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B A Martin, ... , H Thommasen, J C Hogg

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### Research Article

The effect of pulmonary blood flow on the exchange between the circulating and marginating pool of polymorphonuclear leukocytes (PMN) was examined in three sets of experiments. In the first we used the double indicator dilution technique with labeled PMN and erythrocytes (RBC) to calculate the percent extraction and percent recovery of PMN at different levels of cardiac output (CO). In the second group of experiments we took advantage of the wide range of blood flow in the lung to determine the effect of regional blood flow on regional PMN retention, and in the third set we measured total leukocyte (WBC) and PMN counts in simultaneous samples from the pulmonary artery and aorta over a wide range of cardiac output. The studies showed that 80-90% of the labeled PMN were removed in a single pass through the lung and that regional retention of labeled PMN and A-V differences for unlabeled PMN increased with decreasing blood flow. The data for regional retention of labeled PMN and the A-V differences observed for unlabeled cells both fit the equation  $Y = A + Be^{-cx}$  (where  $A + B = 100$ ), which showed that PMN accumulate in the lung as blood flow is reduced. We conclude that a dynamic equilibrium exists between the circulating and marginating pools of leukocytes in the lung and that blood [...]

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# Effect of Pulmonary Blood Flow on the Exchange Between the Circulating and Marginating Pool of Polymorphonuclear Leukocytes in Dog Lungs

BRIDGET ANN MARTIN, JOANNE L. WRIGHT, HARVEY THOMMASEN, and  
JAMES C. HOGG, *University of British Columbia Pulmonary Research  
Laboratory, St. Paul's Hospital, Vancouver, British Columbia V6Z 1Y6,  
Canada*

**ABSTRACT** The effect of pulmonary blood flow on the exchange between the circulating and marginating pool of polymorphonuclear leukocytes (PMN) was examined in three sets of experiments. In the first we used the double indicator dilution technique with labeled PMN and erythrocytes (RBC) to calculate the percent extraction and percent recovery of PMN at different levels of cardiac output (CO). In the second group of experiments we took advantage of the wide range of blood flow in the lung to determine the effect of regional blood flow on regional PMN retention, and in the third set we measured total leukocyte (WBC) and PMN counts in simultaneous samples from the pulmonary artery and aorta over a wide range of cardiac output. The studies showed that 80–90% of the labeled PMN were removed in a single pass through the lung and that regional retention of labeled PMN and A-V differences for unlabeled PMN increased with decreasing blood flow. The data for regional retention of labeled PMN and the A-V differences observed for unlabeled cells both fit the equation  $Y = A + Be^{-cx}$  (where  $A + B = 100$ ), which showed that PMN accumulate in the lung as blood flow is reduced. We conclude that a dynamic equilibrium exists between the circulating and marginating pools of leukocytes in the lung and that blood flow primarily affects the reentry of cells into the circulating pool so that the marginating pool of PMN within the lung accumulates cells when blood flow is reduced below 7 ml/min per g.

## INTRODUCTION

The “marginated pool” of polymorphonuclear leukocytes (PMN)<sup>1</sup> consists of cells that are inside the

microvasculature but do not circulate. This pool of cells plays an important role in leukocyte kinetics because it can be rapidly mobilized into the circulation by stimuli such as exercise (1, 2) or epinephrine (1–3). Whereas the lung is thought to be a major site of the marginating PMN pool (4), there have been relatively few attempts (1, 2) to determine how cells enter and leave it. In this study we report data on three separate but related experiments that were designed to investigate the interaction between the circulating and marginating pool. In the first set of experiments we used the double indicator dilution technique with labeled PMN and erythrocytes (RBC) to try to determine the removal of PMN from the circulation in a single pass through the lung at different levels of cardiac output. In the second set of experiments we took advantage of the wide range of regional blood flow within the lung to measure the effect of blood flow on regional retention of a single injection of labeled PMN, and in the third we measured the number of leukocytes (WBC) in pulmonary arterial and venous blood to determine if lowering pulmonary blood flow could increase the number of WBC in the marginating pool and result in A-V differences in leukocytes across the lung. The studies showed that ~80–90% of labeled PMN leave the circulating pool in one pass through the lung without the appearance of an A-V difference for nonlabeled cells across the lung. They also showed that blood flow modified the retention of labeled cells within the lung and that reduction in pulmonary blood flow was capable of producing A-V PMN differences across the lung. This suggests a significant exchange between marginating and circulating PMN within the lung that remains in balance until blood flow is markedly reduced.

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<sup>1</sup>Abbreviations used in this paper: CO, cardiac output; IVC, inferior vena cava; MAA, macroaggregates; PA, pul-

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monary artery; PBS, phosphate-buffered saline; PEEP, positive end expiratory pressure; PMN, polymorphonuclear leukocytes; RA, right atrium; RBC, erythrocytes; RV, right ventricle; WBC, leukocytes.

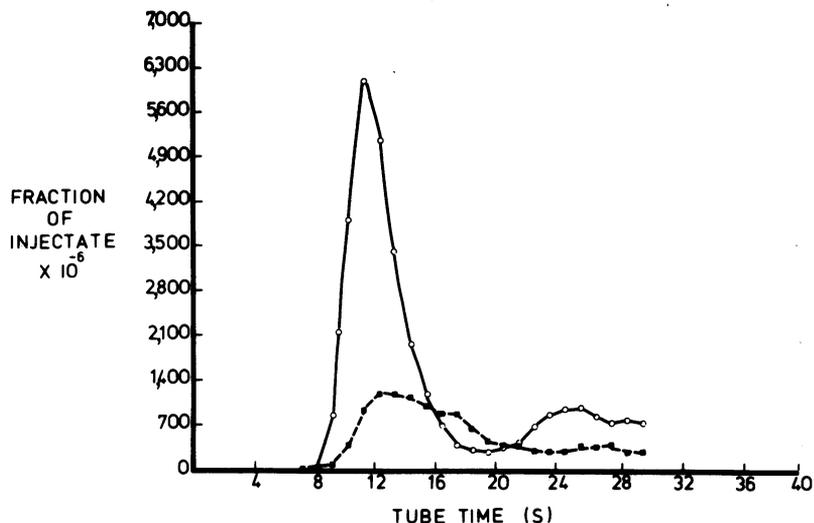


FIGURE 1 Shows two concentration time curves constructed from aortic samples of blood containing labeled RBC and PMN after a bolus of the labeled cells had been injected into the RA. Calculations of percent extraction and percent recovery were made by comparing the PMN curve with the peak of the RBC curve, and the PMN and RBC curves up to the point of RBC recirculation (see text). O, RBC; ■, PMN.

## METHODS

**Animals.** Mongrel dogs weighing  $20.5 \pm 0.73$  kg were used in these studies.

**Cell labeling.** Before anesthesia, 250 ml of unheparinized venous blood were drawn. 10 ml were used for labeling RBC with  $180 \mu\text{Ci } ^{99m}\text{Tc}$  using the Glucoscan kit (NEN NRP-180) (5). 240 ml were divided into six 50-ml sterile polypropylene tubes, each containing 6 ml of freshly prepared acid citrate dextrose (pH 4.45). The blood was spun slowly at 590 *g* for 20 min in a Beckman model TJ-6 centrifuge (Beckman Instruments, Inc., Fullerton, CA). The platelet-rich plasma was removed and discarded. The buffy coat layer and some RBC were drawn off using sterile siliconized pasteur pipets, divided into two equal volumes and put in 50-ml sterile polypropylene tubes. These were each resuspended to a volume of 40 ml using freshly prepared sterile phosphate-buffered saline (PBS), pH 7.47, and spun at 590 *g* for 10 min. The supernatant was removed and discarded and the buffy layer and contaminating RBC were resuspended to a volume of 50 ml with PBS (pH 7.47). The RBC were removed by two hypotonic lyses where the cells were suspended in 30-ml sterile distilled water for 30 s to which immediately was added 15 ml of 2.7% PBS. Each time the suspension was spun at 200 *g* for 10 min, and the supernatant was discarded. The remaining cell pellet, which contained <5% RBC, was resuspended in PBS to a volume of 10 ml, which was divided into two 5-ml aliquots. Each 5-ml sample of WBC was placed over 3 ml of Ficoll-Hypaque (6), and these were spun at 375 *g* for 20 min. The PMN layer, which contained <5% platelets and lymphocytes, was separated, washed twice with PBS, counted, and then incubated with 300–500  $\mu\text{Ci } ^{51}\text{Cr}$ , in a final volume of 3 ml (7) for 45 min at 37°C with occasional gentle agitation. The labeled PMN were then suspended with PBS to a volume of 10 ml and spun at 200 *g* for 10 min. This procedure was repeated two or three times to bring the  $^{51}\text{Cr}$  counts in the supernatant to background levels. The cells were then resuspended in PBS containing 0.25% bovine

serum albumin (Sigma Chemical Co., St. Louis, MO) and were ready to be mixed with the labeled RBC into a common pool. Cell counts (hemocytometer), viability (trypan blue exclusion), and cell sizing (Coulter model ZB1 and Channelyzer 1000) were done on the pre- and postlabeled cells (8). Final PMN count and viability were  $0.618 \pm 0.193 \times 10^8/\text{ml}$  and 98.6%, respectively, and the PMN cell size did not change. The labeling efficiency was  $9.4 \pm 1.2\%$ . All equipment used was sterile and only siliconized glass or polypropylene were used to ensure maximum labeling efficiency (7, 9, 10).

**Surgical procedure.** When the preparation of labeled cells was nearly complete the animals were tranquilized with Acepromazine Maleate D (0.63 mg/kg, i.m.) and then anesthetized with sodium pentobarbital (20 mg/kg), intubated, and placed in the supine position, spontaneously breathing room air. Catheters were placed into the pulmonary artery (PA) and right atrium (RA) via the right jugular vein and into the aortic root via the left carotid artery under fluoroscopic control. Heparin (100 U/kg) was administered at the end of surgery. Arterial blood pressure, pulmonary artery, and pulmonary artery wedge pressures, cardiac output (CO), serum protein concentration, hematocrit, and arterial blood gases were measured. Pressures were measured using Hewlett-Packard transducers (Hewlett-Packard Co., Palo Alto, CA, model no. 1280C) and recorded on an 8-channel recorder (Hewlett-Packard Co., model no. 9800) where the 0 level of the transducers was referenced to the back of the animals. Cardiac output was measured using a modified 7F Swan Ganz flow-directed thermodilution catheter.

**Indicator dilution studies.** PMN extraction and recovery were measured across the lung on 17 occasions in five dogs by injecting a bolus of labeled RBC and PMN into the RA, while blood was rapidly sampled from the aortic root so that time concentration curves could be constructed (11, 12). PMN extraction was calculated by comparing the PMN and RBC curves to the peak of the RBC curve (13), and PMN recovery was calculated by comparison of the labeled PMN

and RBC curves up to the point of RBC recirculation (11, 12). The effect of pulmonary blood flow on these calculated parameters was determined from separate bolus injections of RBC and PMN carried out under control conditions (seven injections) and while pulmonary blood flow was increased by opening femoral A-V shunts (five injections) and decreased by inflating a balloon in the inferior vena cava (IVC) (five injections).

**Effect of regional pulmonary blood flow on PMN retention.** In another five animals a single indicator dilution study was followed immediately by an injection of  $^{125}\text{I}$ -labeled macroaggregates (MAA), (Chas. E. Frosst, Pointe Claire, Quebec, Canada, 30  $\mu\text{Ci}$  in 1 ml) to mark regional blood flow (14). Between 1.5 and 10 min after the indicator dilution study was completed, 40 ml of blood was then taken, and the dogs were killed with an injection of hypertonic potassium chloride into the aortic root. The thorax was rapidly opened, the hila were clamped, and the lungs were quickly removed. With the lungs held above the heart, the major vessels were clamped, the heart was removed, and the lungs were inflated, clamped, and frozen in  $-70^\circ$  ethylene glycol, the whole procedure taking  $<4.5$  min. The lungs were then divided and sliced coronally, posterior to anterior, each slice 2 cm thick. Each slice was divided into small samples that were placed into preweighed gamma scintillation vials and counted in a gamma counter (Beckman model 7000, Beckman Instruments, Inc.). The number of PMN delivered to the lung per unit flow was determined by dividing the number of cells in the injected bolus by the cardiac output calculated from the labeled RBC in that bolus. Regional pulmonary blood flow was calculated using a described technique (14) where the total pulmonary blood flow was set equal to the total  $^{125}\text{I}$  MAA counts in the lung, obtained by summing the counts in all the lung samples. The total PMN retention was calculated by dividing the total number of PMN counts present in the lung by the known total number of PMN counts injected. Regional PMN retention was calculated by dividing the number of PMN present in each sample by the number delivered to that sample. All lung samples were corrected for residual  $^{125}\text{I}$  MAA,  $^{99\text{m}}\text{Tc}$  RBC, and  $^{51}\text{Cr}$  PMN in the blood, and were expressed per gram lung.

To ensure that the injection of labeled PMN did not alter regional blood flow, one experiment was performed where regional blood flow was measured as described above by injecting  $^{99\text{m}}\text{Tc}$ -labeled MAA before and  $^{125}\text{I}$ -labeled MAA after the injection of the  $^{51}\text{Cr}$ -labeled PMN.

**Effect of total pulmonary blood flow on WBC A-V differences.** These studies were carried out on nine animals without the use of labeled cells. In four animals simultaneous blood samples were drawn into dry 5-ml EDTA Vacutainer tubes from the aortic root and PA at 0.5–1-min intervals for a 7-min control period that was followed by periods where CO was decreased either by applying 15–18 cm of positive end expiratory pressure (PEEP) or inflating an IVC balloon (Edwards Fogarty Venous Thrombectomy Catheter, model 32-080-8/10F) or where CO was increased by opening femoral A-V shunts. Corresponding thermal dilution cardiac output determinations were done. Total WBC (Coulter Electronics Inc., Hialeah, FL, model S) and differential counts were performed on these blood samples so that pulmonary A-V differences for WBC and PMN could be calculated.

A final group of five animals were used to examine unlabeled PMN behavior more critically. In these experiments we used a standard 2-min inflation of an IVC balloon to reduce cardiac output. Aortic root and RV samples were taken every 10 s for 30 s before the IVC balloon inflation

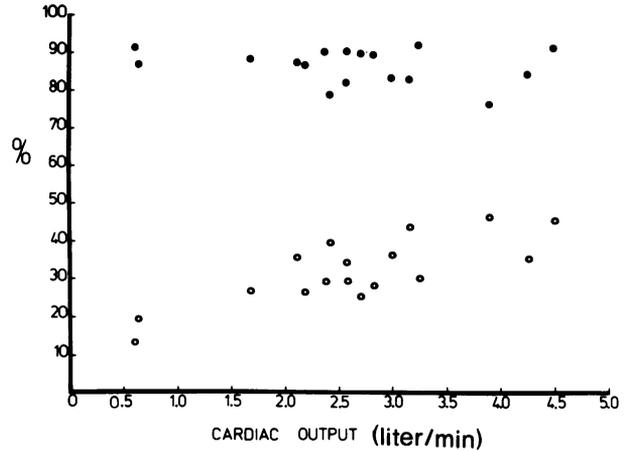


FIGURE 2 Shows the percent extraction (closed circles) and percent recovery (open circles) of labeled PMN calculated from curves such as that shown in Fig. 1. Note that the percent extraction remains constant while the percent recovery tends to rise as cardiac output increases.

and for the entire 2-min balloon inflation. Systemic blood pressure was measured during this run but it was not possible to measure cardiac output because of the 10-s sampling. To establish cardiac output during the collection run we did two separate but similar runs where BP and cardiac output were both measured without collecting blood. These runs were performed before and after the collection run to ensure that the vascular response to the balloon inflation did not change. In this way it was possible to obtain A-V samples at 10-s intervals, while pulmonary blood flow was lowered from 16 to 2 ml/min per g. WBC counts were performed on each sample using a Coulter Counter and differential counts were performed on smears made for each sample to obtain PMN differences across the lung. The data for all five dogs were fit to the equation  $y = A + Be^{-cx}$  using a reiterative process where  $y$  was PMN expressed as  $Rv\text{-aorta}/Rv\%$ , and  $x$  was total blood flow measured by thermodilution and expressed per gram of wet lung weight.

## RESULTS

The data concerning arterial blood pressure, pulmonary artery pressure, pulmonary artery wedge pressure, cardiac output, and serum proteins were similar to those reported from this laboratory (12).

Fig. 1 shows an example of the concentration-time curves that were constructed from the aortic blood samples obtained while a bolus of labeled RBC and PMN was injected into the RA. These data show that the fraction of injected PMN appearing in the aortic sample is much less than the fraction of injected RBC. The percent extraction and percent recovery of PMN calculated from these curves at all cardiac outputs are shown in Fig. 2. The percent extraction (closed circles) averaged  $87 \pm 1.6\%$  (SE) and changed little at cardiac outputs ranging from 0.6 to 4.5 liter/min. The percent recovery (open circles) of PMN on the other hand

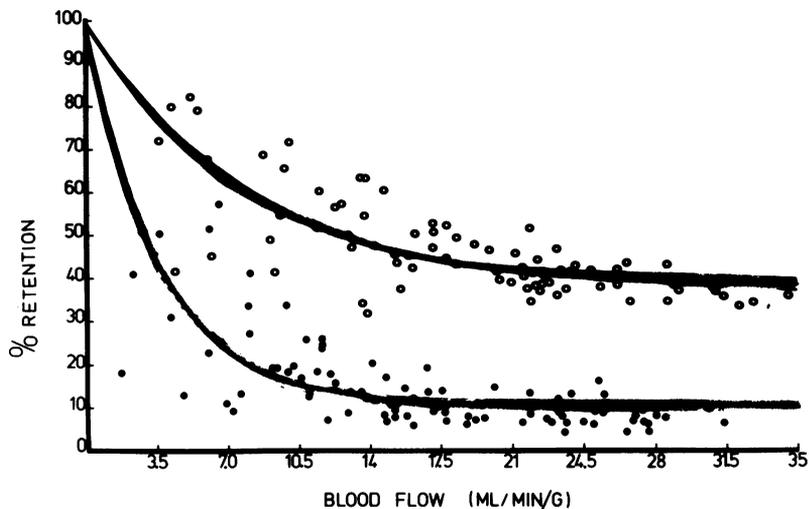


FIGURE 3 Shows data from two experiments where the regional retention of PMN was examined in relation to regional pulmonary blood flow 1.5 and 10 min after the injection of the bolus of labeled cells. Each data point represents a single lung sample where the blood flow received by the sample is plotted against the percent PMN retained in the sample. The solid lines are computer-derived best fit for the equation  $y = A + Be^{-cx}$  (where  $A + B = 100$ ). The stippled area about the lines represents  $\pm 1$  SD  $\circ$ , 1.5 min;  $\bullet$ , 10 min.

ranged from 12 to 18% at a cardiac output of 0.6 liter/min, and rose to 45% when the cardiac output was 4.5 liter/min.

The data from two of the five animals where regional PMN retention was calculated in relation to regional blood flow are shown in Fig. 3. These data show that percent retention was greater in the animal killed at 1.5 min (open circle) after the indicator in-

jection study, than in the animal killed at 10 min (closed circle) postinjection. Fig. 3 also shows that retention is greatest in regions of the lung receiving the least blood flow and falls in an exponential fashion as flow increases. The solid line through each curve is the line of best fit estimated by a reiterative fitting procedure to an exponential function  $y = A + Be^{-cx}$  (where  $A + B = 100$ ). The stippled area around the line rep-

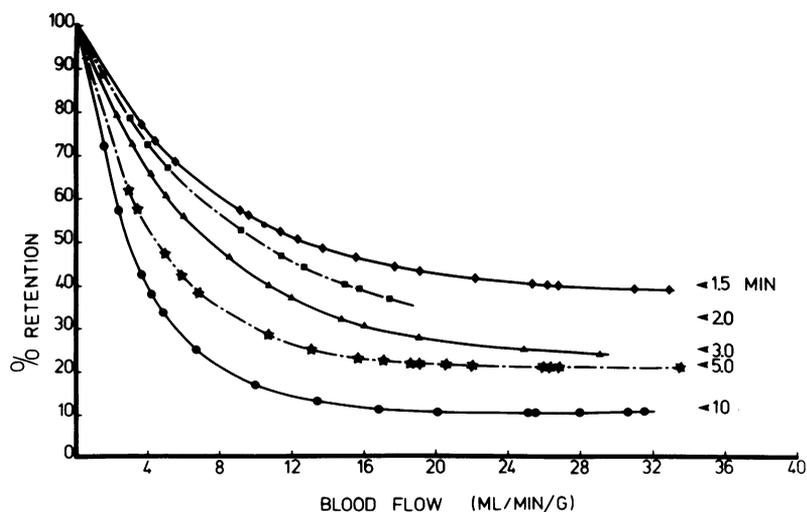


FIGURE 4 This shows the calculated lines of best fit for experiments such as that shown in Fig. 3, where the lines represent the lines of best fit derived for each experiment. Data are shown for experiments where the lungs were removed 1.5, 2, 3, 5, and 10 min after the bolus injection of labeled cells into the RA, and show that fewer cells remain in the lung as time passes.

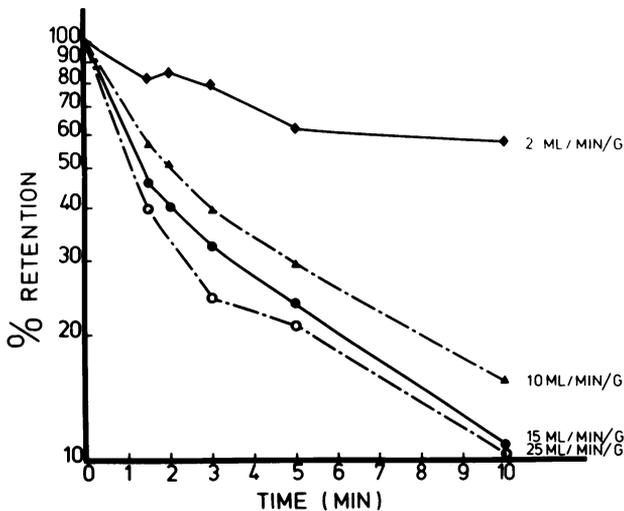


FIGURE 5 The effect of time on percent retention of labeled PMN at flow rates of 2, 10, 15, and 25 ml/min per g. Data points were obtained for each flow rate from the lines of best fit shown in Fig. 4. The data show that 50% of the delivered PMN are gone in 2-3 min at high flow rates but that this requires a longer time (<10 min) when the flow rate is 2 ml/min per g.

been plotted through the data on regional PMN retention in relation to regional pulmonary blood flow. From these five curves it is possible to examine the effect of time on PMN retention at constant blood flow. Fig. 5 shows data for the effect of time on PMN retention at flows of 2, 10, 15, and 25 ml/min per g. One sees that it takes ~1 min for 50% of the cells to leave an area with a flow of 25 ml/min per g, and >10 min for 50% of the cells to leave an area receiving a flow of 2 ml/min per g.

The results for one of the experiments in which simultaneous Pa and aorta blood samples were drawn during a control period, a period where cardiac output was reduced by inflating an IVC balloon, and a period where the cardiac output was allowed to recover by deflating the IVC balloon, are seen in Fig. 6. This shows that marked A-V differences for WBC concentration developed when output was reduced and that these A-V differences returned to control values when cardiac output was allowed to recover.

Fig. 7 shows the mean percent A-V difference  $\pm$ SE for experiments when mean blood flow was increased from  $11.9 \pm 2$  to  $15.7 \pm 4.0$  ml/min per g by opening A-V shunts, decreased from  $16.6 \pm 7.7$  to  $9.4 \pm 3.0$  ml/min per g by applying 15-18 cm of PEEP and decreased from  $13.2 \pm 2.8$  to  $3.7 \pm 0.7$  ml/min per g by inflating the IVC balloon. These data show that an A-V difference developed ( $P < 0.01$ ) only when blood

represents one standard deviation about the predicted line. Fig. 4 shows the data from all five experiments where the computer-derived best exponential fit has

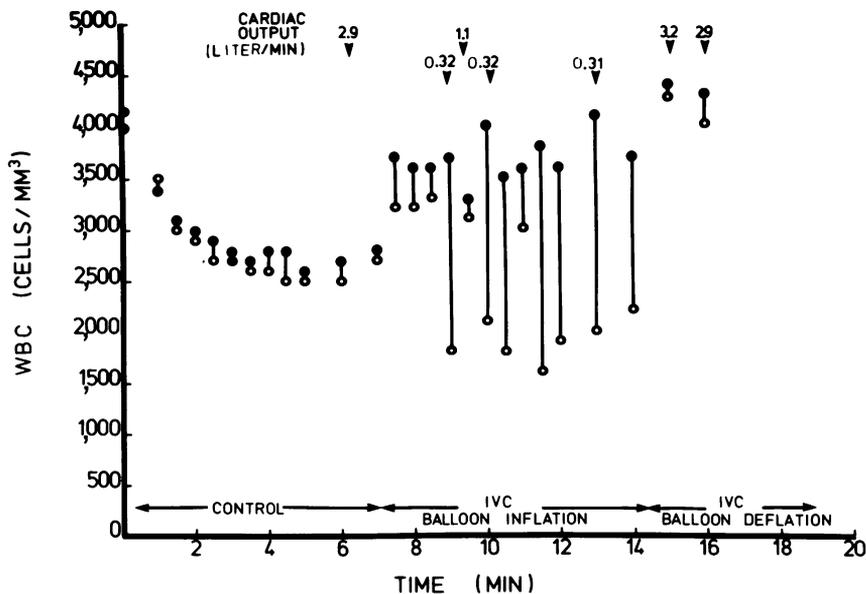


FIGURE 6 WBC concentration (cell/mm<sup>3</sup>) of blood samples drawn simultaneously from the PA (closed circles) and aorta (open circles) for one experiment. The data show that during a 7-min control period where CO averaged 2.9 liter/min there were only small A-V differences and that when cardiac output was lowered to a mean of 0.3 liter/min by inflation of the IVC balloon these differences increased and then returned to control levels when the cardiac output was restored by deflating the IVC balloon.

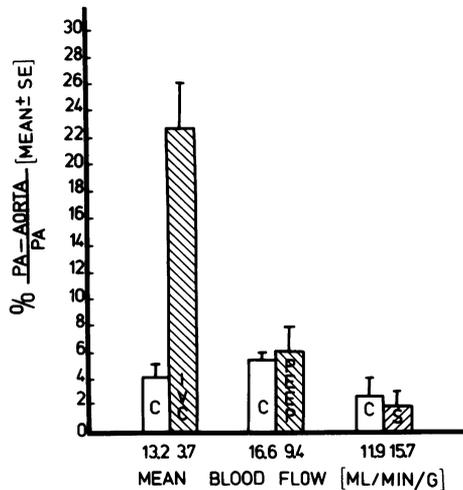


FIGURE 7 Shows A-V differences for WBC as a percentage of pulmonary artery (WBC) count. When blood flow was reduced to a mean of  $3.7 \pm 0.7$  ml/min per g by inflating a balloon in the IVC marked A-V differences appear ( $P < 0.01$ ) while lesser reduction in flow caused by PEEP and increases in flow caused by A-V shunts have no effect.

flow was reduced by inflating the IVC balloon. Although the data showed a trend for the A-V difference to rise above control levels when flow was decreased by PEEP and to fall below control levels when blood flow was increased by opening the shunt, these changes were not significant.

Fig. 8 shows the PMN difference across the lung plotted against total pulmonary blood flow. The PMN difference was expressed as  $R_v - aorta / R_v \%$ , and blood flow is expressed per gram of wet lung. The data has been fit to the same equation used in Fig. 4 where  $y = A + Be^{-cx}$  ( $A + B = 100$ ). These data show that the A-V difference for PMN begins to increase when blood flow falls below 7 ml/min per g and to have increased sharply when blood flow reaches 4 ml/min per g.

## DISCUSSION

WBC flow through the microvasculature has been of interest since the early studies of Cohnheim who noted that PMN were seen to adhere to the capillary walls and roll along in a marginal stream near the vessel walls, while RBC and other cells remained in the axial stream (15). As these marginated cells can also drop back into the axial stream, it follows that the cells within the microvasculature can both circulate and marginate and that an equilibrium exists between these two pools. In 1961, Athens et al. (1, 2) tried to establish the size of the circulating and marginating pools by the use of labeled granulocytes. They noted that after a 15–20-min infusion, 54% of the labeled

granulocytes had disappeared from the circulation and they postulated that this was not due to damage of the cells or elution of radioactive label, but rather to distribution in a total blood granulocyte pool twice as large as the circulating pool. The fact that labeled cells could also be brought back into the circulating pool when Athens et al. (1, 2) exercised their subjects, or gave them epinephrine, showed that there was considerable exchange between the pools.

The analysis of our first set of experiments shows that if we accept the percent extraction to the peak of the RBC curve as evidence of removal of labeled cells from the circulation, ~86–94% of the labeled cells leave the circulating pool in one pass through the lung. This rapid removal of cells from the circulating pool in a single pass through the pulmonary capillary bed is consistent with the recent report of Schmid-Schonbein et al. (16) who showed that 94% of WBC are seen to marginate in the venous end of capillaries in the peripheral circulation. When such large numbers of leukocytes are removed from the circulating pool in a single pass through the lung, it follows that an equal number must leave the marginated pool and reenter the circulation or large A-V differences would result. The reentry of the injected labeled cells into the circulating pool can be partially appreciated by looking at the data concerning the percent recovery of labeled cells, as this analysis includes the downslope of the curves. The fact that the percent recovery increased with increasing total pulmonary blood flow while percent extraction remained the same is interesting. It suggests that increasing blood flow has little effect on the movement of cells from the circulating pool to the marginating pool, but does increase their return to the circulating pool.

The second group of experiments was designed to examine the effect of blood flow on the retention of PMN in the lung more closely. These experiments allowed us to measure the number of PMN retained in small lung samples in relation to the number delivered to the sample by its regional blood flow. The data show (Fig. 3) that the greatest percent retention of PMN occurred when blood flow was  $<7$  ml/min per g and was least when the flow was  $>15$  ml/min per g. The effect of time on PMN retention at constant blood flows (Fig. 5) shows that when the blood flow is 2 ml/min per g, 50% of the delivered PMN remained in the lung at 10 min. However, in regions receiving blood flows of 10–25 ml/min per g, 50% of the delivered cells are gone in  $<2$  min. These data support the notion that retention of PMN in the marginating pool is dependent on blood flow in that regional PMN retention can be either increased or decreased by an appropriate change in blood flow. This observation is consistent with the data from the first set of experiments (Fig.

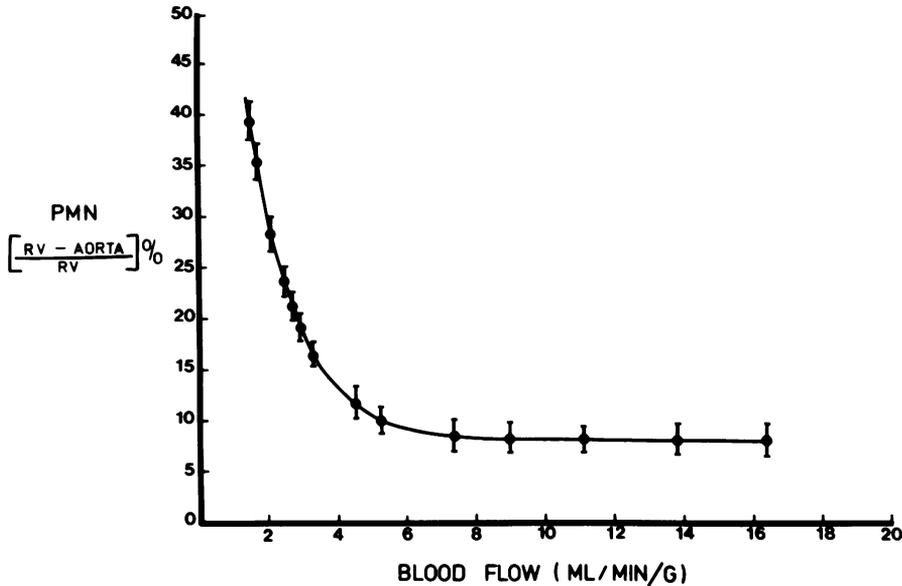


FIGURE 8 Shows the mean computer-derived line  $\pm$ SD for all of the data from five experiments where the unlabeled PMN A-V differences across the lung were plotted against total pulmonary blood flow per gram of wet lung. The data were fit to the same equation  $Y = A + Be^{-cx}$  used in Fig. 4 where  $Y =$  PMN retention and  $x =$  blood flow. These data show that the A-V difference for PMN begins to increase when blood falls below 7 ml/min per g.  $n = 5$ , (mean $\pm$ SD).

2) where the percent extraction is independent of flow while percent recovery increased as total pulmonary blood flow increased. This could be explained by labeled cells entering the marginating pool and remaining there for short periods of time, depending on the local pulmonary blood flow.

To rule out the possibility that injection of labeled cells might alter regional blood flow, we compared regional blood flow before and after the injection of labeled PMN. The data fell along a line of identity with a calculated slope of 0.99, an intercept of 0.01, and a correlation coefficient of 0.97 when blood flow before the PMN injection was plotted against blood flow after the PMN injection.

The experiments measuring the number of unlabeled cells across the lung were performed to rule out the possibility that the label placed on the cell caused it to be removed by the pulmonary circulation. These data (Fig. 6) show that small A-V differences are present in the control periods and these A-V differences increased ( $P < 0.01$ ) when the cardiac output was reduced from 13.2 to 3.7 ml/min per g by inflation of the IVC balloon and returned to control values when the balloon was deflated.

On the basis of present knowledge of leukocyte kinetics, the rate of PMN turnover is of the order of  $1.6 \times 10^9$  cells/kg per d (2, 17, 18). In our animals this would mean that we would expect a total turnover of  $\sim 3.2 \times 10^{10}$  cells/d. As the total pulmonary blood flow

would have been  $4.3 \times 10^3$  liter in 24 h, one could not expect to measure A-V leukocyte differences even if all of the PMN were destroyed in the lungs. However, in several other studies where catheters were placed in the right and left heart to sample blood on both sides of the pulmonary circulation small A-V leukocyte differences similar to those seen in the periods marked control in Fig. 6 have been reported (4, 19, 20). Bierman (19) concluded that these small A-V differences were due to respiratory movements because they increased with Mueller and Valsalva maneuvers. Ambrus et al. (4) attributed them to the presence of catheters in the RA and in the carotid artery. Although we cannot provide any additional insight into the reason for the small A-V differences seen in the control period (Fig. 6), there is no doubt that lowering of the cardiac output below 7 ml/min per g (Fig. 8) caused the A-V differences to increase sharply.

It is of interest to examine the data in Figs. 7 and 8 in relation to that shown in Fig. 3. Fig. 3 shows that the retention of labeled PMN increases sharply at flows below 7 ml/min per g and changes little at higher blood flows. This means that one could predict that A-V leukocyte differences might develop when flow dropped below 7 ml/min per g because more cells would be retained in the lung. The data in Fig. 8 show that lowering flow below 7 ml/min per g increased the A-V differences for unlabeled PMN while flows  $>7$  ml/min per g had no effect. Indeed, the shape of the

curves in both Figs. 3 and 8 provide a clear explanation for the data in Fig. 7. Fig. 7 shows that A-V differences develop with IVC balloon inflation which lowered blood flow to 3.7 ml/min per g but not with PEEP which lowered flow to 9.4 ml/min per g or with A-V shunts that increased flow to 15.7 ml/min per g. This could be predicted from both Figs. 3 and 8 because they show that labeled PMN retention (Fig. 3) and A-V difference for unlabeled PMN (Fig. 8) increase sharply at 3.7 ml/min per g but are not affected by the changes in blood flows caused with PEEP and A-V shunts.

The fact that reduced blood flow can cause PMN to be sequestered in the lung is of interest in relation to other studies. Perlo et al. (21) showed that the ratio of WBC to RBC was greater in zone 2 than in zone 3 in isolated perfused lungs. However, there are several differences in their findings in relation to ours that will have to be reconciled by future experiments. They found that it was primarily lymphocytes that occurred in excess in zone 2 and our data suggest that the PMN are the cells that are primarily retained when flow is decreased. In addition they suggested that the compression of the microvasculature in zone 2 might cause the alveolar vessels to act as a sieve. In our experiments substantial portions of the lung must have been under zone 2 conditions when flow was decreased by inflating the IVC catheter and by the application of 15–18 cm of PEEP. However, as we only saw leukocyte A-V differences with the very low flows produced by inflating the IVC balloon and not with PEEP, our studies suggest that the A-V differences are more sensitive to absolute flow rather than to the fact that portions of the lung enter zone 2. Very recently, Guenter et al. (22) showed that the sequestration of leukocytes caused by endotoxin in dogs could lead to experimental emphysema, presumably due to elastase release from PMN. Our data suggest that the marginating pool of leukocytes may be greatest in the low flow regions of the lung. As this is likely to occur in the upper lung region where emphysema is commonly found (23), it may be that emphysema results from excess elastase in the upper regions because of a large marginating pool of leukocytes there. It is also possible that when cardiac output falls to very low levels in conditions such as anaphylactic, hemorrhagic, and endotoxic shock, the sequestration of leukocytes in the lung due to the reduction in blood flow might play a role in the subsequent development of Adult Respiratory Distress Syndrome (24).

Interpreting the findings from all three sets of experiments, it is reasonable to conclude that a large number (80–90%) of the circulating PMN enter the marginating pool in one pass through the lung. These cells equilibrate with the marginated pool and reenter

the circulation so that the time spent in the lung is dependent on blood flow. The data also show that when blood flow is reduced to very low levels the number of leukocytes in the blood leaving the lung falls below that in the pulmonary artery so that the absolute number of WBC in the lung increases.

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