by incubated red cells.

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Rate of Localization of Anti-Rat Lung Antibody.*† (26470)

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Antibody prepared in rabbits against rat lung tissue has been shown to be fixed in the lung and other organs when injected intravenously into rats(1). A similar situation exists with antibody against rat kidney in that when it is injected intravenously it localizes in the kidney with some cross-localization elsewhere(2,3). Rate of localization in kidney has been determined for the latter case(4) and it was found that for the most part the antibody is removed from the circulation as it passes through that organ. There is a second component which appears to be fixed at a somewhat slower rate. In the experiments reported here, rate of localization of anti-lung antibody in the lung was determined.

Preliminary results with the globulin fraction of anti-lung serum indicated that the lung localizing activity was too low to permit very accurate measurement in view of the relatively high background localization in the lung. Therefore, the study was made primarily with antibodies in which the lung localizing activity had been concentrated and purified by adsorption and elution from lung tissue. The major part of the kidney localizing activity was removed from the purified preparation by treatment with kidney sediment.

Materials and methods. Sprague-Dawley rats were used throughout the studies. *Sera.* Anti-rat lung sera were obtained by injecting perfused rat lung homogenate intraperitoneally into 8 rabbits 3 times a week for 4 weeks and bleeding one week after last injection. The dose given was 0.5 g wet tissue per injection. The γ-globulin fraction of each antiserum (γ G Anti-R Lu) and of normal rabbit serum (γ GNS) (Pentex, Inc.) was prepared by ethanol fractionation(5). The γ G anti-R Lu from a single rabbit showing the highest lung localizing activity relative to localization elsewhere was selected for this study. The proteins were radioiodinated as described previously(6,7). *Preparation of sediments*: Frozen rat lungs previously perfused with saline were minced with scissors and homogenized with 4.5 vol. physiological saline

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and 0.5 vol. borate buffer, pH 8.0, by means of a glass tissue grinder. The homogenate was centrifuged at 1,500 RCF for 15 min. at 5°C and the sediment removed. The supernatant was recentrifuged at 25,000 RCF for 15 min. and the collected sediment was washed several times, then lyophilized. This preparation of lung high speed sediment, referred to as Lu H Sed was found to concentrate lung localizing antibody more specifically than the low speed sediment. Rat kidney sediment (K L Sed) was collected by centrifuging the homogenate at 1,500 RCF, washed and lyophilized. *Preparation of fibrin*: Fibrinogen was prepared from normal rat plasma by fractionation with phosphate buffer similar to that for human fibrinogen(8). Fibrinogen (30 mg) was dissolved in borate buffer, pH 8.0 and mixed with 20 NIH units of bovine thrombin (1.0 mg lyophilized bovine thrombin, Parke, Davis & Co.). After standing at 37°C for 1 hour, fibrin was collected on a stirring rod, chopped in small pieces with scissors and homogenized with a glass tissue grinder to make a fine suspension. After centrifuging and washing, the fibrin was used for adsorption. *Purification of anti-lung antibody*: 16 mg of radiolabeled γ-globulin fraction of anti-lung serum was treated with 16 mg of Lu H Sed in the presence of 0.2 ml of normal rabbit serum at 37°C for 1 hour. The sediment was collected by centrifuging the mixture at 25,000 RCF for 15 min. and washed twice with 10 ml of borate buffer pH 8. The sediment was suspended in 5 ml borate buffer and treated at 60°C for 20 min. to elute the adsorbed antibody. The same procedure was repeated with the residue using 3 ml borate buffer. Both eluates were combined. Of the original radioactivity, 4.5% was recovered in the eluate. The purified anti-lung antibody (eluate) was further treated once with rat fibrin prepared from 30 mg fibrinogen, then twice with 16 mg of K L Sed to remove anti-fibrin(9) and the major part of kidney localizing antibody. The final supernatant was used for injection into donor animals. The final recovery was 3.2% of the original radioactivity. Radioiodinated normal serum globulin was treated in the same manner. Recovery of radioglobulin in the eluate was 0.91%. After adsorption with fibrin and kidney sediment, 0.72% of the original radioactivity was found in the final supernatant. This was used for injection into donors of control groups.

Results. The purified anti-lung antibody described above was diluted with saline containing 2% of normal rat serum, and 2 ml portions, containing 34 μg radio-globulin, were injected intravenously (tail vein) into a series of 14 Sprague-Dawley rats weighing 130-150 g. After a certain period of time blood from a group of 2 donor rats was taken from the abdominal aorta with heparinized syringes and collected plasmas were pooled. The localizing activity remaining in the plasma from each group was determined by injecting 0.5 ml of the plasma (diluted to 2.0 ml with borate buffer), into each of 5 recipient rats. The initial localizing activity of the antibody preparation (zero circulation time) was determined without passive transfer by direct injection of the original material (diluted similarly) into a group of recipient rats. All recipient rats were perfused 3 days after injection, the tissues removed(7) and radioactivity was determined as described previously(6).

A similar preparation from normal serum globulin (11.5 μg/donor rat) was used for donor rats of control groups and the passive transfer carried out as mentioned above.

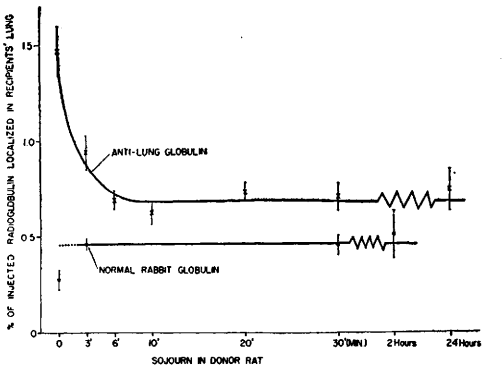

FIG. 1. Disappearance of lung localizing activity of anti-lung antibody from circulation. Purified anti-lung antibody and the similar preparation from normal serum globulin (control) were used. Abscissa: circulation time in donor rats. Ordinate: % of inj. radioglobulin, remaining in donor plasmas, which was fixed by the lungs of assay rats.

TABLE I. Disappearance of Localizing Activity from Circulation (Anti-Lung Antibody). Donor rats were inj. with purified anti-lung antibody and bled at various times after inj. Localizing activity of radioglobulin remaining in circulation at time of bleeding was assayed by inj. of the plasma into 5 assay rats. (3 day assay.)

Time between inj. & bleeding	% of inj. globulin remaining in donor's plasma*	Localization of remaining antibody in assay rats (% dose in total organ)					Net localization of remaining antibody† (% dose in total organ)			
		Lung	Kidney	Liver	Spleen	Blood*	Lung	Kidney	Liver	Spleen
0 min.‡	—	1.47	.48	2.58	.41	12.2	1.04	.36	2.10	.34
3	84.1	.94	.54	2.11	.38	15.0	.51	.42	1.63	.31
6	67.1	.69	.42	1.26	.28	18.1	.26	.30	.78	.21
10	59.6	.62	.32	1.08	.26	18.7	.19	.20	.60	.19
20	51.2	.73	.42	1.18	.24	18.9	.30	.30	.70	.17
30	47.8	.70	.22	.75	.15	15.6	.27	.10	.27	.08
24 hr	18.5	.74	.20	.74	.14	28.2	.31	.08	.26	.07

* Calculated assuming total blood as 1/10 of body wt.
† Localization corrected for nonspecific localization of control preparation from GNS (Table II).
‡ Zero time: direct inj. into 5 assay rats.

TABLE II. Disappearance of Localizing Activity from Circulation (Control Preparation from GNS). Donor rats were inj. with purified GNS and bled at various times after inj. Nonspecific localizing activity of radioglobulin remaining in circulation at time of bleeding was assayed by inj. of the plasma into 5 assay rats. (3 day assay.)

Time between injection and bleeding, min.	% of inj. globulin remaining in donor's plasma*	Localization of remaining radioglobulin in assay rats (% dose in total organ)				
		Lung	Kidney	Liver	Spleen	Blood*
0†	—	.28	.10	.45	.05	15.7
6	64.0	.46	.13	.62	.06	28.9
30	45.7	.45	.13	.47	.09	31.8
120	38.2	.51	.10	.36	.07	32.6
	Avg	.43	.12	.48	.07	

* Calculated assuming total blood as 1/10 of body wt.
† Zero time: direct inj. into 5 assay rats.

Preliminary experiments indicated that the fixation of lung-localizing antibody takes place rapidly, essentially within one hour after injection. Therefore, the experiment was carried out with shorter circulation times of 3, 6, 10, 20, 30 min. and 24 hours. The results are shown in Table I and Fig. 1. Net localizing activity remaining in the donors' plasma was calculated by subtracting the average background values of control groups (Table II). The lung localizing activity of the donor plasma decreased to 50% level after only 3 minutes of circulation and to 25% level in 6 minutes, then remained at this level up to 24 hours of circulation. This indicates that the lung localizing antibody in anti-lung serum is fixed by donor rats very rapidly. Indeed, all the available lung localizing antibody seems to be cleared from the circulation in 6 minutes. Liver, kidney, and spleen localizing antibody although still present in the purified preparation,‡ are also fixed rapidly, but complete clearance from the

‡ The localizing activity of the purified antibody (before adsorption with K L Sed) can be affected by treatment with sediments from various organs. K L Sed was most effective in removing the kidney localizing antibody together with some of the liver and spleen localizing antibody. This did not affect the lung localizing antibody. The sediments from liver and spleen removed parts of the liver, spleen and kidney localizing activity without affecting the lung localizing activity. The high speed sediment from kidneys and the low speed sediment from lungs removed part of the lung localizing antibody as well as part of the other localizing antibodies. The high speed sediment from lungs was most effective in removing the lung localizing antibody.

blood seems to take a longer time than the lung localizing antibody.

Discussion. For the passive transfer method to give an accurate measure of the localization of antibody in a particular tissue, the localizing antibodies should be made as specific as possible against the target tissue in question. If the lung localizing antibodies are fixed by other tissues as well as the lung itself, the disappearance rate of lung localizing activity from the donor's circulation would not indicate the true rate of fixation by lung. However, it has been shown that the anti-lung antibody once localized in the lung does not go to other organs to any appreciable extent, when eluted and reinjected(10). Therefore, we feel that the data obtained here represent a rather accurate estimate of the localization rate occurring in the lung. Thus, the lung localizing antibody seems to be fixed by lungs very rapidly and the responsible antigens must be in close contact with the circulating blood.

The data are not accurate enough to determine whether the trapping efficiency of lung is greater than that of kidney or liver for their respective antibodies. The more rapid clearance by lung may be due to the greater circulation through the lungs which get the full cardiac output as compared with the other organs which get only part.

Summary. Rate of localization of anti-rat lung antibody in lungs was determined by a passive transfer method. The purified anti-lung antibody was injected into donor rats and the lung localizing activity remaining in the circulation was assayed by injecting the plasmas into groups of recipient rats. The lung localizing antibody was cleared from the circulation very rapidly, indicating that the responsible antigens must be in very close contact with the circulating blood.

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An Improved Method for Rapid Laboratory Diagnosis of Poliomyelitis. (26471)

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A common procedure for laboratory diagnosis of poliomyelitis involves first isolation of the viral agent from a stool specimen followed by serological identification of this agent. Attempts to expedite this procedure by carrying out serological identification directly on virus-containing stool extract have not been entirely successful principally because of the presence of non-specific cytotoxins in such material. Various aspects of this problem have been fully described and discussed(1,2).

It will be shown in this report that by differential centrifugation of infected stool extracts, virus can be concentrated and separated from cytotoxic material. Serological