noon	1	0	0	0	0	Control specimen
4:15 p.m.	2	0	0	0	0	4 hrs. and 5 min.
10:45 p.m.	3	0	0	0	0	10 hrs. and 35 min.
8:40 a.m.	4	++	+	Trace	0	20 hrs. and 30 min.
12:15 p.m.	5	++	+	Trace	0	24 hrs. and 5 min.

4:15. Rectal temperature 97.2°F. Lymph specimen 2. Blood specimen 2. 4:35. 60 cc. 20 per cent glucose solution plus 180 cc. of water by stomach tube.

10:45. Lymph specimen 3. Blood specimen 3. 10:50. Rectal temperature 96.6°F. 50 cc. water by stomach tube.

Jan. 29. 8:40 a.m. Lymph specimen 4. Blood specimen 4. 9:05. Rectal temperature 96.3°F. 9:10. 100 cc. physiological saline intravenously. Lymph flowing well. 12:05 p.m. Rectal temperature 95.2°F.

12:15. Lymph specimen 5. Blood specimen 5. 12:20. Bled to death. Blood flowed well indicating a fairly high blood pressure. Duration of experiment from the time of the first injection of horse serum 24 hours and 10 minutes.

Autopsy.—Practically all of the graphite colored injection was in the *right* lower lobe, only a trace having reached the left lung. Microscopic sections of blocks of the right lung taken from serum-containing areas showed much pink stained serum in the alveoli and many particles of graphite both free and in phago-

cytes. There was no perceptible accumulation of graphite in lymphatics. Tracheobronchial lymph nodes showed no accumulation of serum or graphite in the sinuses.

Table I shows the results of serological examination of the blood. Similar examinations of lymph were all negative.

Summary of Experiment 1.—In this case only a small fraction of the injected serum reached the left lung, which is in the main line of lymph drainage in the preparation employed. After 10 hours and 35 minutes no horse protein could be detected in the blood. There followed an interval of 9 hours and 55 minutes, and then the foreign protein was found in the blood. Lymph was consistently negative. The conclusion appears unescapable that the horse protein passed through the pulmonary epithelium and directly into the blood capillaries.

Experiment 2. Intratracheal Horse Serum.—Jan. 19, 1937. Dog weighing 13.5 kilos. Anesthesia and preparations as in Experiment 1. 3:30–3:45 p.m. *30 cc. horse serum plus a small amount of trypan blue intratracheally.*

Jan. 20. 10:00 a.m. Animal bled to death.

Autopsy.—The serum and trypan blue were distributed in both lower lobes, which were dark red and solid. The left side on cross-section contained somewhat more. Microscopical examination showed much intra-alveolar serum with many polymorphonuclear leucocytes scattered through it. The tracheobronchial lymph node sections were normal.

Tables II and III show the results of serological examination.

Summary of Experiment 2.—In dog 2 a large part of the injected serum reached the left lung. Horse protein was found in blood and lymph 17 hours and 5 minutes after intratracheal injection, but the concentration was greater in the blood. This result was obtained on other occasions, and in our opinion expresses the fact that after foreign protein accumulates in the blood one may expect transfer of it to the thoracic duct lymph in the abdominal region, where both in the liver and intestine capillary permeability to protein is high. The rise in concentration in the lymph parallels that in the blood and is a further indication of natural transfer.

Experiment 3. Intratracheal Horse Serum Followed by Plasmapheresis.—Mar. 9, 1937. Dog weighing 13 kilos. Anesthesia and general preparations as in Experiment 1, with the addition of cannulation of the femoral artery and vein in preparation for plasmapheresis.

10:50 a.m. Plasmapheresis started and carried on until 3:15 p.m. when the blood protein was reduced from 7.6 per cent to 4.2 per cent. Under these circumstances thoracic duct lymph flows very freely. 3:35 p.m. 35 cc. of horse serum were given intratracheally. This serum contained 6.6 per cent protein. Specimens of lymph and blood were taken for 3 hours and 10 minutes. None contained detectable amounts of horse protein.

TABLE II

Titration of Blood Samples with Anti-Horse-Rabbit Serum. Dog 2

Time	Specimen	Antiserum dilutions					Saline control	Time after intratracheal injection of horse serum
		1:2	1:4	1:8	1:16	1:32		
3:15 p.m.	1	0	0	0	0		0	Control specimen
10:00 p.m.	2	0	0	0	0			6 hrs. and 30 min.
8:35 a.m.	3	+	+	0	0			17 hrs. and 5 min.
12:00 noon	4	+	+	+	0			20 hrs. and 30 min.
3:45 p.m.	5	++	+	+	0	0		24 hrs. and 15 min.
9:50 p.m.	6	++	++	+		0		30 hrs. and 10 min.

TABLE III

Titration of Lymph Samples with Anti-Horse-Rabbit Serum. Dog 2

Time	Specimen	Antiserum dilutions					Saline control	Time after intratracheal injection of horse serum
		0	1:2	1:3	1:6	1:12		
3:10 p.m.	1	0	0	0	0	0	0	Control specimen
10:00 p.m.	2	0	0	0	0	0	0	6 hrs. and 30 min.
8:35 a.m.	3	+	Trace?	0	0	0	0	17 hrs. and 5 min.
12:00 noon	4	++	+	+	0	0		20 hrs. and 30 min.
3:45 p.m.	5	++	++	+	0	0		24 hrs. and 15 min.
9:50 p.m.	6	++	++	+	+	0		30 hrs. and 10 min.

Summary of Experiment 3.—In this case neither the rapid flow of lymph nor the fact that the intra-alveolar protein had a higher concentration than the blood protein of the dog had any marked effect on absorption from the alveoli. Had the experiment continued longer some acceleration might have been observed, but since at the close of the experiment the blood protein had risen to 6.5 per cent it is improbable that the result would have differed greatly from that observed in Experiments 1 and 2.

Experiment 4. Intratracheal Horse Serum in a Dog Sensitized to Horse Serum.—Jan. 25, 1937. Dog weighing 21 kilos. 50 cc. horse serum intravenously.

Mar. 3. 10 cc. horse serum intravenously.

Mar. 27. Blood shows no horse protein.

Mar. 30. 9:30 a.m. Anesthesia and preparations as in Experiment 1 plus cannulation of femoral artery for blood pressure measurements. 11:45. After taking control specimens, *30 cc. of horse serum were given intratracheally.* There was no increase in lymph flow, and blood pressure tracings taken during the next 9 hours and 45 minutes showed no disturbance of any sort. 9:30 p.m. *10 cc. of horse serum were given intravenously* to find out whether the animal was sensitized. This injection produced a slight fall in blood pressure and an enormous increase in lymph flow,—typical effect of anaphylactic shock in the dog (Petersen and Hughes, 1925).

During this entire experiment no horse protein was found in blood or lymph. The animal was evidently sensitized but did not absorb enough horse protein from the alveolar reservoir to cause shock.

Experiment 5. Intratracheal Horse Serum with a Forced Increase in Breathing.—Apr. 20, 1937. Dog weighing 11 kilos. In this case all preparations were as in Experiment 1 except that the animal was forced to rebreathe, thus greatly accelerating and deepening its respiration. Specimens of blood and lymph were taken during 5 hours and 25 minutes. No horse serum was detected in either fluid, and it is evident that even the most vigorous breathing has no effect on the absorption of horse protein.

Experiment 6. Intratracheal Crystallized Hemoglobin.—Apr. 15, 1937. Dog weighing 14.5 kilos. Anesthesia and preparations as in Experiment 1. 10:35 a.m. *25 cc. of 20 per cent hemoglobin solution injected intratracheally.*

Apr. 16. 10:45 a.m. The dog was bled to death. At autopsy the injected hemoglobin was about equally divided between the right and left lower lobes. Microscopically the hemoglobin-containing portions of the lung show a large number of polymorphonuclear leucocytes. No concentration of hemoglobin in lymphatics or in the sinuses of the lymph nodes from the root of the lung can be detected.

Table IV shows the results of serological examination of the blood. 13 hours and 28 minutes passed before hemoglobin was detected with certainty in the blood. Lymph titrated in a similar manner throughout the experiment never was shown to contain hemoglobin.

Summary of Experiment 6.—The absorption of hemoglobin is directly into the blood vessels and seems to be entirely similar to the absorption of horse serum protein.

Experiment 7. Intratracheal Crystallized Egg Albumin in Physiological Saline.—Mar. 15, 1937. Dog weighing 17 kilos. Anesthesia and preparations as in Experi-

TABLE IV

Titration of Blood Samples with Anti-Hemoglobin-Rabbit Serum

Time	Specimen	Antiserum dilutions			Saline control	Time after intratracheal injection of hemoglobin
		1:2	1:4	1:6		
10:05 a.m.	1	0	0	0	0	Control specimen
12:03 p.m.	2	0	0	0	0	1 hr. and 28 min.
2:10 p.m.	3	0	Trace	0	0	3 hrs. and 35 min.
4:00 p.m.	4	0	Trace	0	0	5 hrs. and 25 min.
5:00 p.m.	5	Trace	0	0	0	6 hrs. and 25 min.
8:00 p.m.	6	0	0	Trace	0	9 hrs. and 25 min.
12:03 a.m.	7	+	0	Trace	0	13 hrs. and 28 min.
8:30 a.m.	8	+	+	0	0	21 hrs. and 55 min.
10:45 a.m.	9	+	+	+	0	24 hrs. and 10 min.

TABLE V

Titration of Blood Samples with Anti-Egg-Albumin-Rabbit Serum

Time	Specimen	Antiserum dilutions				Saline control	Time after intratracheal injection of egg albumin
		1:2	1:4	1:6	1:8		
11:35 a.m.	1	+	0	0	0	0	Control specimen
1:45 p.m.	2	+	+	+	0	0	2 hrs.
3:45 p.m.	3	+++	++	++	+	0	4 hrs.
4:55 p.m.	4	++	++	+	++	0	6 hrs. and 10 min.
7:55 p.m.	5	++	++	+	0	0	9 hrs. and 10 min.

TABLE VI

Titration of Lymph Samples with Anti-Egg-Albumin-Rabbit Serum

Time	Specimen	Antiserum dilutions				Saline control	Time after intratracheal injection of egg albumin
		1:2	1:4	1:6	1:8		
11:35 a.m.	1	0	0	0	0	0	Control specimen
1:45 p.m.	2	0	0	0	0	0	2 hrs.
3:45 p.m.	3	+++	++	++	+	0	4 hrs.
4:55 p.m.	4	+++	+++	++	++	0	6 hrs. and 10 min.
7:55 p.m.	5	+++	+++	++	++	0	9 hrs. and 10 min.

ment 1. 11:45. 39 cc. egg albumin solution given intratracheally. This solution contained approximately 6 per cent of egg albumin. No evidence of irritation.

Mar. 16. Dog was found dead in the morning, the tracheal cannula being plugged with mucus and froth.

Tables V and VI show the results of serological examinations.

Summary of Experiment 7.—In this case absorption into the blood was prompt and appearance in the lymph soon follows. The somewhat higher concentration of egg albumin in the lymph found late in the experiment occurred in another similar experiment and perhaps indicates some degree of absorption by the lung lymphatics, but in any event this was slight.

DISCUSSION

The results of these experiments were not at all in accord with our expectations. It is known that physiological salt solution is absorbed rapidly from the pulmonary alveoli and undoubtedly passes directly into the blood (Winternitz and Smith, 1920). In contrast to this direct route, particles of various sorts which reach the alveoli find their way into the lymphatics and are removed by the lymphatic route. It is generally thought that intra-alveolar phagocytosis is a necessary first step in removal of particulate material from the alveoli, but many experiments have shown that particles so deposited reach the lymph nodes at the root of the lung far too rapidly to permit phagocytosis as a first step (Drinker and Field, 1933). It has also been shown that respiratory movements have an important influence upon particle absorption, it being extremely slow in a motionless lung (Shingu, 1908). By analogy with the fact that protein solutions injected subcutaneously are removed practically entirely by the lymphatics and that this is true for such solutions when deposited in the serous cavities and in practically all parts of the body, it had been our belief that intra-alveolar protein would depart by the lymphatic route and in view of the results with visible particles we were prepared to find this absorption rather rapid and accelerated by deep breathing. Neither of these expectations was realized. Absorption was exceedingly slow and of very small volume. It was directly into the blood vessels and the rate and volume of absorption were not affected by increase in breathing, by sensitization, or by reducing the concentration of the blood proteins by plasmapheresis. Horse serum and horse hemoglobin were absorbed very slowly and at about an equal rate.

A great deal is known about the anatomy and histology of the lung lymphatics, but the reasons for their very extensive distribution are by no means clear. Owing to the fact that the blood pressure

in the pulmonary capillaries is probably much below the colloid osmotic pressure of the blood, a situation exists very favorable to the absorption of water by the blood capillaries, and this in turn is a favorable arrangement in an organ where gas transfer is preeminently important. Elsewhere in the body an important function of the lymphatics is to carry away blood proteins which have passed through the walls of the capillaries to become constituents of the tissue fluid, but in the lungs the situation does not favor tissue fluid production, and one is forced to believe that the very elaborate lymphatic system described by Miller (1937) exists to function in the presence of infection, hemorrhage, foreign body deposition, in short against any process which results in the presence of foreign material in the lung tissue. In order to enter lung lymphatics it is essential to traverse a certain amount of lung tissue since lymphatic capillaries do not extend past the alveolar ducts. Apparently particles of inorganic material and bacteria make this transit in the breathing lung, but fluids actually in the alveoli and appropriate to removal by the lymphatic route do not succeed in it. Even very vigorous breathing, which greatly accelerates the movement of intra-alveolar particles into the lymphatics, fails to bring about lymphatic absorption of fluids.

SUMMARY

Horse serum, crystallized hemoglobin, and crystallized egg albumin have been injected into the lung alveoli of dogs in which the entrances of the right lymphatics have been tied and the thoracic duct cannulated. Samples of blood and lymph have been taken following this injection. Only after several hours in the case of the horse serum and hemoglobin have these proteins been detected by immunological methods and invariably they have appeared first in the blood. Egg albumin also enters the blood capillaries, but much more rapidly than the other two proteins, due probably to the smaller molecular size.

BIBLIOGRAPHY

Baum, H., Das Lymphgefässsystem des Hundes, Berlin, August Hirschwald, 1918.

Drinker, C. K., and Field, M. E., Lymphatics, lymph and tissue fluid, Baltimore, The Williams & Wilkins Co., 1933.

Fox, J. P., *J. Immunol.*, 1936, **31**, 7.

Green, A. A., *J. Biol. Chem.*, 1931, **93,** 495.
Miller, W. S., The lung, Springfield, Illinois, and Baltimore, Charles C. Thomas, 1937. See page 84, Chapter VI, The lymphatics.
Petersen, W. F., and Hughes, T. P., *J. Biol. Chem.*, 1925, **63,** 179.
Shingu, S., *Beitr. Klin. Tuberk.*, 1908, **11,** 1.
Sørensen, S. P. L., *Compt.-rend. trav. Lab. Carlsberg*, 1917, **12,** 1.
Winternitz, M. C., and Smith, G. H., Preliminary studies in intratracheal therapy, from Collected studies on the pathology of war gas poisoning, New Haven, Yale University Press, 1920, 143.