Regional Cerebral Blood Flow in Dogs Using a Particle-Distribution Method* (34079)

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(Introduced by M. W. Meyer)

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Methods of determining cerebral blood flow such as the Kety-Schmidt technique (1) or external monitoring of injected or inhaled radioactive material (2–5) are of limited value since regional cerebral blood flows cannot usually be determined. It has been suggested that when diffusable indicators are used in the fractionation technique the requirement that extraction ratios for all tissues be identical is not generally met (6). Incorporating particle indicators in this technique could satisfy this requirement. In this study, labeled carbonized microspheres were used to estimate regional cerebral blood flow in dogs.

Preliminary studies using nonlabeled microspheres suggest that these particles are distributed in the same manner as the blood flow (7). More recent studies (8–12) have also suggested that labeled microspheres may be distributed in the same manner as the blood flow and that the extraction ratio approaches unity. It was felt that the particle-distribution method might provide a means of comparing and assessing the isotope clearance techniques (2–5) in experimental animals.

Method. Small (7–15kg) dogs lightly anesthetized with sodium pentobarbital were used. Artificial respiration was not provided. To determine cardiac output a known activity of ^{42}K or ^{86}Rb was injected intravenously. Continuous sampling of aortic blood activity for 20–30 sec after injection provided an isotope dilution curve from which cardiac output was estimated. The left ventricle was catheterized via the right brachial artery. Carbonized ^{169}Yb-labeled microspheres with a mean diameter of about $25 \pm 5\mu$ (SD) and a density of about 1.5 g/ml (8) were suspended in dextran to give a concentration from about 0.5 to 1.5×10^6 spheres/ml depending upon the desired activity to be administered. A known activity (A_0) in 1 ml of this suspension was washed from a chamber and injected slowly (80–100 sec) into the left ventricle. The activity injected was estimated by counting standard ^{169}Yb samples as described previously (9). Left ventricular, arterial, and venous blood samples were taken about 40 sec after completing the bead injection. Animals were then killed with saturated potassium chloride. The brain was removed, weighed, and frozen. Samples of cerebral cortex, cerebellum, caudate nucleus, thalamus, and white matter were placed in small vials, weighed, dissolved with nitric acid, and counted in a well scintillation counter to determine the sample activity per unit weight (A/wt.). The remaining brain tissue was weighed, dissolved in saturated potassium hydroxide, and counted to estimate an average activity per unit weight of total brain. The equation: $F = (A/A_0 \cdot wt)\ CO$ (Where $A/A_0 \cdot wt$ is the fractional distribution of the particles per unit weight and CO = cardiac output) was used to estimate flow values (F) in ml/min · g.

The animals were divided into two groups. Group I consisted of 6 old and 10 young dogs breathing air. Group II consisted of 8 young dogs breathing 5% CO_2 in air from a bag

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TABLE I. Regional and Total Brain Blood Flow (ml/min • g).
(Group I: Dogs Breathing Air)

	Regional brain flow[b]					TBF[c]		
Dog no.[a]	Cc	Ce	Wm	Ca	Th	ml/min • g	ml/min	% CO
1	3.48	3.10	1.16	1.72	1.76	1.92	126.7	8.5
2	2.94	1.68	0.93	1.50	0.06	1.77	148.7	5.5
3	1.58	1.61	0.68	1.54	1.30	1.43	99.0	6.2
4	3.09	1.93	0.67	2.27	1.58	1.76	149.6	6.1
5	2.00	2.43	0.87	2.18	2.32	1.73	156.0	5.0
6	1.46	1.30	0.50	1.13	1.27	1.28	115.0	2.6
7	0.79	0.98	—	—	—	0.84	65.9	1.9
8	0.52	0.62	—	—	—	0.52	39.7	1.1
9	1.07	1.03	—	—	—	0.98	73.3	2.5
10	0.84	1.01	0.39	0.93	0.66	—	—	—
11	0.65	0.92	—	—	—	0.51	40.1	1.4
12	1.47	1.11	0.84	1.38	1.13	0.99	71.3	2.3
13	2.05	2.66	1.18	1.77	1.53	1.45	112.4	3.7
14	1.39	1.28	0.81	1.58	0.92	0.95	82.8	2.6
15	0.93	0.94	0.29	0.93	1.02	0.70	72.1	2.0
16	1.76	1.75	0.46	1.44	1.20	1.22	89.0	3.4
Mean								
Old	2.42	2.01	0.80	1.73	1.50	1.65	132.5	5.7
Young	1.15	1.23	0.66	1.34	1.08	0.91	71.8	2.3
All	1.62	1.52	0.73	1.53	1.31	1.20	96.1	3.7

[a] Numbers 1–6, old dogs (12 mo.); 7–16, young dogs (5–7 mo.).
[b] Cerebral cortex (Cc), cerebellum (Ce), white matter (Wm), caudate (Ca), thalamus (Th).
[c] Total brain flow (TBF), and % of cardiac output (% CO).

during the experiment. In some of the Group I animals and in all of those in Group II, arterial (CO_2) was measured in volumes percent. A coronal section 5–6 mm in thickness was cut from the brain of several dogs in each group and prepared for autoradiography. The autoradiographs were superimposed on the brain section to observe the distribution of the microspheres.

Results. Regional cerebral blood flow values for Group I animals are shown in Table I. Six of these animals were approximately 1 year old, whereas the remainder ranged from 5 to 7 months. Blood flow values tended to be

TABLE II. Regional Blood Flow (ml/min • g).
(Group II: Young Dogs Breathing 5% CO_2 in Air)

	Regional brain flow[b]					TBF[c]		
Dog no.[a]	Cc	Ce	Wm	Ca	Th	ml/min • g	ml/min	% CO
1	4.90	4.46	1.67	4.55	3.91	3.76	325.6	9.4
2	3.35	2.96	1.00	3.91	5.13	2.87	254.6	8.0
3	5.71	4.11	1.37	6.76	6.42	5.54	489.2	14.4
4	5.07	5.07	1.26	6.49	8.15	5.17	443.6	16.3
5	1.58	2.06	0.59	2.19	1.20	1.46	121.3	3.5
6	3.79	4.07	1.62	3.60	2.52	3.32	224.1	9.4
7	4.47	3.99	0.76	7.00	7.00	3.66	229.1	8.5
8	2.40	2.32	1.03	2.09	2.28	1.51	129.4	5.4
Mean: (8)	3.91	3.63	1.16	4.57	4.58	3.41	277.1	9.4

For explanation of abbreviations see Table I.

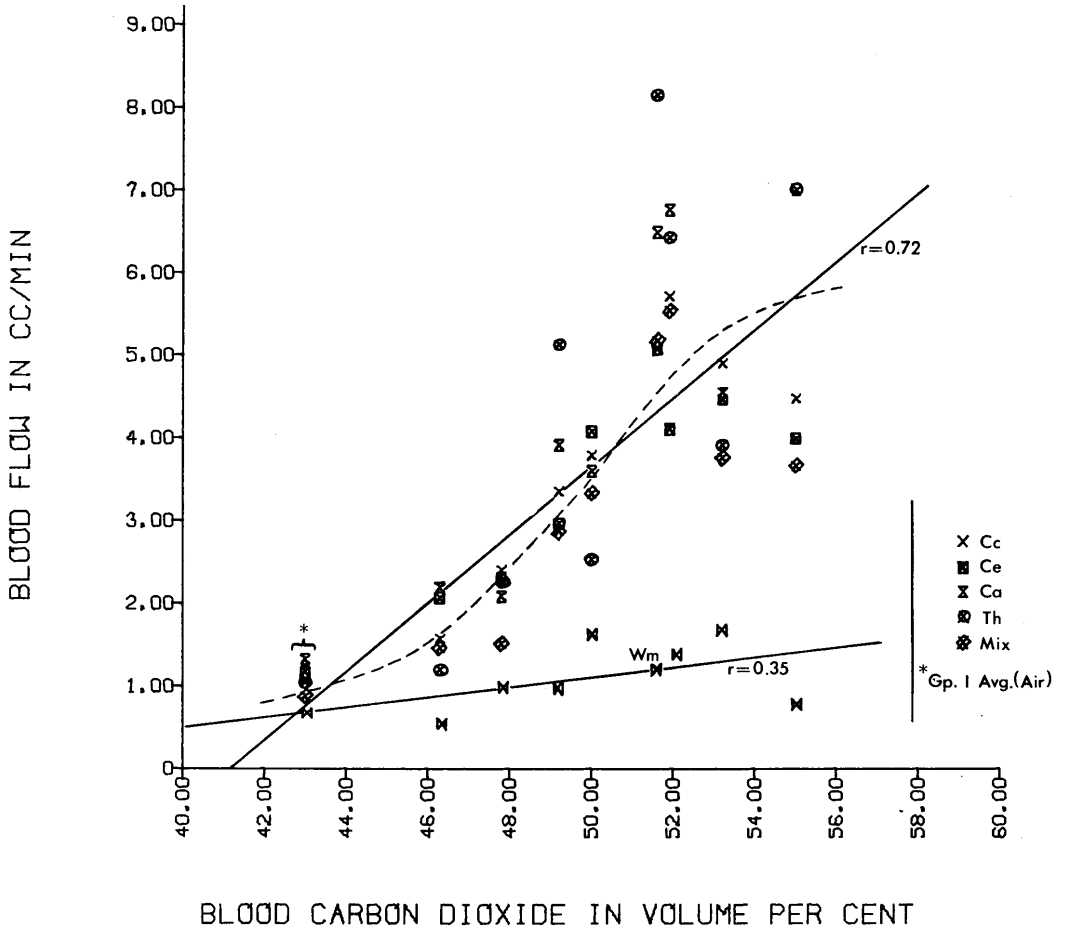

FIG. 1. A computer plot relating flow (ml/min • g) to blood CO_2 in volumes percent for five tissues and mixed samples (See Table I for abbreviations). The regression line for all Group II data points except white matter has a correlation coefficient (r) = 0.72. The regression line for white matter alone has $r = 0.35$. If the average values for Group I animals were included with all Group II data points except white matter, perhaps a sigmoid relationship would exist as shown by the dotted line.

greater in the older animals. This is also shown by the percentage of cardiac output represented by the total brain flow which averaged about 2.5 times greater for older dogs.

The regional blood flows for Group II animals are shown in Table II. Except for the white matter, the flows to the brain and various tissues were 3–4 fold higher in Group II. The percentage of cardiac output was about four times higher in Group II animals than the average for young animals in Group I.

In Fig. 1, regional and total brain flow values for Group II animals are plotted against the arterial (CO_2) in volumes per cent. The "least squares" regression line for white matter has a correlation coefficient (r) of 0.35. An average regression line for the remaining data is also shown ($r = 0.72$). Correlation coefficients were also calculated for individual regions (cerebral cortex, 0.83; cerebellum, 0.79; caudate, 0.86; thalamus, 0.72) and total brain flow (mixed sample, 0.70). Blood flow and arterial (CO_2) are significantly correlated (p values $<.05$) for all regional and total brain flows except the white matter.

Concurrent studies indicated that the per-

centage of cardiac output going to the lung averaged about 1.5% as calculated from the fractional uptake of either labeled particles or the ionic tracer (^{42}K or ^{86}Rb) injected intravenously to obtain the isotope dilution curve. If all shunted particles were trapped by the pulmonary vessels, then the overall shunting would have to be less than 1.5% of the total injected activity. Intravenous injection of a known activity of labeled particles showed that the fractional extraction by the pulmonary capillaries was about 99.5%.

A representative autoradiograph, Fig. 2, demonstrates the distribution of particles in a coronal section of the brain. Comparison of the number of particles per unit area in the cerebral cortex to the number in the white matter would indicate that the cortex had a higher flow. The autoradiograph also suggests that particles may be distributed as the blood flow (number on right side = number on left side). Autoradiographs taken from dogs breathing 5% CO_2 also showed similar bilateral distribution, but the number of spheres were dramatically increased.

Discussion. Regional blood flow in cortical areas and white matter has been determined by compartmental analysis using isotope-clearance techniques (2–5). Only a limited number of studies (3, 14), however, have presented data on brain blood flow to other areas. In this study the particle-distribution method was not combined with the external monitoring approach in the same experimental animal. Our results, however, can be compared to those obtained by others using isotope-clearance techniques. Häggendal and Johansson (2) using the ^{85}Kr clearance technique in small dogs anesthetized with sodium pentobarbital and artificially ventilated found that cerebral cortical flow values ranged from 0.42 to 0.96 ml/min · g. It has been observed, however, that cardiac output in dogs could be reduced 20–35% by artificial ventilation at a rate and tidal volume approximating that during the unanesthetized state (unpublished data). If there is a corresponding 25% reduction for regional and total brain flow in our study, then the range for cortical flow in Group I young dogs would be 0.39–1.54 ml/min · g. Perhaps by using the clearance technique and particle-distribution method in the same animal the results of each method could be better assessed.

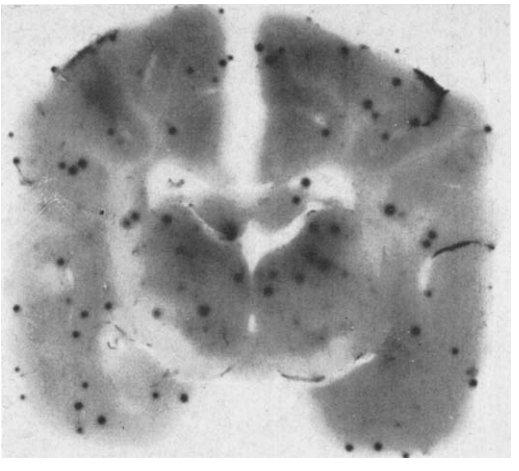

FIG. 2. An autoradiograph of Group I animals obtained with a 4-day exposure. It has been superimposed over the corresponding tissue section about 2 mm thick after it was microtomed. Apparent differences in bead size are due to variation in depth of the microspheres in the tissue section. The majority of particles are in the cortex and thalamus. There are relatively few in the white matter.

The effect of CO_2 on regional brain flow as determined by the particle-distribution method appeared to be much more dramatic than that reported by others (2, 5, 13). The increase in flow values between young dogs in Group I and Group II animals ranged from 76% (white matter) to 324% (thalamus). The cortex showed an increase of 240%. In cats under light thiopental anesthesia breathing 5% CO_2 the mean increases observed by others (13) were 54% for white matter and 67% for the cerebral cortex.

It is assumed that particles trapped initially have little or no effect on blood flow before subsequent microspheres are caught. It is possible that the initial microsphere embolism causes metabolic changes that lead to a marked decrease in resistance to flow. However, since relatively large numbers of microspheres were injected initially, trapping would tend to cause an increase in resistance rather than a decrease. This would tend to offset any such metabolic effects. Further-

more, if vasodilatation occurred, then the probability diminished that all the 25-μ particle would remain caught and flow values may be underestimated. Errors due to determining the amount of injected microsphere antivity averaged $\pm$ 2.5% of the total activity. Such an error cannot account for the large increase in blood flow produced by inhaling 5% CO_2. This increase in flow appears to be at the expense of blood flow to skeletal muscle (unpublished data). The change in regional blood flow is significantly correlated with the arterial (CO_2) as suggested by others (2, 13).

Table III compares the results of this study with those obtained by other workers (10, 12) using 50-μ carbonized microspheres. Their values for total brain flow and percentage of cardiac output represented by total brain flow are of the same range and magnitude as the values reported in this study. The difference in flow values between old and young dogs breathing air may be due to higher arterial (CO_2) in the older dogs. In several of the older animals in which arterial (CO_2) was measured, the (CO_2) was higher than that observed in any of the younger dogs.

It appears that there is a wide variation in blood flow to different regions of the brain. Comparing the results of different methods is difficult. The arterial (CO_2), the anesthetic agent, if one is used, and the difference in techniques must be considered. Methods for testing the validity of potential clinical approaches that provide a qualitative or quantitative measure of regional cerebral blood flow seem essential. The particle distribution technique may provide such a method.

Summary. In this study a known activity of microspheres labeled with ^{169}Yb was injected into the left heart of lightly anesthetized dogs. Prior to injection of microspheres, cardiac output was determined by the isotope dilution technique using ^{42}K or ^{86}Rb. Blood flow to the cerebral cortex, white matter, thalamus, cerebellum, and caudate nucleus was determined in ml/min • g from fractional distribution of particles in tissue samples. Total brain blood flow in ml/min·g was estimated from the radioactivity observed in the remaining brain tissue. Comparison of dogs breathing air with dogs breathing 5% CO_2 showed that flow values were significantly increased ($p<.05$) with 5% CO_2 for all areas except white matter. Old dogs tended to show higher flow values than young dogs. Flow values obtained in this study were compared with values reported by others using different methods. The distribution of the particles in the brain was checked by autoradiography.

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TABLE III. Comparison of Total Brain Flow in ml/min • g and Related Percentage of Cardiac Output in Dogs and Other Animals when Using Radioactive Microspheres.

	Total brain flow		Percentage cardiac output	
	Average	Range	Average	Range
This study				
Group I dogs				
Old	1.65	1.28–1.95	5.7	2.6–8.5
Young	0.91	0.51–1.45	2.3	1.1–3.7
Neutze *et al.* (10)				
Rabbits				
Anesthetized	1.03	0.51–2.62	1.8	0.9–4.1
Unanesthetized	0.75	0.48–1.30[a]	1.2	0.8–1.5[a]
Rudolph *et al.* (12)				
Sheep fetus	1.5	0.3–2.8	4.9	2.0–8.8

[a] Reported as 10–90 percentile.

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