Regional Hematocrit Ratio and Interstitial Fluid Volume in the Normal Rat

Red cells, plasma and extracellular fluid volumes in rats' tissues have been measured by several investigators in the past years[1–4]. In some papers it is possible to deduce from their data the hematocrit ratio of the blood contained in organs or tissues. However the appraisal of the hematocrit ratio depends on how precisely the red cell and plasma volumes have been determined.

For a careful measurement of red cell and plasma volumes by means of the dilution of radioactive tracers, it is necessary to wait a certain time (mixing time) before sampling the blood for measurement of the concentration of indicator. Furthermore variations with time of the concentration of the indicator after complete mixing can give false results; to avoid this error, multiple sampling and semilogarithmic extrapolation to 'zero time' can be used.

Since previous investigators in this field did not employ an appropiate mixing time[1] and/or did not use multiple sampling to avoid errors derived from changes in the concentration of the indicator[2–4] it seemed fit to study red cell, plasma volumes and extracellular fluid volume in several tissues of normal male rats in order to calculate the regional hematocrit ratio and interstitial fluid volume in tissues.

Methods. Seventy-eight male rats from the strain bred at the Instituto de Fisiologia de Buenos Aires, ranging in weight from 183–237 g were anaesthetized with sodium pentobarbital (40 mg/kg body weight) and heparinized (1 mg/100 body weight).

Each animal was injected i.v. (femoral vein) with a radioactive tracer, and the activity injected was determined in cpm as described previously[5]. At a fixed time after injection a sample of arterial blood was taken and testis, kidneys, spleen, liver, heart, lungs and skin were removed without adopting special measures to prevent blood oozing from tissues. Similar aliquots of blood, plasma and organs were placed in plastic containers and their activity determined with a well scintillation counter and a decimal scaler, ensuring a similar geometric efficiency for each reading.

Volume of distribution of the indicator was calculated from the following general equations:

$$\frac{\text{cpm injected} \times 100}{\text{cpm in ml of plasma or blood} \times \text{body weight}} = \text{ml/100 g body weight}$$

$$\frac{\text{cpm in 1 g of tissue} \times 100}{\text{cpm in 1 ml of plasma}} = \text{ml/100 g tissue weight}$$

Simultaneous measurement of red cell and plasma volumes were made with chromium^{51} tagged red cells and radioiodinated serum albumin (I^{131}), in 3 groups of 7–10 animals, 15, 30 and 45 min after injection of the tracer, since mixing time for these tracers is less than 10 min, as previously determined[5].

Extracellular fluid volume was measured as the volume of distribution of sodium, utilizing $Na^{22}Cl$ as tracer, in 3 groups of 8–10 animals, 30, 45 and 60 min after injection, since mixing time for this tracer is less than 20 min as previously determined[6].

From the results obtained at each time of sampling the actual volume of distribution was calculated for each tracer by extrapolation to 'zero time' by means of the semilogarithmic regression line equation.

Hematocrit ratio was calculated from the equation

$$\frac{\text{Red cell volume} \times 100}{\text{Red cell volume} + \text{plasma volume}}$$

Interstitial fluid volume was calculated as follows: extracellular fluid volume – plasma volume. Blood volume was equal to red cell volume plus plasma volume. From the regression line the rate of variation of concentration for each indicator in tissues was calculated and expressed as % of change each 30 min (Table I).

Results. Table II shows the volume of distribution in tissues of each indicator employed: the hematocrit ratio, blood volume and interstitial fluid volume calculated are shown in Table III.

From our results it is possible to deduce that regional variations of concentration of the indicator does not follow the sense and rate of change of the indicator in arterial blood. This can be attributed to local changes in the vascular bed and/or metabolization of the indicator in tissues. Furthermore the differences of concentration of red cells and plasma in tissues and arterial blood are so wide, that no inference of the local hematocrit ratio

Table I. Variation of concentration of indicator in tissues, expressed as % of change each 30 min

	Tagged red cells chromium^{51}	Radioiodinated serum albumin iodine^{131}	Sodium chloride sodium^{22}
Blood	− 1.12	− 0.56	− 7.80
Testis	− 2.16	− 2.58	+ 0.55
Kidney	− 24.62	− 2.66	+ 7.69
Spleen	− 102.30	+ 22.66	+ 3.53
Liver	− 76.35	+ 8.66	+ 2.74
Myocardium	− 3.74	− 6.35	+ 5.64
Lung	+ 1.25	+ 5.12	+ 8.10
Skin	+ 20.28	+ 44.20	+ 1.37

Table II. Red cell, plasma and extracellular fluid volume in tissues in ml/100 g (mean ± S.D.)

	Red cell volume	Plasma volume	Extracellular volume
No. of animals	23	29	26
Average weight g	219	224	193
Whole body	3.13 ± 0.44	4.31 ± 0.12	24.99 ± 1.09
Testis	0.35 ± 0.04	2.05 ± 0.06	14.40 ± 1.39
Kidney	1.50 ± 0.20	10.98 ± 0.29	45.62 ± 2.12
Spleen	14.88 ± 7.20	10.97 ± 1.81	20.98 ± 0.46
Liver	2.04 ± 0.86	8.44 ± 0.52	17.54 ± 0.61
Myocardium	5.06 ± 0.27	9.16 ± 0.10	32.61 ± 1.45
Lung	6.48 ± 0.59	16.50 ± 0.83	48.67 ± 3.30
Skin	0.62 ± 0.21	4.55 ± 1.76	37.35 ± 1.45

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can be obtained through measurements performed in arterial blood.

Our results are very different, and not comparable, with those obtained by other investigators who did not use an adequate mixing time of the indicator[1]. They also differ from those obtained by D'AGOSTINO[2], EVERETT et al.[3] and BOCCI et al.[4] in the rat, who employed a single sample after mixing was completed.

Regional plasma volume was measured in mice by FRIEDMAN[7], KALISS and PRESSMAN[8] and WISH et al.[9], and regional hematocrit calculated on the assumption that there is no difference between arterial blood hematocrit and organs' blood hematocrit. As we have shown in our results their assumption can not be sustained and the regional hematocrit ratio expressed in their results can not be accepted as accurate.

GIBSON et al.[10] measured regional red cell and plasma volumes and hematocrit ratio in dogs; their results are higher than ours in rats, this being due probably to their technique of single sampling of blood and tissues, besides the difference in species.

Table III. Hematocrit ratio, blood volume and interstitial fluid volume in tissues (mean ± S.D.)

	Hematocrit ratio	Blood volume ml/100 g	Interstitial fluid volume ml/100 g
Whole body	42.08 ± 2.55	7.44 ± 0.55	20.68 ± 0.97
Testis	14.63 ± 1.22	2.41 ± 0.11	12.35 ± 1.33
Kidney	11.99 ± 1.11	12.48 ± 0.49	34.64 ± 1.83
Spleen	57.56 ± 6.95	25.85 ± 9.00	10.01 ± 1.35
Liver	19.44 ± 5.79	10.48 ± 1.38	9.10 ± 0.10
Myocardium	35.59 ± 0.97	14.21 ± 0.37	17.54 ± 0.61
Lung	28.19 ± 0.83	22.98 ± 1.42	32.16 ± 2.47
Skin	11.93 ± 0.54	5.16 ± 1.96	32.80 ± 0.30

It should be emphasized that variations of the concentration of indicators are very important, so it is desirable to apply multiple sampling in every determination of total or regional volumes of distribution whenever precision is required.

Conclusions. Determination of regional hematocrit ratio and red cell, plasma and extracellular fluid volume should be done allowing an appropriate mixing time for the indicator and avoiding errors due to changes in local concentration of tracers. Normal values show that hematocrit ratio and volumes are different for each tissue and can no be deduced from arterial hematocrit ratio or corporal red cell plasma and extracellular fluid volumes.

Résumé. La détermination, dans les tissus, du volume globulaire et plasmatique, ainsi que de l'hématocrite, doit être pratiquée après un temps de mélange approprié et en évitant les erreurs dues à la variation locale de la concentration d'indicateur. Les valeurs normales sont fort différentes pour les divers organes étudiés et ne peuvent pas être calculés à partir des résultats obtenus par la mesure de l'hématocrite ou des volumes plasmatiques et globulaires corporels dans le sang artériel.

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Degeneration Activity after Sympathetic Denervation of the Submaxillary Gland and the Eye

While their parasympathetic postganglionic nerves are degenerating, salivary glands have been found to show a 'degeneration secretion'[1-5] mainly caused by increased leakage of acetylcholine[6]. Transmitter release from degenerating sympathetic fibres could also be demonstrated, but only when the gland cells had been highly sensitized and even then only in 10 cats out of 20. These experiments started about 2 days after denervation, but this may not be the optimal time in the case of sympathetic denervation[7]. Recent experiments show that 'degeneration contraction' of the nictitating membrane starts already 18–30 h after sympathectomy[8].

In 10 cats the right submaxillary gland was sensitized by section of the chorda-lingual nerve, and 2–6 weeks later the right superior cervical ganglion was excised; ether anaesthesia was used; 19–25½ h after sympathectomy, chloralose was given, both submaxillary ducts were cannulated and both nictitating membranes connected to frontal writing levers.

Degeneration contractions of the denervated membranes as previously observed in unanaesthetized[8] or spinal cats[9] were seen in all the cats. In 4 cases they started 20½–24 h after ganglionectomy, to proceed to a maximum and disappear gradually within 7–10 h. In 2 cats the contraction had begun, in 3 it had reached a maximum and in 1 cat the membrane was relaxing when the experiment started (24–25½ h after ganglionectomy). Sometimes small contractions were superimposed on the denervation contraction, as described in the spinal cat[9], and they could occasionally occur when the membrane had relaxed also (see Figure).

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