Longitudinal Distribution of Blood in the Rabbit in Relation to the Heart, with Observations on the Contribution of Different Organs


ABSTRACT
The distribution of blood along the length of an animal is relevant to the stability of cardiac filling pressure during tilting. We investigated the longitudinal distribution of blood in conscious, anesthetized, and dead rabbits by a scanning technique, after labeling red blood cells with *Cr. The mean longitudinal distributions of blood were computed after standardization for body size and radioactivity. The longitudinal distribution of blood showed a large main peak near the level of the tricuspid valve. General anesthesia produced very little change in blood distribution, but after death blood left the lower abdomen and accumulated in the upper abdomen, probably in the liver. The liver made much the greatest contribution (25 to 30%) to the total pool of blood, but the blood contained within it was labile and could be largely removed by bleeding the animal, or transferred to the lungs during pulmonary congestion.

ADDITIONAL KEY WORDS  whole body scanning labeled red cells  radio-active chromium  liver blood volume  cardiac filling pressure

We have been trying to understand what happens to cardiac filling pressure and to cardiac output during tilting. Dickinson (1) constructed a digital computer model of the heart and venous circulation to study the factors governing the location of that point in the venous system at which pressure changed least with tilting. The results of preliminary computations with this model (Dickinson, unpublished observations) showed that if venous capacitance was assumed to be evenly distributed over the whole length of the body, it was not possible for a stable cardiac filling pressure, and consequently a stable cardiac output, to be maintained during body tilting, whatever functional characteristics might be given to the heart itself. This conclusion differs from that of Guyton and Greganti (2), who ascribed the remarkable stability of cardiac filling pressure during tilting to the operation of Starling's law of the heart. Whenever the cardiac filling pressure tends to rise, the heart should pump more blood out and thus hold down filling pressure; when cardiac filling pressure tends to fall, the heart should pump less blood and therefore tend to keep central venous pressure up.

Even when a more realistic distribution of blood was used in the computer model, and the largest capacitance reservoirs were placed within the abdomen, the results were still unrealistic. Cardiac filling pressure could of course be maintained in life by active reflex constriction of venous reservoirs during tilting, but this is known to be unnecessary because Guyton and Greganti (2) have observed stability of cardiac filling pressure during tilting in the dog with complete spinal anesthesia.

We concluded that the distribution of circulatory capacitance must be very different from the one we had imagined. To provide realistic data for the computer model we used the technique of radioisotope scanning to determine the longitudinal distribution of blood in an animal.

We here report our observations on living...
and dead animals and analyze the contribution of different organs to the total longitudinal blood profile.

**Methods**

We studied 18 rabbits weighing 2.3 to 3.9 kg, including four males. Approximately 6 ml of blood was withdrawn from an ear vein and the red cells labeled with 0.5 to 1.0 mc 51Cr, after the technique of Grey and Sterling (3). Specific activity varied from 50 to 150 mc/mg between batches, and quantities used were 4 to 8 /ig/ml for 0.5 to 1.0 mc. About 3 ml of cells were labeled with 1 mc of 51Cr, and the chromium concentration was 1.0 to 2.0 /ig/ml. The toxic level is generally thought to be about 2.0 /ig/ml. Since nearly 80% of the labeled red cells could be removed by bleeding at the end of a 6-hour experiment, we obtained no evidence to suggest appreciable toxic damage to cells. All experiments were performed within 6 hours after labeling. Anesthesia was induced, when required, by sodium pentobarbital, 30 to 40 mg/kg iv, supplemented as necessary by small intramuscular doses of 5 to 10 mg/kg. A weighed amount of labeled blood was kept as a standard and the rest was reinjected. After 10 minutes for equilibration, a sample was withdrawn from the opposite ear, and the hematocrit and radioactivity were determined. From these data, total cell mass and approximate total blood volume were calculated. These calculations were incidental to the main experiments, and we did not therefore take successive samples. However, a single sample at 10 minutes should be accurate within 5% of the true value (3).

The rabbits were placed prone and gently stretched on a flat board, with the head resting between the forepaws and the tips of the paws about 6 cm in front of the snout. The animals were afterwards disturbed as little as possible.

The longitudinal distribution of radioactivity in the rabbits was determined by scanning the whole animal with a rectilinear scanner (Picker Magnascanner III) with a 7.5 cm x 5.0 cm thallium-activated sodium iodide crystal and a 19-hole focused collimator. The official isocount profile showed that the collimator used did not exclude all radiation from adjacent segments, but did so almost completely from segments 1 cm either side. We checked this for our collimator by scanning a glass rod containing 131I (γ-energy close to 51Cr). The pulse height analyzer was set at 300 to 340 kev and its output fed into a scaler. For the standard prone scans, the detector was placed 2.0 to 2.5 cm above the dorsal surface of the rabbit and, while scanning, traveled at a uniform speed in evenly spaced rows at right angles to the long axis of the animal. A complete scan took about 35 minutes with the detector moving at 100 cm/min with a row spacing of 0.5 cm.

![Graph](image-url)  
**Figure 1**  
Typical longimeter output of a scan from a single (living) rabbit. The head is to the left, the tail to the right. The distance of each X from the baseline (dotted line) is proportional to the amount of blood present in that body segment.
Reproducibility of scan profiles. Four successive scan profiles of the same (dead) rabbit were performed, and the original values, less background, were plotted for each of the 114 segments into which the scan of the animal was divided. Each scan is given a different graphical representation: dots, broken line, continuous line, line of short bars. Note close similarity between successive scans.

At least 20 background rows were counted and then subsequent rows covering the entire animal. The counts for each row were recorded automatically by the scaler and the results transferred to IBM cards for later analysis.

Since we wished to establish mean longitudinal blood profiles from a number of animals of different size and length, containing different total amounts of blood and of radioactivity, further analysis was carried out with the aid of programs written in Fortran V and run on the University of London Atlas computer. The first program calculated the mean background count and subtracted this from each individual row count. To allow for animals of different lengths, the longitudinal count profile was then standardized by compressing it into 101 equal segments. To avoid loss of information from omitted rows, the value of counts at each 101 equally spaced points along the length of the animal was taken to be the mean of the two adjacent rows on either side of the ideal location. The total counts over the whole animal were then computed and adjusted to a standard value. To avoid errors arising from inaccuracies in measurement of landmarks from the paw tips and from the different degrees to which different animals were stretched, the position of two well-defined landmarks, at the back of the head and at the proximal ends of the femora (the "hips"), were also recorded during the scanning process. The final computer standardization was run in three sections, from the tip of the forepaws to the back of the head, from the back of the head to the hips, and from the hips to the tips of the hindpaws.

Finally, each scan, standardized for total radioactivity and for animal length, was graphically displayed on a lineprinter (Fig. 1). The total area under the curve corresponded to the total blood volume and was made the same for all animals, regardless of the actual blood volume. In addition to the graphical printout, the computer punched a series of IBM cards corresponding to each rabbit scan. These cards had a format corresponding to the graphic printout of Figure 1. Repeated scans of the same rabbit were reproducible within narrow limits (Fig. 2).

By means of a second program, the mean
Mean profile scan from nine living animals. X’s mark the mean profile and the other points indicate the approximate positions of ±1 se of the mean for values at each of the 101 segments.

Validation of Technique.—The theory underlying the use of scanning with a focused collimator to determine the quantities of radioactivity in various parts of an object is complex (4) because of the inevitability of some degree of overlap between successive lines of scan, and we have had recourse to phantom and other studies to determine the accuracy of our methods. The 19-hole focused collimator, which has a focus 7.5 cm from its face, consists of a symmetrical pattern of converging truncated cone-shaped holes in lead. This design enables the crystal to detect an almost constant emission from a radioactive source as the source is moved up to 20 cm away from the collimator in air.

A 2-ml source of red cells labeled with 51Cr was scanned at increasing distances from the collimator, both in air and in water. In air, the total counts in the scan were only 2% less at 10 cm than they were at 4 cm from the collimator. When the same source was scanned in water, with a 2-cm air gap between the collimator and water surface, the fall off in counts, expressed as a percent of the counts in the scan at 4 cm in air, was as follows: 2 cm of water, 94.5%; 3 cm, 88.5%; 4 cm, 90.0%; 5 cm, 67.8%. It therefore seems that a reasonably constant rate can be recorded to a depth of 4 cm in water.
LONGITUDINAL BLOOD DISTRIBUTION

TABLE 1

Comparison of Blood Content of Six Main Regions in Living and Dead Rabbits

<table>
<thead>
<tr>
<th>Region</th>
<th>Living (9 animals)</th>
<th>Dead (10 animals)</th>
<th>Difference with death</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head and forelimbs</td>
<td>13.8(0.2)</td>
<td>13.6(0.2)</td>
<td>+0.2*</td>
</tr>
<tr>
<td>Neck</td>
<td>27.8(0.1)</td>
<td>28.2(0.1)</td>
<td>-0.4</td>
</tr>
<tr>
<td>Thorax</td>
<td>44.1(0.3)</td>
<td>44.2(0.3)</td>
<td>-0.1</td>
</tr>
<tr>
<td>Abdomen</td>
<td>11.1(0.1)</td>
<td>11.2(0.1)</td>
<td>+0.1</td>
</tr>
<tr>
<td>Pelvis</td>
<td>85.1(0.3)</td>
<td>84.7(0.3)</td>
<td>-0.4</td>
</tr>
</tbody>
</table>

All contents are expressed as percent of the total blood volume, and the standard error of the mean is shown in parentheses.

*Significant at P < 0.05 level.

This apparent similarity masks an accumulation of blood in the upper abdomen and a loss of blood from the lower abdomen after death.

Organ phantoms made by filling cylindrical plastic bottles of 0.8, 1.0, 1.5, and 3.0 cm diameter with 5, 10, 20, and 50 ml respectively, of a suitable diluted solution of $^{51}$Cr were placed in random orientations on a board, and scanned with the collimator 10 cm above the board. After background subtraction, the total counts were as follows: 620, 1540, 2415, and 5004, giving a mean value of 113 counts/ml. The scanned phantoms gave individual values of 124, 124, 128, and 100 counts/ml. The reduction in the count rate for the largest bottle is probably due to increased depth of liquid in the bottle, with increased internal absorption. A nonradioactive rabbit carcass was interposed between bottles and collimator, with its chest and abdomen, including the spine, covering the bottles. Repeat scans of the 3-, 10-, and 50-ml bottles gave counts of 384, 596, and 1121, corresponding to individual values of 124, 124, 121, and 106 counts/ml. The reduction in the mean scan from the same animal scanned four times while supine, apart from a slightly accentuated central peak in the supine scan, there was little difference in the profile. The total counts (less background) in the four successive prone scans were 4720, 4694, 4668, and 4778, giving a mean value of 4715 counts (less background). There seems therefore to be little significant difference in attenuation of $\gamma$-rays from $^{51}$Cr when they pass through small bones, compared to soft tissues of the same depth.

The method was also evaluated by scanning a dead rabbit, both prone and supine, after freezing to prevent shift of organs with gravity. Figure 4 compares the mean scan computed from four successive scans of the same prone animal with the mean scan from the same animal scanned four times while supine. Apart from a slightly accentuated central peak in the prone scan, there was little difference in the profiles. The total counts (less background) in the four successive prone scans were 4800, 4804, 4608, and 4778, giving a mean value of 4715 counts (less background). The differences are clearly small and insignificant. To check that the radioactivity was still in the blood stream and had not become fixed in tissues, the abdomen of one rabbit was opened and the lower aorta and the inferior vena cava were cannulated in both antegrade and retrograde directions at the level of the celiac axis. We also cannulated the portal vein. While the animal was still alive, the total radioactivity in the body was counted by a Sorensen scan performed in the standard way. The animal was then bled as rapidly as possible through all the cannulas, and after all the blood had come out passively the animal was killed head up and then head down while the body was gently squeezed to extract as much blood as possible. We were able, by this means, to remove 70% of...
the calculated blood volume of the animal. This volume was measured in a cylinder and the blood put into a thin plastic container which was scanned in standard fashion. The total radioactivity was measured both in the removed blood, and in the blled body of the animal. The results showed that the volume of blood removed estimated by scanning was within 4% of the measured volume. It therefore seems unlikely that there is much trapping of red blood cells, and we think that this observation can be taken to mean that at least 90%, and probably almost 100%, of the labeled red blood cells are freely mobile in the circulation.

Removal of the major viscera in six animals was used as a further opportunity to test the accuracy of the scanning technique. After killing the animals with pentobarbital, the heart, lungs, liver, spleen, kidneys and the whole gut were removed after ligating the blood vessels. The blood contained in each organ immediately after death was dissected by laying the organs on a board and measuring them consecutively; the eviscerated body was also scanned. The sum of the counts recorded by the scanning technique from all the organs and from the eviscerated body was compared with the total radioactivity recorded in the preliminary scan. In addition, the completely eviscerated animal mentioned above was eviscerated, and its organs and the carcass also scanned. In no case was there a discrepancy greater than 6% between the values obtained from the whole animal and that obtained from the total of all its parts.

We feel therefore that the technique we have used to determine longitudinal distribution of red blood cells in the rabbit is valid and gives a good estimate of the blood contained in any particular segment along the length of the animal.

Results

The Living Animal—Mean red cell mass derived from the 14 animals in which it was determined was 17.6 g/kg (SE 1.2) and mean total blood mass 45.2 g/kg ± 2.5. The figure for total blood mass was determined from venous and not whole body hematocrit and is therefore only approximate. Figure 3 is a mean scan of the longitudinal distribution of blood, derived from scans in total living animals. It is evident that the distribution is far from symmetrical about the center of the animal, and the peak of the blood volume lies very close to the level of the incisura valve, which in the standardized scan lay approximately at segment 33, measured from the tip of the
Comparison of the mean blood distribution profiles for 10 dead and 9 living animals. Separation of the shaded areas of the two scans for more than two segments (1/50 of the total length of the scan) is unlikely to be due to chance.

outstretched forelimbs. The small hump on the left side of Figure 3 is contributed by the head. Almost the whole of the large central peak lies within the confines of the rib cage although it is by no means all within the pleural cavity. Remarkably little is present in the abdomen, but there is a small peak over the pelvis. A complete outline scan was performed with the ears spread out laterally, and this was not significantly different from the normal scan with ear tips lying over the chest. The amount of blood in the ears is therefore very small, even though the flow may be large.

Comparison of Living and Dead Animals. —Figure 5 compares the mean scan, with shading indicating 1 SE at each point along the length, from 10 dead animals (killed with pentobarbital) with that from 9 living animals. Table 1 shows the blood percent contained in each body region. There is a small difference in the head which is significant—the head of the dead animal contained about 3 ml more blood than that of the living one. The central peak is significantly increased in the dead animal, and it appears that about 3% of the total blood volume moved from the lower abdomen and pelvis toward the rib cage after death.

Comparison of Conscious and Anesthetized Animals.—Conscious rabbits will not tolerate stretching but can be induced to remain reasonably still for long periods by wrapping them tightly in a cloth. Figure 6 compares the mean profile of four conscious animals scanned from above, in the usual way, while they remained quietly prone, with similar scans obtained from the same animals after induction of anesthesia with sodium pentobarbital, as described. The standard error of the second set of scans is somewhat large because the animals changed position slightly during administration of the anesthetic. However, it is clear that anesthesia per se makes very little difference to the mean profile of longitudinal blood distribution.

Contribution of Individual Major Viscera to the Total Scan.—In six animals killed with...
pentoobarbital, the heart, lungs, liver, spleen, kidneys, and entire stomach and intestines were removed separately, and the total blood content of each organ was estimated. Care was taken to ligate all blood vessels without trauma or squeezing before removal. The results are shown in Figure 7, in which the average scan of these organs with the standard errors is indicated. The organs were laid out on a board in standard positions, and sequentially scanned in exactly the same way as for the whole animal. The volumes expressed as a percent of the total blood volumes are shown in Table 2. It is apparent that the liver contains much the largest proportion of the total blood volume. However, this blood is not fixed within the liver. Table 2 indicates that draining off the blood through the venae cavae, portal vein, and aorta removes most (about 80%) of that in the liver. Table 2 also gives values for two animals dying with gross pulmonary congestion following prolonged infusion of norepinephrine. In one of these animals most of the blood in the lungs had

TABLE 2
Relative Blood Content of Different Organs as Percent of Total Blood Volume

<table>
<thead>
<tr>
<th></th>
<th>Heart</th>
<th>Spleen</th>
<th>Kidney</th>
<th>Gut</th>
<th>Lung</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>9.1(0.3)</td>
<td>0.9(0.05)</td>
<td>3.2(0.1)</td>
<td>6.0(0.1)</td>
<td>5.6(0.1)</td>
<td>20.0(0.3)</td>
</tr>
<tr>
<td>Bled to death</td>
<td>3.4</td>
<td>0.1</td>
<td>1.0</td>
<td>2.8</td>
<td>1.9</td>
<td>5.8</td>
</tr>
<tr>
<td>Pulmonary congestion</td>
<td>12.5</td>
<td>0.4</td>
<td>1.9</td>
<td>4.0</td>
<td>21.3</td>
<td>28.4</td>
</tr>
<tr>
<td>(n = 2)</td>
<td>15.2</td>
<td>1.4</td>
<td>2.8</td>
<td>5.0</td>
<td>21.3</td>
<td>14.8</td>
</tr>
</tbody>
</table>

Data are means with standard error in parentheses.
Mean scans of excised organs from six animals. As in Figures 1 to 3, area corresponds with volume of labeled red cells. The total area has been reduced for each animal by an amount related to the ratio of total counts from the viscera to total counts from the eviscerated carcass.

In the animal bled as completely as possible, the viscera were removed and

been transferred from the liver; in the other, it had been mobilized mainly from other sites.
Schematic representation of the approximate contribution of each organ to the mean profile of blood distribution in the dead rabbit. Organs have been given their approximately correct position and correct areas.

Discussion

Most of the possible technical errors have already been discussed under Methods, and we are satisfied that the scanning technique used gives a reasonably accurate estimate of the distribution of red cells. In principle, estimates of regional hematocrit variation could be obtained by repeating this scanning technique with labeled plasma proteins. Recording by gamma camera might be necessary so that a quick estimate could be obtained by plasma protein distribution before the inevitable leakage from the vascular compartment altered the pattern. However, the size of object covered by most present gamma cameras is small, and although the scanning method is somewhat tedious, we believe that it is more accurate because the use of a traveling focused collimator at least partly overcomes problems of variable tissue depth.

The choice of animal size is somewhat crucial for this technique. Scanning a small...
animal such as a rat would magnify the inaccuracies of the method in relation to animal size; however, scanning a dog would take a long time, and absorption by the greater depth of tissue would pose severe problems. It would therefore seem that our technique can be easily applied only to animals of the size of rabbit or cat.

Isotopic methods have been used before to detect regional blood distribution. For example, a previous investigation (5) in dogs used unfocused counters above and below the thorax and the abdomen to estimate splanchmic blood pooling during shock. However, no attempt was made to identify different parts of the abdomen with respect to blood content, and the use of an unfocused collimator means that the limits of the region counted cannot be accurately defined.

It is surprising to find such a high proportion of the total blood in the liver, which appears to contain very nearly 30% of all the red blood cells of dead rabbits and probably at least 25% of the total blood of the living animal. The location of this large reservoir very close to the tricuspid valve is likely to contribute to the stability of the circulation during head-up and head-down tilting. When the actual longitudinal distribution of blood volume derived from the mean scan of dead animals was inserted in the computer model previously mentioned (1), there was no difficulty in reproducing in the model the observed behavior of central venous pressure stability during tilting (Dickinson, unpublished observations). This gives us further reason to suppose that the longitudinal distribution which we have shown is approximately correct and suggests that it may have physiological significance.

References
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