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Quantitative Determination of Pulmonary Microembolism in the Dog

Comparison of In vivo and In vitro Methods

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ABSTRACT

External monitoring and in vitro measurement of pulmonary microembolism using ^{51}Cr-labelled platelets and ^{125}I-labelled fibrinogen were compared. The microenbolism was induced in dogs by intravenous infusion of thrombin and the radioactivities were recorded by an external detector as well as at intervals in biopsy material. External detection proved to give lower estimates of the amount of trapped radioactivity than in vitro measurements. This was particularly so for ^{51}Cr. Thus only about 2/13 of the in vitro amount of trapped ^{51}Cr and about 1/3 of the ^{125}I radioactivity were revealed by external detection. External detection, however, records the changes continuously and therefore offers advantages over the biopsy technique, which requires open chest surgery and artificial respiration with consequent alterations in intrathoracic pressure and circulatory conditions.

INTRODUCTION

In previous communications from this laboratory, methods for the quantitation of intravascular platelet and fibrin deposition in various organs of rats, rabbits, dogs and humans have been presented (3, 4, 5, 6, 7, 12, 13). Isotope-labelled platelets and fibrinogen were injected in advance and the organ deposition following intravenous infusion of thrombin was measured *in vivo* by external detectors or *in vitro* by radioactivity measurements in tissue specimens.

The *in vivo* technique was used for continuous registration of deposition of fibrin and platelets in, and their elimination from, the lung after intravenous (i.v.) infusion of thrombin in normal dogs with inhibited fibrinolysis. The method proved to be very useful in elucidating the dynamics of pulmonary microembolism, thought it can be postulated that *in vitro* measurement of lung tissue radioactivity should give more accurate quantitative information.

The aim of the present study was to determine the quantitative differences between the *in vivo* and the *in vitro* techniques and hence to define the applicability of each of them. Thus, the pulmonary distribution of radioactivity before and after an i.v. infusion of thrombin into dogs given ^{99m}Tc-albumin, ^{51}Cr-platelets and ^{125}I-fibrinogen was studied by the two techniques.

MATERIAL AND METHODS

Animals. Twenty-four healthy German pointer dogs of both sexes, weighing 15–24 kg, were used.

Labelling of platelets. The day before the experiment autologous platelets were labelled with ^{51}Cr by a modification (4) of the method described by Aas & Gardner (1). Each dog was given a suspension of labelled platelets containing 15–40 μCi ^{51}Cr. by i.v. injection.

Labelled fibrinogen. Human fibrinogen prepared according to Blombäck & Blombäck (2) was used. The labelling was performed according to Rosa et al. (11). Each dog was given an i.v. injection of about 60 μCi of ^{125}I-labelled fibrinogen (5 μCi/mg fibrinogen) 20–40 min before commencement of the thrombin (or saline) infusion. In previous experiments no differences in the kinetics of human and dog fibrinogen were observed (4).

Labelled albumin. Human serum albumin labelled with ^{99m}Tc (AB Atomenergi, Studsvik) was used to determine the plasma volume per gram of lung tissue. Immediately after passage through an ion exchange column (Dowex 1-X8, 50–100 mesh) about 100 μCi was injected i.v. (1 mCi/mg). This was allowed to mix with the plasma volume for 5 min before the animals were killed (9).

Thrombin. Bovine thrombin (Topostasin®, Roche) dissolved in saline was used.

Blood samples were collected through a catheter inserted in the femoral artery (see below). One-ml portions were taken into small Ellermann plastic tubes for determination of radioactivity.

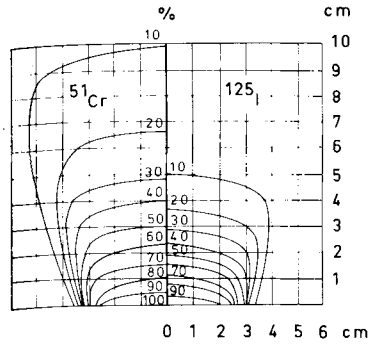


Fig. 1. Absorption of ^{51}Cr-radioactivity (*left*) and of ^{125}I (*right*) in water at various distances from the orifice of the collimator. Isocount lines are expressed as percentage of the counting rate at the orifice of the collimator.

Haematocrit (hct) was determined in triplicate in microhaematocrit tubes after centrifugation at 10 000 *g* for 5 min. No correction for trapped plasma was made.

Calculations. In both *in vivo* and *in vitro* methods the amount of radioactivity of each isotope in the lung (IL(t)) was calculated as

$$IL(t)=L(t)-C(t)$$

where
$L(t)$ is the counting rate over the lung (or the radioactivity per gram lung tissue) at time t and
$C(t)$ the amount of circulating radioactivity recorded by the detector (or in the lung tissue sample) at time t, estimated as

$$C(t)=\frac{L(o)}{P(o)}P(t)$$

where
$P(o)$ and $P(t)$ are the plasma radioactivities at times o and t, calculated from whole blood activities with the aid of the haematocrits.

To allow comparison of results from different experiments the following standardization was introduced:

$$IL(t)_{rel}=\frac{IL(t)}{L(o)}\cdot 100$$

Thus, the amount of radioactivity estimated with either of the described methods was expressed in per cent of the preinfusion radioactivity over (or in) the lung.

This calculation requires two assumptions, first that the pulmonary blood volume does not change during the experiment, and second that the extravascular radioactivity can be neglected. These assumptions are discussed later.

Morphological methods

Light microscopy. Specimens were taken from different parts of both lungs. Formalin fixed 5 μ thich paraffin sections were stained with haematoxylin-eosin and the Mallory PTAH method for demonstration of fibrin.

Radioautographs were prepared from sections adjacent to those used for light microscopy; they were dipped in Ilford G5 emulsion and the film was exposed for 3 weeks at 4°C. The sections were stained with haematoxylin-eosin.

Statistical methods

Mean values, standard deviations, linear regressions and correlation coefficients were calculated by conventional statistical methods. Differences between mean values were tested by means of Student's t-test.

General experimental procedure

The dogs were anaesthetized by intravenous administration of thiopental sodium (Pentohalsodium®, Abbott), 10 mg per kg body weight (b.w.). A cuffed endotracheal tube was inserted and the dogs were placed supine. A polyethylene catheter was advanced into the right atrium via the right external jugular vein and another was inserted in the femoral artery. In previous experiments the dogs were anaesthetized with α-chloralose dissolved in 10 ml of saline per kg b.w. (4) and to obtain comparative conditions in the present experiments each dog was given this amount of saline before the thrombin infusion (see below). In addition the dogs were pretreated i.v. with 100 mg per kg b.w. of tranexamic acid (AMCA, AB Kabi, Stockholm) to prevent fibrinolysis. An infusion of thrombin, 310 NIH units per kg b.w., was given in 30 min through the jugular vein catheter. The dogs submitted to a left-sided thoracotomy were given only 150 NIH units per kg b.w. because with the higher dose the mortality rate in open chest conditions is high.

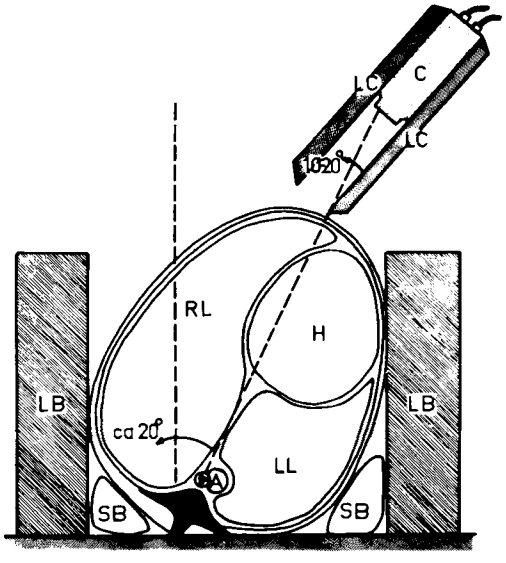


Fig. 2. Schematic diagram of the position of the dog's chest and of the detector. *LB*=lead bricks, *SB*=sand bags, *LL*=left lung, *RL*=right lung, *H*=heart, *LC*=lead collimator, *C*=NaI (Tl) crystal, *A*=aorta.

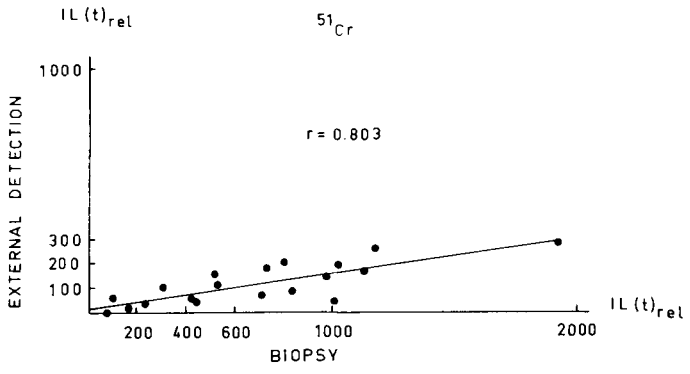


Fig. 3. Relationship between the estimates of the amount of ^{51}Cr-radioactivity trapped in the lungs as measured *in vivo* and *in vitro*.

Arterial blood samples were drawn at regular intervals for determination of radioactivity, plasma fibrinogen and platelet count.

Radioactivity measurements

Recording by external detector. The detector consisted of a 1″×1″ sodium iodine crystal with a photomultiplier (Harshaw) and a 24 mm lead cylindrical collimator with an inner diameter of 50 mm. The distance between the crystal and the collimator orifice was 63 mm. The sensitivity of ^{51}Cr- and ^{125}I-radioactivity in water at various distances from the front of the collimator was measured using a point source of radioactivity, giving the isoresponse curve as shown in Fig. 1.

With this detector the radioactivity was monitored continuously over the right lung as described earlier (4, 5). The dogs were kept immobile by lead bricks. The detector was placed immediately cranial to the edge of the intercostal angel and immediately to the right of the midline and was directed 10–20° laterally to avoid disturbance from the heart and major vessels. The chest was also rotated about 20° to the left to shift the mediastinal structures from the area used for detection (Fig. 2). The signals from the detector were passed to a linear amplifier, analysed by a discriminator and recorded by a scaler (Canberra Industries, Connecticut, USA).

Two or three channels were used simultaneously and set for ^{125}I, ^{99m}Tc and ^{51}Cr respectively. Normally more than 400 counts per 100 seconds were recorded. The background was less than 1/6 of this value. The counting rates in each channel was corrected for overlapping between the channels and for background. For ^{99m}Tc correction was also made for decay; this was not done for ^{125}I and ^{51}Cr, with their long halflives (60 and 28 days respectively).

At autopsy the position of the organs in relation to the detector was checked.

Radioactivity in tissue and blood samples was measured in a gamma counting system (Gammatrix C, Stockholm) with a well crystal and three windows set for ^{99m}Tc, ^{51}Cr and ^{125}I. Normally more than 2000 counts were registered in each channel. The background was usually less than 1/10 of the total counting rate. Corrections for overlapping between the channels, for decay of ^{99m}Tc and for background were made as described above.

Recording by gamma camera.[1] One dog was placed as described above (supine with the thorax rotated

[1] This part of the investigation was performed with the kind and skilful assistance of K.-J. Vikterlöf and K.-W. Beckman, Department of Radiophysics, Regional Hospital, Örebro, Sweden.

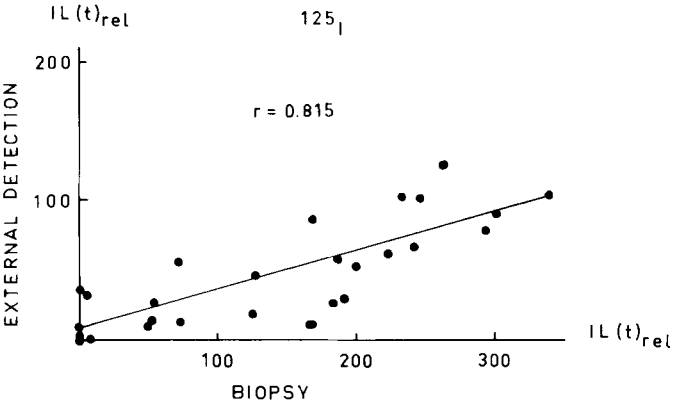
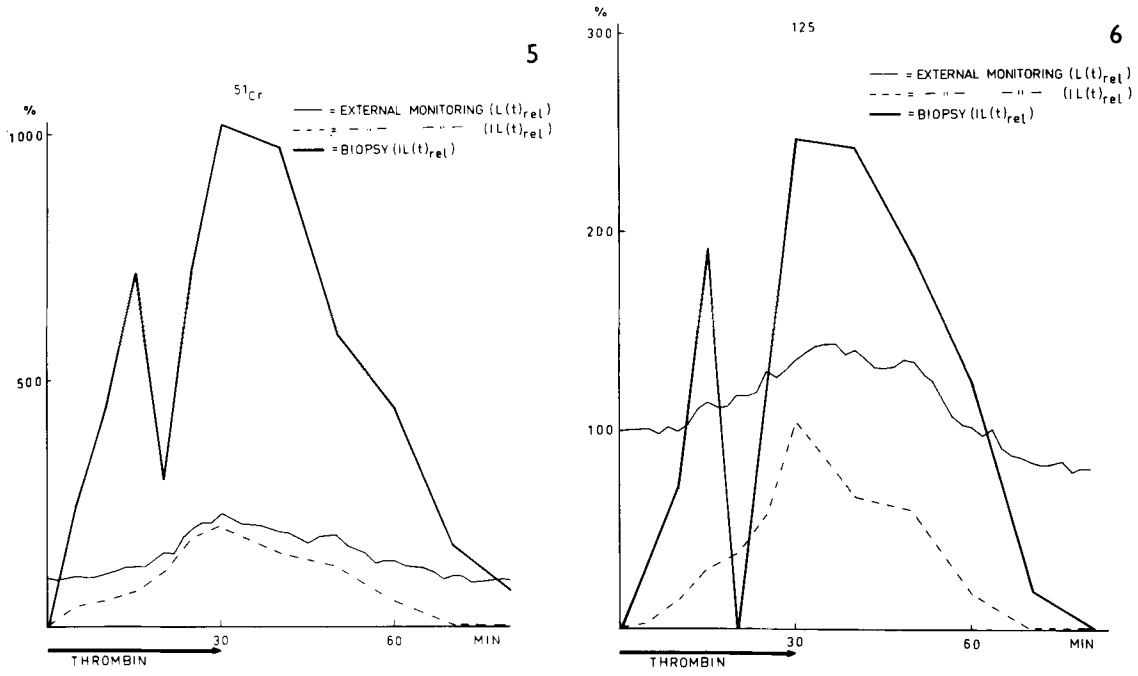


Fig. 4. Relationship between the estimates of the amount of ^{125}I-radioactivity trapped in the lungs as measured *in vivo* and *in vitro*.



Figs. 5 and 6. Pulmonary deposition of ^{51}Cr (Fig. 5) and ^{125}I (Fig. 6) $(IL(t)_{rel})$ as measured *in vitro* (—) and *in vivo* (– – –) as well as the percentual changes recorded by the detector without correction for plasma decrease of the radioactivity (——).

about 20° to the left) and was given about 150 μCi of ^{131}I-labelled human fibrinogen i.v. A 1000-hole, divergent collimator was placed over the thorax in the same direction as the detector (described above).

The radioactivity distribution was recorded by a gamma camera (Selektronik, Denmark). The information was stored, treated and presented by an on-line computer system (NUCAB 2530) incorporating a PDP 8/e computer with 12 k core memory and a cartridge memory for storing programme and pictorial information. The activity distribution was registered as consecutive 64×64 matrices during 100-second intervals. The entire right lung, the base of the same lung and the heart were selected as "regions of interest" and the radioactivity of these regions detected during the 100-second intervals was listed digitally and analogously plotted by the computer.

EXPERIMENTS AND RESULTS

I. Comparison of in vivo and in vitro determination of pulmonary microembolism

A. Six dogs were given ^{51}Cr-labelled platelets and ^{125}I-labelled fibrinogen as described. They were ventilated by an Engström respirator and a left-sided thoracotomy was performed so as to expose the base of the left lung for biopsies. The radioactivity over the right lung was recorded continuously and after administration af AMCA the thrombin was infused in 30 min. Small biopsies (0.3–1.0 g) were taken repeatedly at intervals of 5–10 min during the infusion and in one dog for a further 50 min. The biopsies were taken from the peripheral parts of the lower lobe with the aid of vessel clamps and ligatures. The samples were weighed and the radioactivities determined.

As can be seen in Figs. 3 and 4, an almost linear relationship between the estimates of trapped radioactivity of both ^{51}Cr and ^{125}I was obtained. The correlation coefficient was 0.803 for ^{51}Cr ($n=19$) and 0.815 ($n=27$) for ^{125}I. The uptake registered by external detection was considerably lower than that measured *in vivo*. Thus, for ^{51}Cr, the *in vitro* values were about 6.5 times higher than the *in vivo* estimates for ^{125}I about times higher.

In Figs. 5 and 6 the estimates of pulmonary deposition of ^{51}Cr and ^{125}I $(IL(t)_{rel})$ in one of the dogs as measured *in vitro* and *in vivo*, as well as the percentual changes recorded by the external detector without correction for decrease of plasma radioactivity, are plotted against time. The quanti-

Table I. *The ratios between the ^{51}Cr-radioactivity per gram tissue at the end of the thrombin infusion and that per gram plasma before the infusion (mean ± S.D.)*

Tissue	Dog no. 1	2	3	4	5	No. of samples
Skin	0.064±0.005	0.038±0.010	0.007±0.003	0.052±0.014		5
Skeletal muscle	0.014±0.003	0.040±0.016	0.001±0.007	0.008±0.007		5
Bone-cartilage	0.153±0.021	0.092±0.022	0.065±0.012	0.162±0.053		5
Lung, ventral part	6.559±1.086	3.885±0.615	5.759±0.335	2.163±0.195	3.291±0.955	15
Lung, dorsal part	5.138±1.347	3.692±0.495	5.618±0.394	2.135±0.283	3.576±1.117	15

tative differences between the techniques are shown, as well as the unexplained deviation of the amount of label in the tissue specimens.

B. Five dogs were treated in the same way as those under "A" except that they were allowed to breathe spontaneously and a higher dosage of thrombin (310 NIH units per kg b.w.) was used. After completion of the thrombin infusion the dogs were killed with an overdose of thiopental sodium. Small pieces (0.3–1.0 g) were taken from the ventral and the dorsal parts of the right lung (15 samples from each region) as well as from the skin, skeletal muscle (intercostal) and bone and cartilage from the ribs (5 samples from each tissue). Samples were also taken from different parts of the lung for morphological studies. The radioactivities of the tissue and corresponding plasma samples were determined. The ratio between tissue radioactivity per gram and the pre-infusion plasma activity per gram was calculated for each sample.

The radioactivity of both isotopes was seen to be evenly distributed in different parts of the lung (Tables I and II) and the microemboli were found to be localized in small pulmonary arterioles and capillaries (Fig. 7 and 8). No emboli were found in the large vessels or in the heart at autopsy. The amount of radioactivity in the thoracic wall tissues did not increase during the thrombin infusion (Tables I and II).

II. *Distribution of ^{99m}Tc-albumin, ^{51}Cr-platelets and ^{125}I-fibrinogen before the thrombin infusion*

Three dogs given ^{51}Cr-platelets and ^{125}I-fibrinogen were injected i.v. with 100 μCi ^{99m}Tc-albumin. Ionogenic ^{99m}Tc was removed by an ion exchange column (Dowex 1-X8, 50–100 mesh) immediately before the infusion. After 5-min mixing, which was followed by the external detector, the dogs were killed with an overdose of thiopental sodium. At this time the ^{99m}Tc-albumin should have been distributed homogeneously in the vascular compartment (9).

The lungs were removed and 15 small pieces from the ventral and 15 from the dorsal parts of the right lung were transferred into plastic tubes after gentle drying on filter paper. Furhermore, tissue samples from the thoracic wall (0.5–1.0 g), liver, kidney and spleen were collected analogously with those mentioned above, and the radioactivity was measured. The content of each label per gram was determined in the tissue specimens and in plasma.

Table II. *The ratio between the ^{125}I-radioactivity per gram tissue at the end of the thrombin infusion and that per gram plasma immediately before the thrombin infusion (mean ± S.D.)*

Tissue	Dog no. 1	2	3	4	5	No. of samples
Skin	0.018±0.001	0.014±0.002	0.011±0.002		0.043±0.004	5
Skeletal muscle	0.014±0.001	0.015±0.002	0.011±0.001		0.025±0.002	5
Bone-cartilage	0.059±0.013	0.030±0.009	0.044±0.004		0.019±0.010	5
Lung, ventral part	2.089±0.284	1.115±0.176	1.499±0.170	2.651±1.402	1.326±0.220	15
Lung, dorsal part	1.783±0.326	1.141±0.151	1.346±0.362	2.926±0.965	1.574±0.242	15

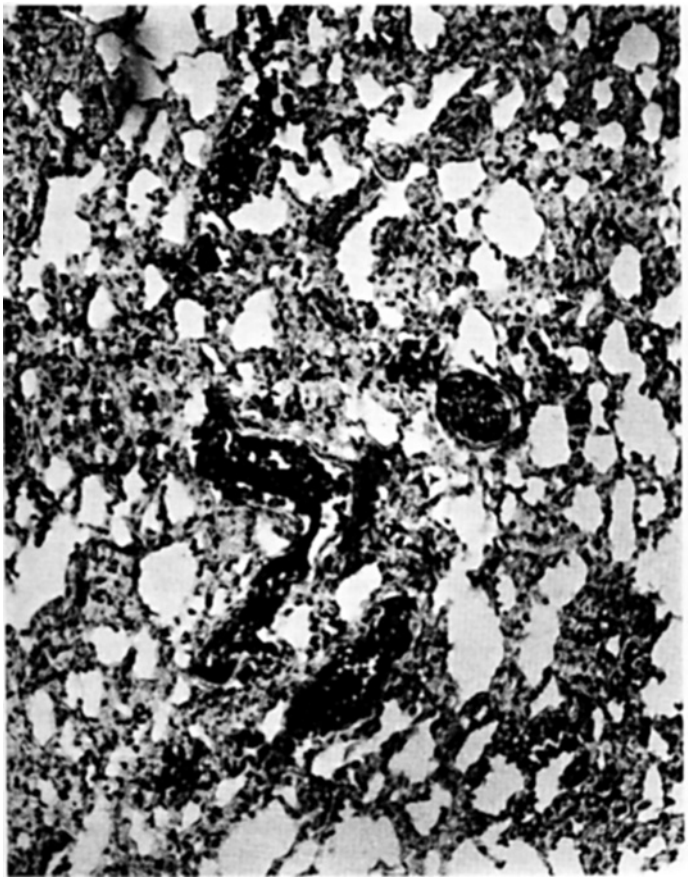


Fig. 7. Fibrin microemboli in pulmonary arterioles and capillaries (PTAH, ×150).

For none of the labels did the tissue/plasma ratios show a significant difference between different parts of the lung (Tables III–V). The ratio for ^{125}I were lower than those of ^{99m}Tc in the lungs, liver and kidneys. The ^{51}Cr ratios were higher than those for ^{125}I and ^{99m}Tc in the spleen, liver and bone-cartilage as well as in the lung (Table III).

III. *Statistical evaluation of in vivo external detection of pulmonary microembolism*

A. Three dogs[1] were injected with ^{51}Cr-labelled platelets and ^{125}I-labelled fibrinogen as in previous experiments. After an injection of 20 ml saline thrombin was infused as before into the jugular vein in 30 min (310 NIH units per kg b.w.). External detection was carried out over right lung for a further 5 1/2 hours. During this time the animals were given another 50 ml of saline.

B. Five dogs[1] were injected with AMCA 100 mg per kg b.w. about 15 min before an infusion of thrombin identical to that above. After the 30-min infusion another 100 mg of AMCA per kg b.w. dissolved in 50 ml of saline was given over 5 1/2 hours. The radioactivity was monitored as in group A.

The amount of ^{51}Cr and ^{125}I radioactivity trapped in the lung as measured by the *in vivo* method ($IL(t)_{rel}$) was plotted against time (Figs. 9 and 10).

C. Two dogs[2] were given 20 ml saline and another two dogs 50 mg AMCA per kg b.w. as a single, rapid i.v. injection. ^{125}I-fibrinogen was also given in advance and external detection was then performed as described (Fig. 11).

One of the main purposes of the *in vivo* method was to measure differences in the rate of elimination of the labelled microemboli from the

[1] The results of these experiments have been presented in detail elsewhere (4).

[2] The results of these experiments have been presented in detail elsewhere (5).

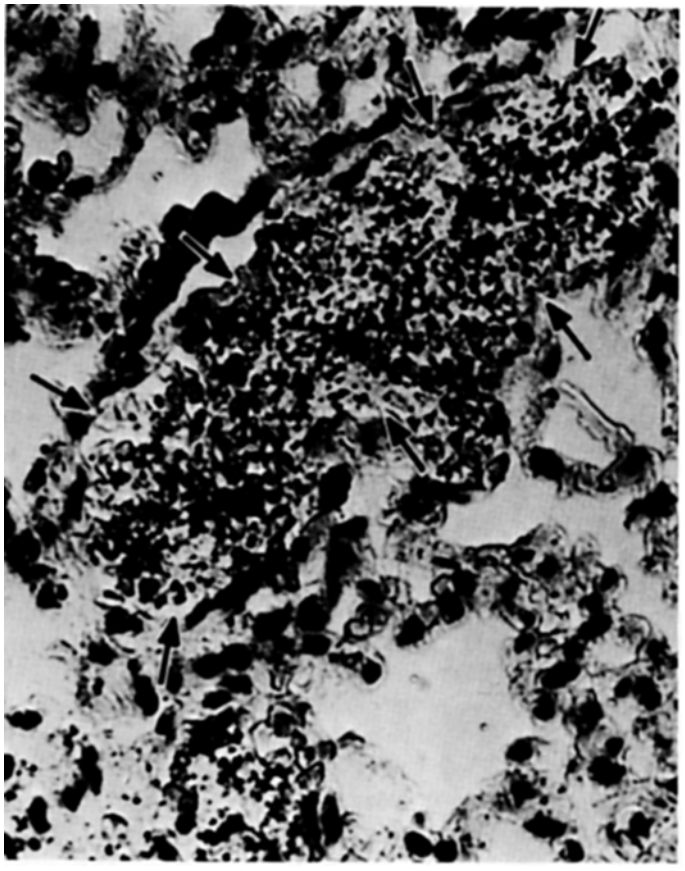


Fig. 8. Radioautograph of microemboli (within arrows) in small pulmonary vessels (haematoxylin-eosin, ×300).

lung. In Figs. 9 and 10 it is visually evident that AMCA treatment delayed the elimination of both ^{125}I-fibrin and ^{51}Cr-platelets. This effect was most pronounced from one to 3 hours after completion of the thrombin infusion. The tails of the curves show high coefficients of variation and/or low numbers of observation.

Fig. 11 shows that even a lower dose of AMCA than was used in previous experiments and given as a single rapid injection caused retardation of the elimination as compared with dogs with normal fibrinolysis. In dogs given 200 mg AMCA per kg b.w. the pre-infusion counting rate was usually not regained during the 6-hour observation period.

Table III. *The ratios between ^{51}Cr-radioactivity per gram tissue and per gram plasma before the thrombin infusion (mean ± S.D.)*

Tissue	Dog no. 1	2	3	No. of samples
Skin	0.031±0.021	0.014±0.007	0.017±0.003	5
Skeletal muscle	0.031±0.014	0.015±0.003	0.013±0.002	5
Bone-cartilage	0.195±0.160	0.046±0.018	0.064±0.023	5
Liver	3.924±0.643	0.697±0.006	0.563±0.018	2
Kidney	0.262±0.019	0.174±0.013	0.134±0.019	2
Spleen	25.074	12.364	17.823	1
Lung, ventral part	0.491±0.193	0.191±0.038	0.262±0.046	15
Lung, dorsal part	0.660±0.405	0.163±0.050	0.225±0.023	15

Table IV. *The ratios between ^{125}I-radioactivity per gram tissue and per gram plasma before the thrombin infusion (mean ±S.D.)*

Tissue	Dog no. 1	2	3	No. of samples
Skin	0.025±0.002	0.020±0.004	0.023±0.002	5
Skeletal muscle	0.015±0.003	0.016±0.003	0.018±0.002	5
Bone-cartilage	0.027±0.001	0.031±0.002	0.051±0.012	5
Liver	0.137±0.001	0.108±0.001	0.191±0.014	2
Kidney	0.176±0.013	0.226±0.004	0.162±0.011	2
Spleen	0.097	0.170	0.157	1
Lung, ventral part	0.160±0.024	0.115±0.011	0.113±0.013	15
Lung, dorsal part	0.156±0.022	0.115±0.019	0.115±0.013	15

Differences in the elimination rate can also be expressed as differences in the percentual amount of the trapped radioactivity at various time points after the maximum value. Such figures ($IL(t)_{rel}$/$IL(0.5)_{rel}$) for groups A and B are shown in Table VI.

Thus, significant differences in the ratios were found at 60, 90 and 120 min after commencement of the thrombin infusion for both isotopes, while the tail values did not differ significantly.

IV. *Recording by gamma camera*

One dog was given 150 μCi ^{131}I-fibrinogen and a 30-min thrombin infusion. After about 1 hour's registration of the radioactivity changes about 1 mCi of ^{99m}Tc-labelled macroaggregated human serum albumin (AB Atomenergi, Studsvik) was injected into the jugular vein catheter so that the contours of the lungs and heart were visualized. The entire right lung, the base of the same lung and the heart were taken as "regions of interest" and the radioactivity recorded in these regions during the 100-second intervals was listed digitally and plotted analogously by the computer.

The results are given in Fig. 12. It is evident that the gamma camera technique was less efficient in revealing the true increase of pulmonary radioactivity than the lead collimated detector placed over a smaller field of the right lung. The pictures also show that the shifting of the chest to the left also shifts the mediastinal structures, thus giving a large free field for detection at the base of the right lung.

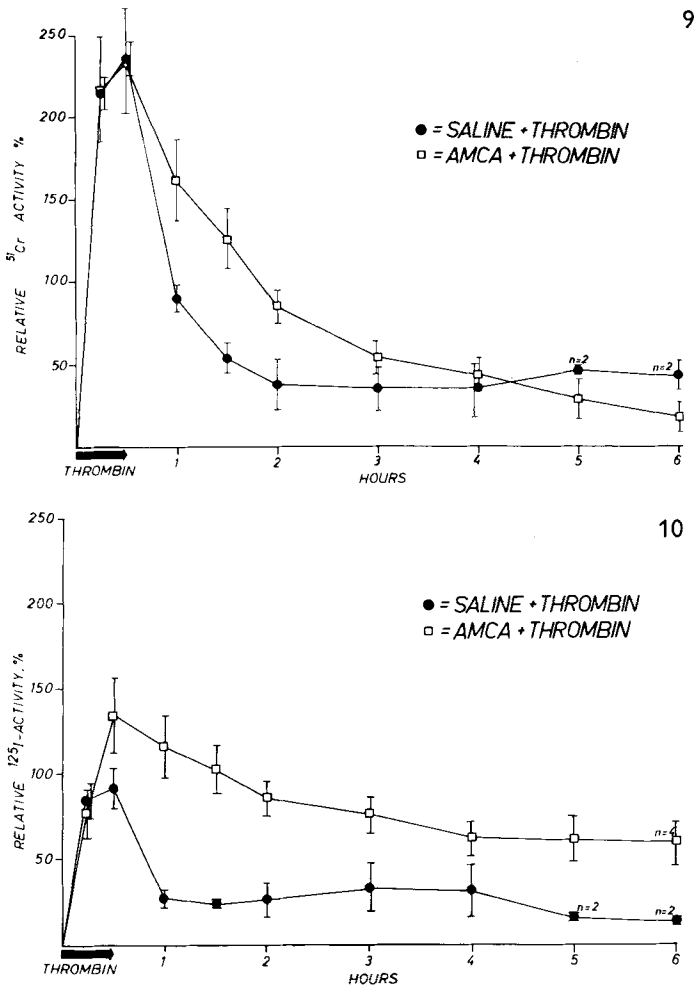
DISCUSSION

The present study has shown that quantitative determination of labelled pulmonary microemboli by external detection in dogs gives lower estimates of the trapped radioactivity than *in vitro* measurement.

The differences are probably due to the fact that the radioactivities measured emanate from

Table V. *The ratios between ^{99m}Tc-radioactivity per gram tissue and per gram plasma before the thrombin infusion (mean ±S.D.)*

Tissue	Dog no. 1	2	3	No. of samples
Skin	0.018±0.003	0.041±0.018	0.022±0.002	5
Skeletal muscle	0.015±0.004	0.021±0.005	0.019±0.001	5
Bone-cartilage	0.032±0.014	0.040±0.004	0.056±0.012	5
Liver	0.127±0.002	0.127±0.001	0.217±0.013	2
Kidney	0.292±0.010	0.335±0.010	0.314±0.014	2
Spleen	0.070	0.149	–	1
Lung, ventral part	0.180±0.025	0.155±0.010	0.145±0.016	15
Lung, dorsal part	0.161±0.018	0.150±0.009	0.140±0.013	15



Figs. 9 and 10. Pulmonary deposition ($IL(t)_{rel}$) of ^{51}Cr-radioactivity *(above)* and ^{125}I-radioactivity *(below)* in dogs pretreated with AMCA, 200 mg per kg *b.w.* (□) and dogs with normal fibrinolysis (●).

different sources in the two techniques. Thus, the external detector records activity originating in all thoracic tissues, the limits and extent of the tissue field depending on the collimating properties of the detector and on the energy of the radioactive isotope employed (Fig. 1). The counting rate is an expression of the sum of radioactivity from the chest wall, peripheral lung tissue (where the deposition actually occurs), large vessels and perhaps also from the right heart (in spite of the preventive measures).

The biopsy specimens consist of peripheral lung tissue only, thus with no disturbance form the blood of large vessels. Thur the decrease of radioactivity in the blood of large vessels gives a smaller net accretion when measured with the external technique than when measured *in vitro* on tissue samples.

This difference was more pronounced for ^{51}Cr than for ^{125}I. Only about 2/13 of the *in vitro* estimate was recorded externally, as compared with about 1/3 for the ^{125}I. It is conceivable that the ^{51}Cr radioactivity with its higher gamma energy (0.320 MeV), emanates to a greater extent from the large central vessels than the radioactivity of ^{125}I (0.035 MeV) (Fig. 1).

The pulmonary microemboli were shown to be homogeneously distributed in the arterioles and capillaries, while at autopsy no emboli were found in the heart or large pulmonary arteries. Furthermore, no significant increments of the ^{51}Cr or ^{125}I radioactivities were observed in the various parts of the chest wall. Thus an uneven distribution of deposits in the tissues mentioned did not influence the measurements.

The related differences are also illustrated in

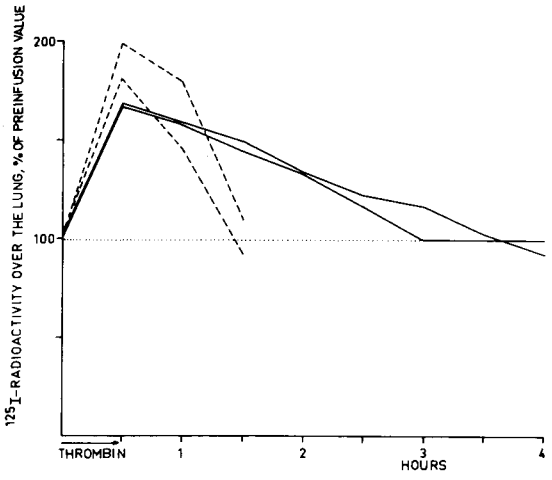


Fig. 11. Pulmonary uptake and elimination of ^{125}I in dogs pretreated with AMCA, 50 mg per kg b.w. (—) and in dogs pretreated with saline (- - -).

Figs. 7 and 8, where the estimates of pulmonary trapping of the radioactivities are presented as well as the changes recorded by the detector without correction for plasma decrease. In spite of the occasional variations in the tissue sample radioactivities the *in vitro* and *in vivo* methods are seen to describe the same time course, which further indicates that they reflect the same pathophysiological events.

The distribution of the different isotopes before the thrombin infusion in the present study revealed some interesting facts. Firstly, the tissue/plasma ratios for all isotopes were homogeneously distributed in the different parts of the lung. Secondly, the ratios of ^{99m}Tc were higher than those of ^{125}I in the lungs, kidneys and liver. This could be taken as an indication of extravascular leakage of small-molecular ^{99m}Tc-compounds in spite of the preventive ion exchange step. This in turn would indicate, though indirectly, that no substantial extravascular leakage of ^{125}I-fibrinogen had occurred. In rats given ^{125}I-fibrinogen 2 days before the experiment the amount of extravascular radioactivity in the lung was estimated to be about 30% (6). In the present experiments this amount should be considerably smaller, since only 20–40 min elapsed between the administration of the labelled fibrinogen and the thrombin infusion. A large amount of ^{51}Cr radioactivity was recovered in the spleen, liver and bone-cartilage. Furthermore, the lung/plasma ratio of ^{51}Cr was higher than that of the two other isotopes indicating that this isotope and/or labelled platelets or fragments of platelets are retained in these organs on re-infusion of the suspension of ^{51}Cr platelets. The accumulation of ^{51}Cr in the liver, spleen and bone might be due to the action of the reticuloendothelial system, while the accretion in the lung might indicate trapping of aggregates formed during the labelling procedure. These high lung/plasma ratios introduce an error in the estimation of the amount of circulating ^{51}Cr activity before the thrombin infusion ($P(t)_0$). Since, however, the tissue/plasma ratio after the thrombin infusion ($L(t)/P(t)_0$) was about 14 times that before the infusion ($L(t)_0/P(t)_0$), and the correction made for circulating radioactivity ($C(t)$ was rather small because of the decreased plasma radioactivity ($P(t)$), this error was neglected.

The increase in counting rate over the lung during the thrombin infusion might theoretically be due to an increase in the pulmonary plasma volume. In an earlier study using ^{125}I-labelled

Table VI. *The ratio between the amount of ^{51}Cr and ^{125}I trapped in the lungs at various time points after the thrombin infusion at the amount at the end of the infusion* ($IL(t)_{rel}/IL(0.5)_{rel}$ *(mean ±S.D.)*

Minutes after start of the thrombin infusion	^{125}I			^{51}Cr		
	Saline+ thrombin	AMCA+ thrombin		Saline+ thrombin	AMCA+ thrombin	
	$\bar{X}_3$±S.D.	$\bar{X}_4$±S.D.	*P*	$\bar{X}_3$±S.D.	$\bar{X}_4$±S.D.	*P*
60	0.327±0.122	0.869±0.046	<0.001	0.427±0.052	0.698±0.069	<0.01
90	0.274±0.082	0.781±0.066	<0.001	0.314±0.045	0.542±0.097	<0.02
120	0.312±0.146	0.672±0.099	<0.02	0.152±0.092	0.364±0.062	<0.05
180	0.397±0.200	0.597±0.123	Non-sign.	0.154±0.058	0.244±0.037	Non-sign.
240	0.379±0.245	0.574±0.133	Non-sign.	0.132±0.070	0.212±0.055	Non-sign.

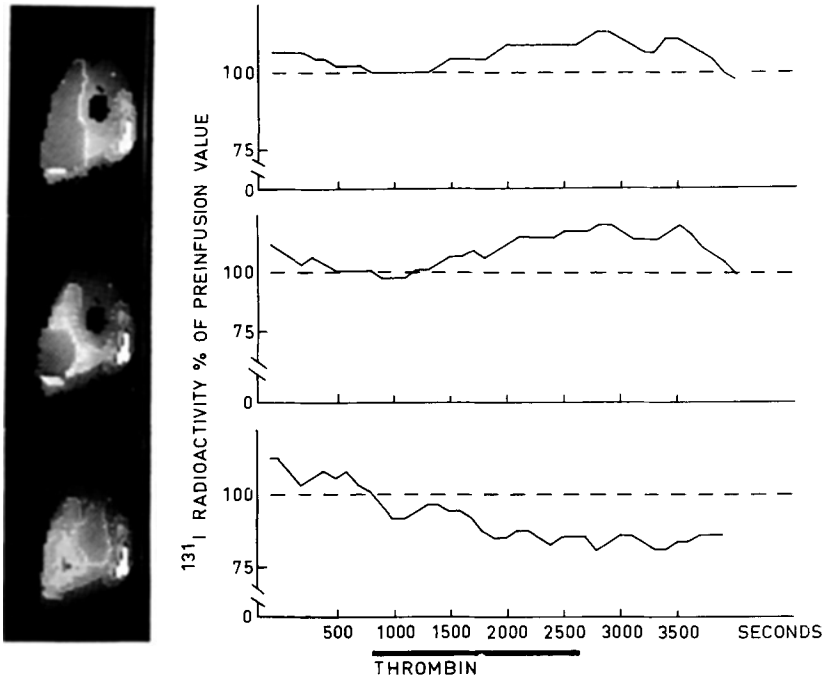


Fig. 12. Gamma camera pictures of the anatomical relationship in the dog's chest and the "regions of interest" (, left). The radioactivity changes in each region (% of preinfusion values) are shown to the right.

albumin and external detection, no such increment was observed, however (4).

The gamma camera system did not prove to be more sensitive than recording by the conventional detector. The former requires high doses of high energy radioisotopes for adequate discrimination and again the influence of decreasing plasma radioactivity, especially in the large vessels, must affect the recordings. This is supported by the fact that some improvement was obtained by selecting only the base of the right lung as the "region of interest".

^{131}I is not an ideal radioisotope for studies with gamma camera. It is possible that the use of more suitable isotopes such as ^{123}I which has not been available to us could increase the sensitivity and hence make monitoring by gamma camera suitable and possible for study also of human microembolism.

The results of the external detection in this study are in accordance with those of Saldeen (13), who used a similar technique on rabbits given ^{131}I-labelled fibrinogen and thrombin i.v. Only a small, though significant increase was detected over the lung. Coombey & Tyler (8), using ^{125}I-fibrinogen, infused 50 units of thrombin via a sublingual vein in 2½ min into rats. The radioactivity was recorded externally over the lung. The increase was about 30% above the baseline level. According to our experience from rats (6), however, such a thrombin infusion will give a true *in vitro* increase in lung radioactivity of several hundred percent, which is another example of the differences between *in vitro* and *in vivo* techniques.

In conclusion, external determination is a reproducible method particularly suited for continuous study of the dynamics of pulmonary microembolism. The method can also be used for simultaneous registration of the radioactivity in other organs such as the liver and kidneys (5). It provides definite advantages over the biopsy technique, which limits the number of observations and occasionally shows considerable variations between different samples. These variations might be due to altered intrathoracic pressure and circulatory conditions following the thoracotomy and artificial respiration.

The sensitivity of the external method is limited, however, and in states with an expected low degree of intravascular coagulation the biopsy technique should be used.

In investigations of humans with intravascular coagulation and microembolism the biopsy method

is not practicable. Hence the external method has been employed in the study of pulmonary fibrin and platelet deposition associated with trauma. In these cases evidence of transient (10) or progressive (3) accumulation of isotope has been observed.

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