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# THE EXPERIMENTAL STUDY ON THE TRANSBRONCHIAL ADMINISTRATION OF CARCINOSTATIC AGENTS IN PULMONARY CARCINOMA

Preliminary Report

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As compared with cancers of other organs the incidence of lung cancer shows a remarkable increasing trend in recent Japan. However, it is extremely regretable that therapeutic results are appallingly low. At present in spite of various progressive therapeutic means, the main treatment consists of surgery and it may be said that postoperative long term cure rate are mainly influenced by lymphatic metastasis. Thus it is suggested that if it is possible to prevent postoperative lymphatic metastasis the post-operative long term results may be improved.

Hitherto, concerning this point, attempts for selective administration of agents for the pulmonary and mediastinal lymphatic system have not been made and no reports can be found. In the present paper basic experiments were conducted and it was shown that

agents administered transbronchially, under a given condition, are specially uptaken by lymphatic tissue adjacent to the bronchial wall and from there are transported to the pulmonary and mediastinal lymph nodes. Based on this new idea, it was suggested that if carcinostatica or radioisotope therapeutic agents are administered transbronchially pre- and postoperatively, to lung cancer patients, a minimal dose of therapeutic agents may be delivered at a high concentration to the pulmonary or the mediastinal lymphatic system. Moreover, no side effects would be seen in other organs and as a result this method may be promising in the prevention of lymphatic metastasis leading to an improved therapeutic means for lung cancer.

Naturally, since it cannot be expected that by this administrative method the cancer lesion itself can reached with effect, the administration should be followed by radical operative procedure of the lesion to ensure a thorough cure of lung cancer.

Method

Mongrel dogs of 10 Kg or thereabouts were used and anesthetized with intravenous injection of pentobarbital sodium at a rate of 30 mg/Kg. Artificial respiration was conducted by a pump after tracheal intubation.

The angulus venosus were exposed on both sides of the neck and retrograde thoracic duct cannulations using polyethylene tubes were made at the sites where bilateral thoracic ducts empty into the the

vein. Polyethylene tubes with an inner diameter of 0.7 - 1.1 mm were used and coated with heparin to prevent coagulation of lymph fluid.

The thoracic duct was ligated at the venous side and the tube was inserted retrogradely towards the periphery for 1 - 2 cm and the thoracic duct lymph was collected.

The right thoracic duct instead of opening directly into the vein, branches off and the lymph passes into the vein via these numerous narrow branches. Thus it is difficult to insert the polyetylene tube via these branches. Hence the tube was inserted into the vein directly above the flow-in site, in a such a way as to collect lymph samples. A method of ligation of vein as shown in Fig. 1 was innovated to prevent mixing of the venous blood and right thoracic duct lymph was collected.

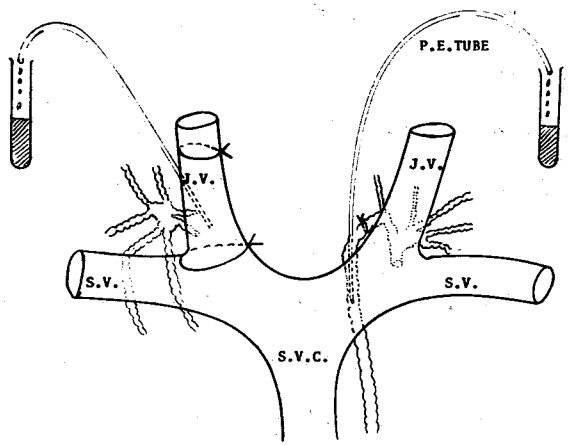


Fig. 1. Method for collection of right and left thoracic duct lymph

As reported by Louvier (21), Baum (2), Correl (6), Warren (28) and various other workers, the pulmonary lymph flow is mainly collected in the lymph duct along the bronchus after which it finally collects in the right thoracic duct via the hilar and mediastinal lymph nodes. Therefore, the concentration of the chemical agent appearing in the right thoracic duct lymph after transbronchial administration was considered to reflect the concentration of the agents in the pulmonary lymph flow.

It was also considered that inasmuch as the left thoracic duct lymph mainly represents the lymph flow of the lower extremities and the abdomen, the influence of the pulmonary lymph flow would be small.

The administration of the agents was done after completion of the right and left thoracic duct cannulation. Two methods of transbronchial administration, inhalation and instillation, were attempted. The inhalation method was conducted by connecting the tracheal tube to an I. P. P. B. circuit and after filling the nebulizer with the desired agents, the agent was given by nebulization. The instillation method consists of inserting a Nelation's catheter to the bifurcation through the tracheal tube, which is followed by a gradual instillation of the agent after which artificial respiration is conducted by a pump.

After the completion of agent administration, right and left thoracic duct lymph samples were collected periodically by the cannulation.

Simultaneously, blood samples were taken from the femoral artery and the concentrations of the agents in the samples was measured. The dogs used in the experiment were later sacrificed and parenchymatic tissue slices were taken from the intrathoracic lymph nodes and the lung, liver, spleen, kidney and bone marrow, and a comparison was run between the respective concentrations of the agent.

For the convenience of measurements the administered agents in the present study were labelled with readily measurable radioactive isotopes and its radioactivity was measured. Of the carcinostatica used today, two agents $P^{32}$ - Thio-TEPA and $Co^{60}$ - Protoporphyrin were used. In addition to these a radioactive isotope cancer therapeutic agent $Au^{198}$colloidal solution which has an affinity to the reticulo-endothelial system was used while as a control $P^{32}$ phosphate saline solution was used for a tracer and the uptake of each respective agent by the pulmonary lymphatic system was measured.

The radioactivity of the lymph and blood samples were assayed by the beta activity for $P^{32}$ and respective gamma activity for $Co^{60}$ and $Au^{198}$ measured with a Geiger- Müller counter and Well type scintillation counter. They were expressed by counts/min/ml.

For the measurement of beta activity of the tissue samples, the animals were sacrificed by evacuating blood. Tissue samples of organs were taken and after a thorough washing with water, the weight was measured, and after mincing this was digested by conc. $HNO_3$.

This was further homogenized and spread out evenly in stainless steel counting caps and dry ashed and counts/min/g was measured. For the measurement of gamma activity in tissue samples, the samples were homogenized in the same manner as described above. Instead of dry ashing the homogenate was diluted in 10 ml of water and measured with a well type scintillation counter.

By the assay back counts were decreased, and were corrected for natural decay up to the time of the assay from administration. Further, prior to the experiment the counts of the experimental apparatus was measured in order to determine the absence of radio-isotope contamination and in the case of the inhalation method the respirator circuit was protected by a vinyl sheet to cut outside interference.

Results

1) $P^{32}$ transbronchially administered group

A preliminary transbronchial administration experiment was conducted using a small particle size substance. 300$\mu$c of $P^{32}$ - labelled sodium phosphate saline solution was administered trans-bronchially into 5 dogs. Three of them were instilled with the agent transbronchially in a supine position. The $P^{32}$ solution was gradually instilled after which positive pressure breathing was done by a pump for one hour and was returned to spontaneous breathing thereafter. In the remaining two dogs the tube inserted into the

trachea was connected with a Bennet Respirator. The $P^{32}$ solution was poured into the nebulizer and inhalation was conducted over a period of 30 minutes after which blood and lymph sample collecting was started.

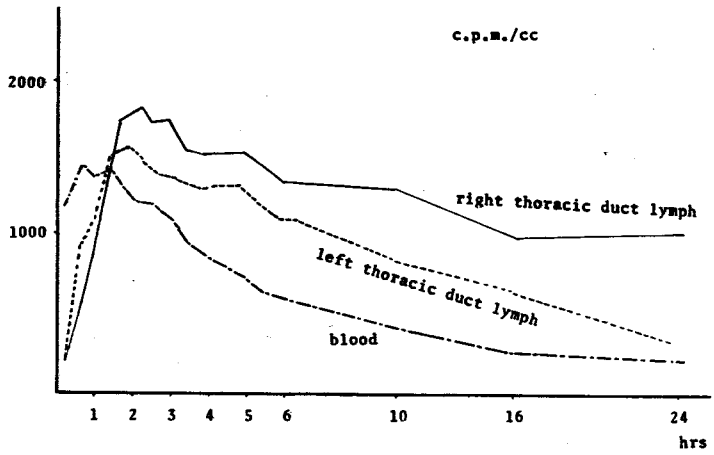


Fig. 2. Radioactivity of blood and lymph after transbronchial administration of $P^{32}$-phosphate solution (300 $\mu$c)

As shown in Fig. 2 the radioactivity assay of blood and lymph with the lapse of time after $P^{32}$ administration is given. In the case of instillation $P^{32}$ radioactivity appeared prominently in the circulating blood 10 - 20 minutes after, which is comparatively early. Somewhat later at 30 - 60 minutes, a rise in concentration of radioactivity was seen in both the right and left thoracic duct lymph.

The assay values were somewhat higher in the lymph of the right

thoracic duct lymph, with the left thoracic duct lymph values coming next and that of the blood coming last. However, very little difference was seen between individual samples. This may be atributed to the fact that the particles of $P^{32}$ are small and a considerable amount may be assumed to pass readily via the alveolar capillaries into the blood stream. Thus, a selective absorption by the lymphatic tissue cannot be expected. In the inhalation group only a slight time difference in appearance of the radioactivity in the blood and lymph samples were seen. Otherwise, no difference was seen between the instillation group.

Three to ten days after $P^{32}$ transbronchial administration the distribution of concentration in the intrathoracic lymph nodes and organ tissue was examined. As shown in Table 1 while a high concentration is seen in the lung and peribronchial lymph nodes, a considerable leakage to other organs via the blood stream was seen. Especially in the liver, bone marrow and kidneys a high concentration of radioactivity was seen.

2) $Au^{198}$ colloidal solution transbronchially administered group

$Au^{198}$ colloidal solution which has an affinity to the lymphatic system and the reticuloendothelial system and which is widely used at present as a radioisotope therapeutic agent for various malignant tumors was administered transbronchially to 6 dogs. Three dogs were administered transbronchially in the same manner as described above, and the remaining three dogs were given nebulization

Table 1. Tissue Distribution of Radioactivity after Transbronchial Administration of $P^{32}$ Phosphate Solution (300 $\mu$c)

Counts/min./g

| Dog No. | 21 | 22 | 25 | 26 | 28 |
|---|---|---|---|---|---|
| Way of Admin. | Instill. | Inhall. | Instill. | Inhall. | Instill. |
| Time interval | 3 days | 3 days | 5 days | 5 days | 10 days |
| L.upper mediast.L.N. | 72 | --- | 112 | --- | 71 |
| R.upper mediast.L.N. | 137 | 57 | 146 | 31 | 46 |
| L.paratracheal L.N. | --- | --- | 75 | --- | 38 |
| R.paratracheal L.N. | 540 | 360 | 286 | 151 | --- |
| Botallow's L.N. | 480 | 426 | 385 | 296 | 252 |
| Subcarinal L.N. | 724 | 531 | 456 | 345 | 240 |
| L. bronchial L.N. | 636 | 211 | 328 | 146 | 126 |
| R. bronchial L.N. | 586 | 425 | 256 | 320 | 193 |
| R. upper lobe | 134 | 228 | 111 | 198 | 38 |
| R. middle lobe | 86 | 137 | 87 | 158 | 26 |
| R. cardiac lobe | 115 | 106 | 105 | 94 | 44 |
| R. lower lobe | 247 | 186 | 126 | 124 | 73 |
| L. upper lobe | 108 | 97 | 81 | 112 | 29 |
| L. lingula | 111 | 86 | 50 | 78 | 58 |
| L. lower lobe | 77 | 138 | 113 | 96 | 70 |
| Liver | 631 | 726 | 456 | 248 | 191 |
| Spleen | 222 | 287 | 137 | 185 | 186 |
| Kidney | 487 | 525 | 212 | 196 | 113 |
| Bone marrow | --- | 718 | 579 | 388 | 105 |

---- no specimen

inhalation using a respirator.

After 2 mc of $Au^{198}$ colloidal solution was administered, radioactivity assay was conducted periodically in lymph and blood samples.

As may be seen in Fig. 3, $Au^{198}$ radioactivity appeared in the right

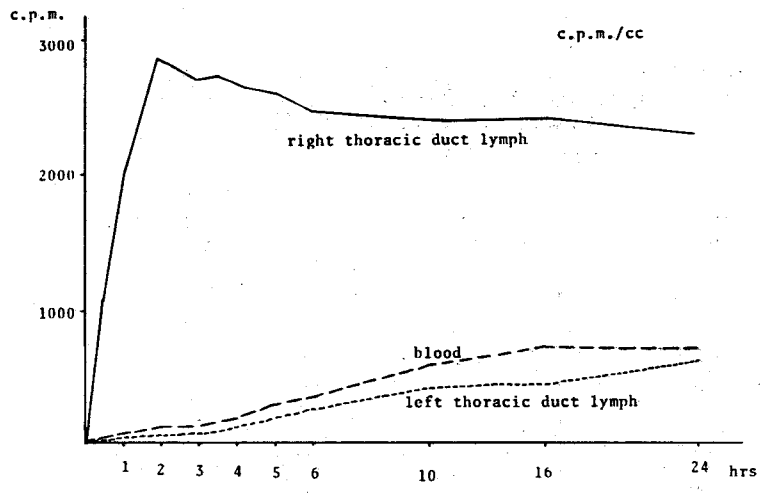


Fig. 3. Radioactivity of blood and lymph after transbronchial administration of $Au^{198}$ colloid (2 mc)

thoracic duct lymph at an early period, specifically and in a large amount. Moreover, a lasting high concentration was seen. In contrast, the blood concentration showed extremely low values at the beginning and thereafter a gradual increase was seen. The left thoracic duct lymph showed the same tendency as that in the blood.

In other words, in the administration of $Au^{198}$ which is a comparatively large particle colloid, the absorption by the bronchial wall shows a considerable selectivity and results in an uptake by the lymphatic tissue. At the same time leakage into the blood stream is minimal. Thus the requirements of selective administration to the pulmonary lymphatic system are met. Further $Au^{198}$ radioactivity in the left

thoracic duct lymph which collects the abdominal and lower extremity lymph flow is scarce.

The fact that the concentration of radioactivity in blood and the left thoracic duct lymph increases with the lapse of time may be attributed to the fact that the return flow from the left and right thoracic ducts into the angulus venosus is blocked resulting in the formation of a lymphatico-venous shunt at another site.

The lymph node and organ tissue distribution of $Au^{198}$ 3 to 5 days after administration is as shown in Table 2. A specific high concentration of $Au^{198}$ is seen in the hilar and mediastinal lymph nodes. A slightly high concentration is seen in the lung parenchym and while the concentration is extremely low in other organs, the kidney alone shows a somewhat higher concentration which may be considered as a selective uptake by the pulmonary and mediastinal lymphatic system.

3) $P^{32}$ -TSPA transbronchially administered group

Triethylene thio- phosphoramide (Thio-TEPA or TSPA) was administered transbronchially to 6 dogs. This is one of the alkylating agents and its phosphor is labelled with $P^{32}$ ($P^{32}$ -TSPA). The $P^{32}$ -TSPA used here was synthesized by the SUMITOMO Atomic Energy Kogyo Co. $P^{32}$ -TSPA, corresponding to 600 $\mu$c of $P^{32}$ and 20 mg of Thio-TEPA, was made into a polyethylene glycol solution to which 3 ml saline solution was added. This was administered transbronchially into 3 dogs by instillation while the remaining 3 dogs were administered with the nebulized inhalation method.

Table 2. Tissue Distribution of Radioactivity after Transbronchial Administration of $Au^{198}$ Colloidal Solution (2 mc)

Counts/min./g

| Dog No, | 31 | 32 | 33 | 34 | 36 | 37 |
|---|---|---|---|---|---|---|
| Way of Admin. | Instill. | Inhall. | Instill. | Inhall. | Instill. | Inhall. |
| Time interval | 3 days | 3 days | 4days | 4days | 5 days | 5 days |
| L.upper mediast.L.N. | 45,840 | 23,125 | —— | 19,664 | 38,625 | 10,205 |
| R.upper mediast.L.N. | 88,741 | 65,221 | 96,103 | 27,862 | 74,262 | 35,440 |
| L.paratracheal L.N. | 82,703 | - | 46,845 | 41,627 | - | 29,650 |
| R.paratracheal L.N. | - | 33,450 | 78,951 | 59,195 | 59,865 | 46,219 |
| Botallow's L.N. | 284,856 | 95,921 | 103,672 | 43,863 | 116,425 | 43,115 |
| Subcarinal L.N. | 463,277 | 113,543 | 425,884 | 88,217 | 279,187 | 68,249 |
| L.bronchial L.N. | | 45,326 | 75,918 | 31,258 | 21,056 | 51,445 |
| R.bronchial L.N. | 467,120 | 83,296 | 125,123 | 91,250 | 119,284 | 91,241 |
| R.upper lobe | 21,118 | 2,456 | 10,946 | 2,010 | 2,480 | 2,363 |
| R.middle lobe | 4,163 | 3,168 | 8,746 | 1,937 | 4,170 | 1,835 |
| R.caediac lobe | 2,837 | 2,763 | 3,456 | 2,166 | 1,515 | 1,962 |
| R.lower lobe | 21,595 | 4,865 | 9,487 | 4,236 | 7,650 | 3,096 |
| L.upper lobe | 5,689 | 1,962 | 6,542 | 1,748 | 3,303 | 2,480 |
| L.lingula | 3,130 | 1,118 | 3,116 | 1,960 | 1,862 | 1,107 |
| L.lower lobe | 1,698 | 2,563 | 4,027 | 2,010 | 3,949 | 1,644 |
| Liver | 1,010 | 865 | 960 | 1,040 | 2,187 | 450 |
| Spleen | 1,076 | 462 | 1,017 | 850 | 1,116 | 552 |
| Kidney | 6,137 | 3,569 | 4,458 | 3,817 | 5,680 | 3,583 |
| Bone marrow | 1,115 | 459 | 1,304 | 741 | 1,241 | 760 |

—— no specimen

A periodical assay of radioactivity in lymph and blood was made after administration of 600 $\mu$ c of $P^{32}$ -TSPA. The results are as seen in Fig. 4. While at an early period of 10 minutes after a comparatively high concentration is seen in the blood, a gradual decrease is seen. The $P^{32}$ activity in both left and right thoracic duct

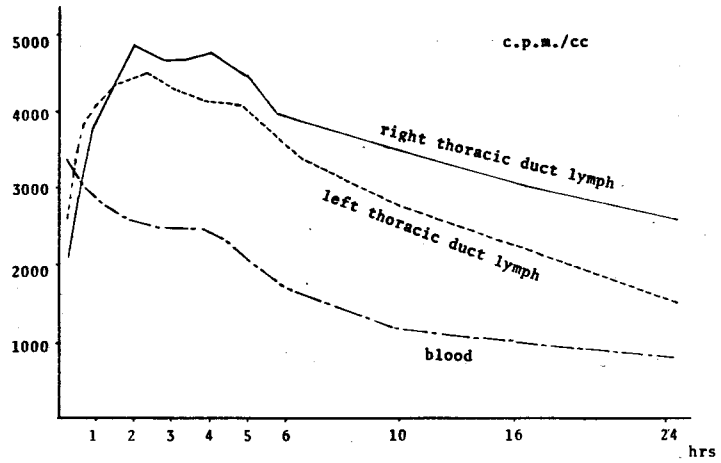


Fig. 4. Radioactivity of blood and lymph after transbronchial administration of $P^{32}$ -Thio-TEPA (600 $\mu$ c)

lymph shows a delayed rise compared with that of blood, it reaches its peak at 1 to 2 hours after and thereafter maintains a high concentration. Whereas the concentration in the right thoracic duct lymph shows higher values, the difference in concentration between the right and left thoracic duct lymph is not so large.

The distribution in the lymph nodes and organ tissue at 3 to 10 days after transbronchial administration of 600 $\mu$c of $P^{32}$ -TSPA is as shown in Table 3. No selective absorption by the pulmonary lymphatic system was seen and a considerable distribution in the liver, spleen, kidneys and bone marrow was evident. Likewise a considerable

Table 3. Tissue Distribution of Radioactivity after Transbronchial administration of $P^{32}$-Thio-TEPA (600 $\mu$ c)

Counts/min./g

| Dog No. | 51 | 53 | 54 | 55 | 56 | 57 |
|---|---|---|---|---|---|---|
| Way of Admin. | Instill. | Inhall. | Instill. | Inhall. | Instill. | Inhall. |
| Time interval | 3 days | 3 days | 5 days | 5 days | 10 days | 10 days |
| L.upper mediast.L.N. | ----- | 449 | ---- | 337 | ---- | 56 |
| R.upper mediast.L.N. | 386 | 560 | ---- | 555 | 215 | 186 |
| L.paratracheal L.N. | 339 | --- | 290 | 237 | 112 | 245 |
| R.paratracheal L.N. | 526 | 450 | 392 | 501 | 187 | 183 |
| Botallow's L.N. | 925 | 788 | 916 | 726 | 423 | 240 |
| Subcarinal L.N. | 1,871 | 800 | 1,156 | 920 | 825 | 459 |
| L. bronchial L.N. | 856 | 380 | 530 | 285 | --- | 111 |
| R. bronchial L.N. | 1,123 | 496 | 1,013 | 521 | 521 | 180 |
| R. upper lobe | 926 | 435 | 635 | 315 | 122 | 71 |
| R. middle lobe | 901 | 557 | 527 | 289 | 91 | 105 |
| R. cardiac lobe | 725 | 550 | 436 | 330 | 231 | 80 |
| R. lower lobe | 1,021 | 335 | 863 | 321 | 178 | 121 |
| L. upper lobe | 817 | 535 | 614 | 329 | 364 | 85 |
| L. lingula | 1,212 | 411 | 578 | 276 | 216 | 91 |
| L. lower lobe | 1,192 | 408 | 1,020 | 359 | 403 | 93 |
| Liver | 2,015 | 1,573 | 1,543 | 1,213 | 627 | 314 |
| Spleen | 1,721 | 902 | 702 | 708 | 611 | 206 |
| Kidney | 998 | 1,602 | 887 | 966 | --- | 280 |
| Bone marrow | 760 | 737 | 713 | 659 | 315 | 272 |

---- no specimen

transfer into the blood stream was seen. Viewed from the mode of administration, radioactivity was generally higher in the instillation method, and in the inhalation method since part of the agent is expelled out of the expiratory circuit as a mist with expiration, a slight lowering in the radioactivity is seen. With consideration to the expelled radioactive substance, care must be taken to cope with radioactive contamination. The distribution of $P^{32}$ activity at 5 to 10 days after

shows that as compared against other tissues the lymph node shows a tendency to maintain the activity for a comparatively long period (Table 3).

$P^{32}$ -TSPA in such a solution form, administered transbronchially, because of its small particle size, in addition to its absorption by the pulmonary lymphatic system, shows a high absorption by the blood via the peripheral vessels of the alveolar wall. Thus it was shown that the initial objective of transbronchial administration was not attained.

Further it is known that $P^{32}$ -TSPA in the body is metabolized rapidly and that TSPA changes into TEPA. Thus in the present assay of $P^{32}$ radioactivity, it may be assumed that in addition to $P^{32}$ -TSPA activity a liberated $P^{32}$ activity was measured together. However, analytical work in this direction was not done.

4) $Co^{60}$ -COPP transbronchially administered group

Based on the findings that liver catalase shows a decrease in tumor bearing bodies, Figge (1948) et al (10) studied on porphyrin which is the reacting base connected with catalase. And based on its affinity to liver and cancer tissue a new carcinostatica, cobalt-protoporphyrin (COPP) was developed. When this cobalt is labelled with $Co^{60}$, the resulting $Co^{60}$ -protoporphyrin ($Co^{60}$ -COPP) shows the following characteristic. With in the living body the cobalt resists separation and remains in the living body as a stable metallo-porphyrin. Thus, it is convinient for tracer work.

$Co^{60}$-COPP powder synthesized by the Japan Blood Bank Co. was disolved in a pH 7.4 phosphate buffer solution and was adjusted in such a way as to contain 4 $\mu$ c of $Co^{60}$ and 2 mg of COPP in 4 cc of the solution. This was instilled into the lobar bronchus of 3 dogs.

4 $\mu$c of $Co^{60}$-COPP was administered transbronchially and the gamma activity in the lymph and blood was assayed. As shown in Fig. 5 , the activity was extemely low in both lymph and blood with

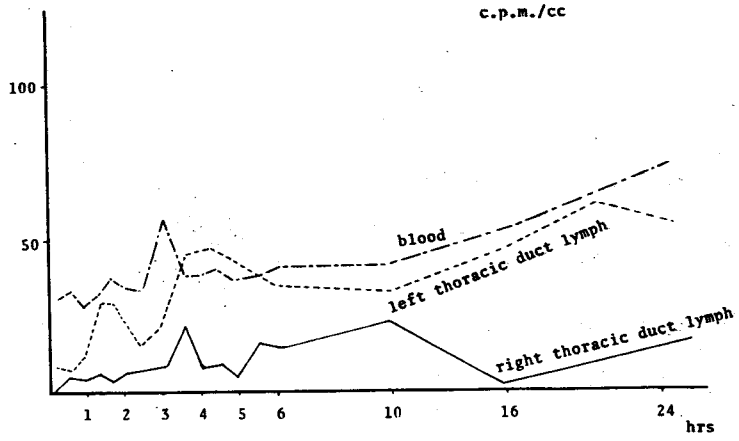


Fig. 5. Radioactivity of blood and lymph after transbronchial administration of $Co^{60}$-protoporphyrin ( 4 $\mu$c)

especially low figures seen in the right thoracic duct lymph. While this may be due to the limited $Co^{60}$ radioactivity of $Co^{60}$-COPP

however as shown in Table 4 at 1 to 5 days after transbronchial administration, the distribution in lymph nodes and organ tissue shows a high concentration of radioactivity in the hilar and mediastinal lymph nodes together with a high concentration of radioactivity in the

Table 4. Tissue Distribution of Radioactivity after Transbronchial Administration of $Co^{66}$-Protoporphyrin (4 $\mu$ c)

| | Counts/min./g | | |
|---|---|---|---|
| Dog No. | 71 | 72 | 73 |
| Lobe of Instill. | L.upper lobe | L. lower lobe | R.lower lobe |
| Time interval | 1 day | 3 days | 5 days |
| L.upper mediast.L.N. | 2,720 | 3,088 | 2,824 |
| R.upper mediast.L.N. | 2,666 | 30,066 | 51,920 |
| L. paratracheal L.N. | 1,856 | 63,350 | 12,346 |
| R. paratracheal L.N. | 2,360 | 19,350 | 35,291 |
| Botallow's L.N. | 6,076 | 82,108 | 7,496 |
| Subcarinal L.N. | 8,857 | 4,234 | 93,256 |
| L. bronchial L.N. | ----- | ----- | 130 |
| R. bronchial L.N. | 2,700 | 0 | 123,485 |
| R. upper lobe | 37 | 320 | 320 |
| R. middle lobe | 102 | 21 | 248 |
| R. cardiac lobe | 0 | 0 | 340 |
| R. lower lobe | 20 | 50 | 429* |
| L. upper lobe | 0 | 570,910* | 58 |
| L. lingula | 44 | 12,131 | 30 |
| L. lower lobe | 37,064* | 160 | 152 |
| Liver | 167 | 767 | 25,887 |
| Spleen | 51 | 148 | 63 |
| Kidney | 129 | 533 | 286 |
| Bone marrow | 48 | 8 | 38 |

* instilled lobe

---- no specimen

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parenchym of the instilled lobe. In contrast, in other lobes and other organs hardly any radioactivity was seen with a small amount in the liver. This may be because the small amount leaking into the blood flow is exereted into the bile from the liver. Based on these facts it may be said that owing to the large particle size of $Co^{60}$-COPP a transport into the blood via the alveolar capillary is extremely small. Thus the majority of the particles remain in the lung parenchym and owing to the phagocytosis of lymphatic tissue of the bronchial wall the particles are gradually transported towards the hilar and mediastinal lymph nodes. Thus it may be surmized that the particles are filtered here and settle in the nodes.

Aside from the carcinostatic effect of the agent, this distribution of the agent in the body is in line with the initial objective of the present work. However, in the present study due to the insufficiency of agents available, sufficient evidence was lacking. It is hoped that further work along this line will give better results.

## Discussion

Inhalation therapy of agents for respiratory diseases have been in long use. Especially in the case of special diseases such as bronchial asthma this therapy is in actual use and is known to have considerable effect.

From the physiological point of view the respiratory way as an administrative route is not favorable inasmuch as, with the exception of certain non irritable gaseous substances or mists, foreign particles are coughed out by the living body defense mechanism. Compared with other administrative routes such as oral, intravenous, subcutaneous, intramuscular administration or enteroclysis, the respiratory may has this as a disadvantage which may be the cause of its not being in common use.

That the healthy lung has an absorbing effect has been pointed out long ago by Claude Bernard (1857) (4). In 1877 Nothnagel (20), in 1878 Ruppert (23) showed experimentally that carbon particles and erythrocytes inhaled into the respiratory way are carried to the hilar lymph nodes via the bronchial endothel. As to the course taken by such substances inhaled into the respiratory pathway various work was done by Lubenau (1924) (16), Carleton (1924) (5), Gross (1927) (13) and others. Thus it was gradually clarified that the course taken by these substances through the respiratory pathway, via the peribronchial lymphatic tissue, being ever absorbed lymphward finally arrives at the hilar lymph nodes Fig. 6. Still later, it was shown that the route of absorption of substances through the bronchial wall differs with the individual size of the particles. Two routes were established, one through the alveolar wall directly into the alveolar capillary and the other via the bronchial endothel, moving ever lymphward through the bronchial wall lymphatic tissue and finally arriving at the hilar

lymph nodes. The small size particles follow the former route while comparatively larger particles pass through the latter route. This was reported by Drinker et al (1941) (7), Umeda (1939) (26) and Nishikawa (1940) (19).

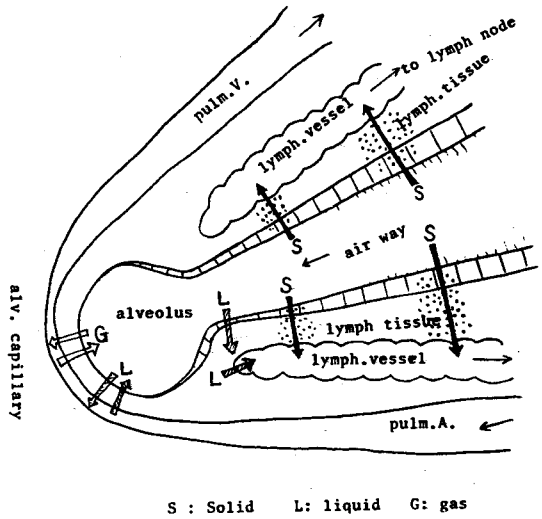


Fig. 6. Absorption of intrabronchial substance

On the other hand clinically from a point of view of administrating chemical agents directly or selectively into the affected lung transbronchial mist administration was developed which the advances in chemotherapy became widely used in the administration of antibiotics for pulmonary tuberculosis, pulmonary gangrene or bronchitis. Numerous work on this point was done by such workers as Lammer

and Herol (1951) (15), Gaensler et al (1949) (11), Watanabe (1953) (29) and Abramson (1950) (1).

However, among the lung diseases with special regards to the therapy of lung cancer which have been showing a rapid increase in various countries no attempts have been made to develop transbronchial treatment. The only work in this direction was done by Meneely (1951) (17), Hahn (1951) (14) and Berg (1951) (3). These three groups, from a standpoint of irradiation therapy, and with the intent of conducting local irradiation to the lung, administered radioisotope therapeutic agents transbronchially on an experimental basis.

In the present paper the authors, from an entirely different point of view, utilizing the characteristics of the absorbing mechanism of substances via the broncial wall described above, attempted a selective local administration into the pulmonary lymphatic system of carcinostatica for lung cancer.

In other words, with the intent of preventing lymphatic metastasis which has a strong bearing in postoperative lung cancer, the authors attempted to administer a high concentration of carcinostatic agents selectively into the pulmonary and mediastinal lymph nodes transbronchially. When the particle size of the transbronchially administered agent is large it is surmized that the bronchial absorption of the substance is conducted via the bronchial endothel into the peribronchial lymphatic tissue and is transported through he hilar and the mediastinal lymph nodes.

Therefore, the true objective of transbronchial administration of carcinostatic agents for lung cancer, is not in the administration to the lung parenchym or tumor tissue itself but is in the prevention or therapy of lymph node metastasis or a selective administration to the pulmonary lymphatic system. As for the tumor tissue itself it is understood that the tumor is to be removed surgically.

Therefore, in this experimental work, attention was centered on the achievement of the objective described above and experiments were conducted to determine the substance and the mode of administration by which the objective may be attained.

To determine the distribution of the agents in the pulmonary lymphatic system, thoracic duct lymph fluid was collected by bilateral thoracic duct cannulations (9, 27) and the radioactivity in the pulmonary and mediastinal lymph nodes were assayed. These were compared against the concentration in the blood and other organs.

In the case of $P^{32}$ tracer, when such a low molecular weight substance is given in the form of solution, a considerable amount passes through the alveolar wall capillary and is directly absorbed by the blood. Thus, there is no essential difference between intravenous administration except that the former method shows a higher distribution in the pulmonary field.

Concerning $Au^{198}$, colloidal solution administered transbronchially, experiments have already been done by Meneely (17), Hahn (14), Berg (3) in 1951 for the purpose of local irradiation. As compared

with $P^{32}$, $Au^{198}$ has a higher molecular weight, a larger particle size and further because it is a colloidal solution, transfer into the blood is limited and moreover since $Au^{198}$ has an affinity to the reticuloendothelial system and to the lymphatic system, in our present work it was reascertained that an extreme concentration was seen in the pulmonary lymph fluid and lymph nodes. Thus, it meets with the requirements of the primary objective. However, inasmuch as a radioisotope itself is used, some problems such as damage to the pulmonary tissue or administrative conditions must be cared in its clinical application.

Among known carcinostatica with special regards to $P^{32}$-TSPA and $Co^{60}$-COPP used in our present work, the distribution in the body after administration was traced respectively by their radioactivity. It was impossible to obtain an accurate $P^{32}$-TSPA distribution in the body because the analysis of the assayed $P^{32}$ radioactivity was not done whether it came from liberated $P^{32}$ or from $P^{32}$-TSPA itself. However, the approximate trend of distribution was almost the same as that of $P^{32}$ phosphate solution. The molecular weight was 189.22 and in a saline solution the leakage into the blood was considerable rendering it impossible for local administration.

The molecular weight of $Co^{60}$-COPP is 620, and at the time of purification the original powder had a particle distribution size of 1 - 200$\mu$ or thereabouts. In a solution from since it could not pass a cellophane membrane it may be considered to be 0.05 - 50$\mu$ in

particle size. Judging from this, it may be surmized that $Co^{60}$-COPP instead of passing the alveolar wall capillaries, is transfered via the bronchial endothel and is entrapped by the bronchial wall lymphatic system from which it passes into the lymph flow and is settled in the hilar and mediastinal lymph nodes. Thus, these tissues alone show a high intensity resulting in a minimal leakage to the entire body. In this case it was noted that almost no activity was seen in the right thoracic duct lymph which may be attributable to the large particle size and may be explained to be a result of being filtered by the lymph node and thus did not appear in the thoracic duct lymph. This, to us is an ideal situation.

From the above results, it seems clear that the particle size of the agents to be administered is the first condition to be met in order to have the agent selectively absorbed by the pulmonary and mediastinal lymph nodes after transbronchial administration, which is our primary objective.

In regard to this some previous work has been done. Abramson (1) (1950) reported that with special regard to the relation between particle size and the depth of its reach in the lungs, in the case of 30 $\mu$ particles the depth of reach was the bifurcation, in the case of 10 $\mu$ respiratory bronchus and in the case of 1 - 3 $\mu$ particles the depth of reach was the alveolus. Taplin (24) (1950) using rabbits conducted inhalation tests with 2 - 3 $\mu$ sized baliumsulphate particles. As a result of microscopic investigation, the particles

were found at the alveolar depth and in the case of 5 - 10 $\mu$ particle size he reported that these were seen only in the trachea and large bronchus.

A limit in particle size for the absorption by the bronchial wall exsists. Gillin et al (12) (1938) reported that the maximal particle size absorbable by the snake lung was 2 $\mu$ , Drinker et al (8) (1947) reported that as a result of instillation of pyrex glass particles 4 $\mu$ in diameter into dog bronchus, no absorption was seen.

Thus, in inhalation studies the obtaining of an even particle size becomes a problematic point. However, due to its difficulty various attempts have been made. Taplin et al (25) (1951) tagged B. subtilis spores with $P^{32}$ and conducted experiments. The spores are 0.5 - 1.5 $\mu$ in size and have a uniform size distribution.

At present in our team we are conducting experiments along this line using $I^{131}$ - aggregated albumin as a tracer in uniform particle size. In the heating process of its production, the desired particle sizes were produced for this purpose. These were administered transbronchially to check the distribution for each particle size in the body.

At the same time the various agents adsorbed by ion exchanger resin which has particles of 0.5 - 2$\mu$ in size is used for the same purpose. These experimental work is underway at present and will be purlished later.

The conditions to be met in the clinical application of transbronchial administration of carcinostatica are as follows:

1) The agent must have a uniform distribution size of 0.5 - 2 $\mu$ or thereabouts. Well soluble agents should be adsorbed by solid fine particles of this size and used.
In the case of larger size particles, they are used in suspension form.

2) The nebulizer apparatus should deliver a narrow range of size distribution.

3) The agent at the desired therapeutic dose, should be non irritating to the mucous membrane of the bronchus.

4) The agent should have a strong carcinostatic action and should be retained by the local lymph node for a considerable length of time and also have a long lasting effect.

In this administrative method the above points are to be carefully studied. It is expected that clinical application of this method will have a favorable bearing on the improvement of lung cancer therapy.

## Summary

Using adult mongrel dogs $P^{32}$ saline solution, $Au^{198}$ colloidal solution, $P^{32}$ -Thio-TEPA and $Co^{60}$ -Protoporphyrin were administered transbronchially by inhalation or instillation and an assay was conducted on the radioactive intensity of each agent in the right

and left thoracic duct lymph, blood, hilar and mediastinal lymph nodes and various other organs. The distribution of the agents in the body after administration was investigated.

1) In $P^{32}$ and $P^{32}$ -TSPA which have a small particle size, a larger amount was absorbed into the blood through the alveolar wall capillaries and selective administration to the pulmonary lymphatic system was not achieved.

2) In $Au^{198}$ administration, because of its lymphatic affinity and because it is a colloidal solution, the agent appeared specificially and at a high concentration in the pulmonary lymph system.

3) Since the particle size of $Co^{60}$-COPP is large the absorbing into the blood via the alveolar capillaries was extremely small and through the bronchial endothel a high distribution into the hilar and mediastinal lymph nodes was seen.

4) Based on these facts, it was suggested that it would be possible to selectively administer carcinostatic agents with an even particle size distribution transbronchially, to the pulmonary and mediastinal lymph nodes of clinical lung cancer patients and that an improvement in the therapy of lung cancer may be expected.

5) The problematic points in this mode of clinical administration which remain are the influence or side effects of the agents on the living body especially on the respiratory organs, together with the clinical indications for this administration.

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