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Determination of Uterine Blood Volume and Correlation with Ovarian Function[1] (38287)

M. J. BRODY,[2] K. E. CLARK, L. EDVINSSON, CH. OWMAN, AND N.-O. SJOBERG

Departments of Histology and Obstetrics and Gynecology, University of Lund, Lund, Sweden, and Department of Pharmacology, College of Medicine, University of Iowa, Iowa City, Iowa 52242

Changes in blood flow to the uterus as well as alterations in the intrauterine distribution of blood flow play important roles in the cyclic changes in morphology which the uterus undergoes. A primary example is the increased vascularization which is associated with endometrial proliferation. Several methods have been applied for quantitative evaluation of uterine hemodynamics. In larger animals, such as sheep, the ideal can be achieved with implanted electromagnetic flow probes which allow for continuous measurements of uterine blood flow in conscious animals (1, 2). As is the case with many measurements of cardiovascular function, the situation is greatly complicated in small animals, *e.g.*, rodents, in which continuous measurements of blood flow in an organ such as the uterus are not technically feasible at the present time. Since rodents are so widely used for endocrinological purposes, it would be very useful to have methods available for convenient estimation of changes in the hemodynamic status of the uterus.

The present study documents and validates a simple technique for demonstrating changes in uterine blood volume. This technique appears to provide a reliable index of hemodynamic changes in a variety of experimental situations known to be associated with alterations in uterine vascular resistance.

Methods. Commercially available radioiodine-labeled human serum albumin (RIHSA) was used for the measurement of uterine blood volume. The technique is similar to that applied for example for measurement of cerebral blood volume (3). The principle underlying the method (4) is that the amount of plasma (*i.e.*, the amount of blood at unchanged hematocrit) in an organ can be determined by simultaneous measurement of the concentration in blood of a tracer (presumed to be nondiffusible) and the total content of the tracer in the tissue to be studied.

In mice (NMRI strain, weighing approximately 25 g), a solution of RIHSA (1 μCi) was injected during 10 sec into a tail vein in a volume of 0.1 ml (0.3–0.5 mg albumin per ml of 0.9% saline). Rats (Sprague–Dawley, 180–200 g body wt) were lightly anesthetized with ether, the femoral vein exposed, and RIHSA (10 μCi) in a volume of 0.1 ml (3–4 mg albumin per ml of 0.9% saline) was injected. The incision was closed with a wound clip and the animal allowed to regain consciousness. It had previously been determined that radioactivity in the blood reached a stable level within 5 min following administration of the tracer and remained stable for periods up to 60 min (3). In order to insure that radioactivity in the tissue at any moment was a reflection of that in blood, the animals were killed instantaneously 5 min following administration of RIHSA: mice by immersion into liquid nitrogen and rats by cervical dislocation followed by immersion in liquid nitrogen. Care was taken to limit immersion time to only that required to stop the blood circulation but not to freeze the internal organs. Blood samples (25 μl in mice, 100 μl in rats) to be used as internal reference standard were removed from the left ventricle in mice or the vena cava in rats after the animal was opened. The uterus was exposed by a midline abdominal incision and was dissected free. Both uterine horns were cut at the vaginal

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and tubal junctions, all loose parametrial tissue being stripped away from the organ. The uteri of mice were weighed on tared wax paper and placed at the bottom of a plastic counting tube, while the uteri of rats were placed directly in preweighed glass counting tubes and then weighed. Blood samples were placed in counting tubes containing 2 ml of distilled water for rinsing the transferring pipette.

In some experiments, samples of intestine were removed for comparison with uterus: small segments of ileum were dissected free from their vascular and mesenteric connections, weighed, and counted. Radioactivity was determined with a gamma spectrometer (Packard). Volumes of blood were calculated by dividing cpm/g tissue by cpm/ml blood, and the data on blood volume are given as mean (± SEM) values of μl blood per g wet wt in mice and both wet and dry weight of tissue in rats.

Some of the mice were ovariectomized (through bilateral incisions in the back under ether anesthesia) 2–4 weeks prior to blood volume measurements. Other animals were studied during the estrus cycle, the stages being monitored in standard fashion by microscopic evaluation of stained vaginal smears. Ovariectomized mice (operated 4 weeks prior to hormone injections) were treated with estrogen in two different types of experiments. In the first series, estrogen (17-β-estradiol benzoate) was administered in a dose of 0.3 μg subcutaneously on each of 2 days prior to measurement of uterine blood volume. Control animals received the same amount of solvent (peanut oil). In the second series of experiments, ovariectomized animals received 1 μg of estradiol intravenously and uterine blood volume was measured 30, 60, and 120 min after administration of estrogen. Control animals were injected with the same amount of alcohol–saline vehicle. In a third series of experiments, blood volumes were measured in animals in three phases of the estrus cycle, namely, diestrus, proestrus and estrus.

Rats were bilaterally ovariectomized, and on the 7th day following ovariectomy, they received an estrogen supplement in the form of a subcutaneous injection (0.1 μg/100 g body wt) of estradiol given as estradiol benzoate dissolved in corn oil. On the 14th day, animals were lightly anesthetized with ether and the femoral vein was exposed for injection. Half the animals received 0.5 μg/100 body wt of 17-β-estradiol (0.5 μg/0.1 ml) and the other half received the vehicle (0.1 ml/100 g). The femoral incision was closed with a wound clip and the animals allowed to regain consciousness. In a first series of experiments on the rats, uterine blood volume was determined 2 hr after administration of estrogen. In a second experiment, the uterine blood volumes were measured at 5, 30, 60, 120, and 240 min after estrogen administration. In a third series of experiments, the validity of estimating uterine blood volume with RIHSA (MW 70,000) was determined. This was done by comparing the uterine blood volume measurements made with RIHSA-^{131}I (RIHSA space) with those obtained by using a protein of much larger size, radioiodine-labeled bovine gamma globulin-^{125}I (RIBGG^{125}I, MW 160,000). The RIBGG-^{125}I was prepared using the method of Hunter and Greenwood (5). In a subsequent experiment, RIBGG-^{125}I (10 μCi) and ^{51}Cr-labeled rat red blood cells (30 μCi) were administered simultaneously to the same rat. Red blood cells were labeled with 1 mCi of Chromitrope Sodium in Modified A-C-D Solution (Medotopes, E. R. Squibb and Sons, Inc.). In all experiments designed to test the validity of the method, uterine blood volume was determined 2 hr after either estrogen or the vehicle was administered.

Results. Changes in uterine blood volume and weight associated with the estrous cycle are depicted in Fig. 1 (left side). There was a similar increase in blood volume and organ weight as the cycle progressed from diestrous to estrous. A

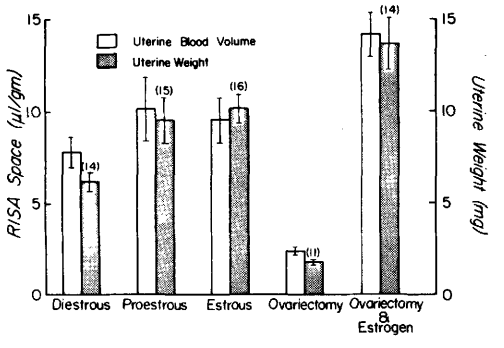

FIG. 1. Uterine blood volume (RIHSA) and uterine wet weight in mice: variations associated with the diestrus, proestrus, estrus phase and the effect of 0.3 μg of 17-β-estradiol benzoate (E) given subcutaneously once daily for 2 days to ovariectomized animals. Mean ± SEM, number of determinations within parentheses.

peak effect on volume was reached during proestrous. Visually the uteri were small and pale during diestrous whereas they appeared enlarged and hyperemic in proestrus and estrus.

The effect of protracted estrogen treatment on ovariectomized mice is shown in Fig. 1 (right side). It should be noted that blood volume in ovariectomized animals was lower than in normal animals during diestrus. The former uteri were noticeably small and pale. Estrogen treatment induced a large change in both uterine blood volume and uterine wt. The increase was in the magnitude of 5–6 times in each parameter.

The acute effects of estrogen administration to ovariectomized mice are summarized in Fig. 2. This figure includes the effects of the vehicle in which estrogen was dissolved. No changes in uterine blood volume were observed over the 2-hr period of observation when the animals received an intravenous administration of the same volume of vehicle. However, uterine blood volume increased dramatically 30 min after the administration of estrogen, appeared to peak at 60 min, and returned to control level 2 hr after administration. In contrast to the chronic effects of estrogen administration, no significant changes in uterine weight occurred. In the same experiment, the effects of estrogen on intestinal blood volume were compared with those of uterine blood volume. No statistically significant changes in intestinal volume or wt were observed during the period when uterine blood volume increased dramatically.

The acute effects of estrogen administration on ovariectomized rats were similar to those seen in mice (Table I). The uterine blood volume (RIHSA space) measured 2 hr after estrogen administration was 94 ± 3 μl/g (mean ± SEM) of tissue as compared to 52 ± 2 μl/g in animals receiving only the vehicle. These values are significantly different and are based on a wet tissue weight. The values on a dry weight basis were 217 ± 17 μl/g in animals receiving vehicle and 416 ± 26 μl/g in animals receiving estrogen. Thus uterine blood volume nearly doubled and the uteri appeared visibly hyperemic. Although blood volume (RIHSA space) increased significantly in the uterus, there was no statistically significant change in intestinal blood volume.

The time course of estrogen-induced hyperemia in the rat can be seen in Fig. 3. This figure shows that there is no significant change in uterine blood volume in control animals during the 4-hr period. However, after the administration of estrogen, uterine blood volume was increased by 60 min, appeared to peak at 2 hr and returned toward control levels 4 hr after administration. As in the mouse, no significant changes occurred in either uterine wt or intestinal blood volume.

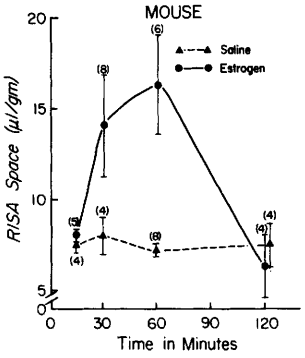

FIG. 2. Uterine blood volume (RIHSA) in mice at different time periods after a single injection of 1.0 μg of 17-β-estradiol benzoate or vehicle (corresponding amount of alcohol–saline). Mean ± SEM, number of determinations within parentheses.

Since several authors (7, 8) have suggested that increased uptake of RIHSA by uterine tissue following estrogen is the result of increased permeability, it was necessary to validate the method as a reliable indicator of changes in the magnitude of the uterine vascular space. In Table I are shown the mean values (± SEM) for uterine blood volumes of the rat on a wet and dry basis, measured with RIHSA-^{131}I, RIBGG-^{125}I, and ^{51}Cr red blood cells. The uterine blood volume measured with labeled plasma proteins, RIHSA-^{131}I or RIBGG-^{125}I, are not significantly different from each other for either the control or estrogen treated animals.

Since it is conceivable that even gamma globulin might pass through uterine capillary pores, whose size have been increased by estrogen, the last experiment was designed. Rat red blood cells labeled with ^{51}Cr were injected into rats simultaneously with ^{125}I-labeled gamma globulin. The uterine blood volume determinations for both methods are shown in Table I. Although the blood volume determined by the ^{51}Cr red cell method is smaller than the ^{125}I-labeled gamma globulin (RIBGG space), both values are increased to approximately the same extent following estrogen administration.

TABLE I. Uterine Blood Volume in Rats[a].

(a) Wet weight

Method	N	Vehicle	N	Estrogen
RIHSA-^{131}I	37	52 ± 2	38	94 ± 3
RIBGG-^{125}I	33	50 ± 3	34	100 ± 6
RIBGG-^{125}I	10	56 ± 4	11	125 ± 8
^{51}Cr red blood cells		24 ± 3		47 ± 4
Ratio ^{51}Cr/RIBGG-^{125}I		0.44 ± 0.02		0.38 ± 0.03

(b) Dry weight

Method	N	Vehicle	N	Estrogen
RIHSA-^{131}I	37	217 ± 17	38	416 ± 26
RIBGG-^{125}I	33	164 ± 12	34	398 ± 29
RIBGG-^{125}I	10	180 ± 22	11	498 ± 39
^{51}Cr red blood cells		76 ± 11		184 ± 14
Ratio ^{51}Cr/RIBGG-^{125}I		0.44 ± 0.02		0.39 ± 0.04

[a] Values (μl/g tissue weight) are mean ± SEM, N = number of determinations. Vehicle = 0.1 ml/100 g body wt of 1% ethanol in saline, estrogen = 0.5 μg/100 g body wt of 17-β-estradiol.

Most importantly, the ratio of the ^{51}Cr red cell space to RIBGG space did not significantly change in the presence of estrogen (Table I). This demonstrates that significant amounts of plasma proteins are not moving into the extravascular space under the conditions of these experiments.

Discussion. A simple convenient technique for the measurement of uterine blood volume was evaluated as a possible quantitative indicator of changes in uterine hemodynamics associated with the estrous cycle and administration of estrogen. Visually, uteri in estrus and those treated with estrogen were noticeably larger and redder than their corresponding controls. The purpose of the study was to determine if a quantitative correlate to this obviously hyperemic state could be found. Measurement of uterine blood volume using RIHSA appears to satisfy the criterion of providing a simple and precise indication of changes in the circulatory state of the uterus.

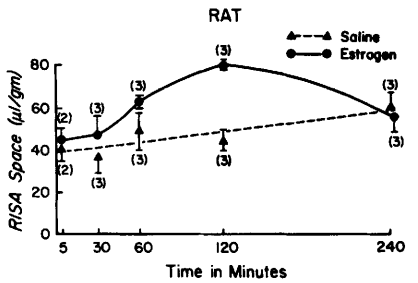

FIG. 3. Uterine blood volume (RIHSA) in rats: effect of 0.5 μg/100 g body wt of 17-β-estradiol or the corresponding amount of vehicle (alcohol–saline). Mean values, number of determinations within parentheses.

A number of investigators have used radioiodinated serum albumin (RISA) to measure tissue blood volumes. Friedman (4, 10) measured the rate of vascular–extravascular equilibration of RISA in mice and found that iodinated albumins move out of the vasculature in an exponential manner with time. Kalman and Lowenstein (7) showed increases in uterine blood volume with RISA-^{131}I following estrogen administration, but were unable to demonstrate this increase with ^{51}Cr-labeled red cells. This suggested to them that radiolabeled albumin was not confined to the vascular compartment and moved out into the extravascular space.

In a later report, Kalman (9) injected radioactive tracers, *e.g.*, ^{22}NaCl or Na^{131}I, followed by immediate sacrifice of the animal. In these experiments it was determined that the percent of tracer in the uterus of ovariectomized rats was increased threefold following estrogen administration and that this indicated an increase in

uterine blood flow. Based on his earlier work, Kalman (9) stated that estrogen did not appear to affect vascular pool size.

Dewey (11) using ^{51}Cr-labeled rat red blood cells and ^{131}I-labeled albumin in different animals found that the rate at which labeled plasma proteins penetrated the vascular compartment differs greatly from tissue to tissue and is roughly proportional to the vascularity of the tissue. Dewey's measurements of plasma volume and red cell volumes in small intestine and ovary indicated that tissue hematocrit can differ from tissue to tissue in the rat.

Davis (12) used ^{59}Fe-labeled rat red blood cells and RISA-^{131}I to measure estrogen-induced increases in uterine blood volume and observed a greater increase in plasma volume than in red blood cell volume; however, these experiments were not performed in the same animal. Peterson and Spaziani (13), Cecil *et al.* (14), and Ham *et al.* (8) all measured increases in RIHSA space following estrogen, and all suggested that increased permeability allowed the iodinated plasma protein or dye used as a tracer to move out into the extravascular space.

Ham *et al.* (8) combined an isotopic and electron microscopic study of the response of the rat uterus to exogenous estradiol. These experiments suggested that 17-β-estradiol produced a massive escape of fluid, with a protein content similar to that of plasma, into the extravascular tissue of the uterus. Electron microscopic examination of these tissues confirmed the increase in permeability on a morphological basis. It should be noted that in these experiments radioiodinated protein (^{131}I) was allowed to circulate for periods of between 15 and 75 min.

Peterson and Spaziani (15) demonstrated that the uterus becomes more permeable to iodinated albumin as a result of increased duration of estrogen stimulation. This observation was made using autoradiographic techniques. In addition, it was observed that increasing the circulation time of iodinated albumin resulted in increased accumulation of the protein in the uterine extravascular space.

Thus numerous reports have suggested that changes in the space occupied by a radiolabeled protein in a hyperemic uterus result from permeability changes rather than from an increase in vascular volume. Most of these reports however, involved protracted exposure of the uterus to the tracer. Since it would be predicted *a priori* that a uterus which becomes visibly engorged with blood should exhibit a measurable increase in vascular space, experiments were designed to test the validity of the use of RIHSA as a convenient index of changes in uterine blood volume.

The increased uptake of RIHSA by uterine tissue following estrogen does not appear to be due to increased permeability under the present experimental conditions, at least in rats. The following findings support this conclusion: (a) RIHSA-^{131}I was present in the animals for only 5 min, (b) in the 5-min period, the uptake of radioiodine-labeled gamma globulin (a much bigger protein) bythe uterus was identical to that of RIHSA-^{131}I, (c) after estrogen the uptake of gamma globulin, ^{125}I was increased to the same extent as RIHSA, (d) uterine blood volume measured with ^{51}Cr-labeled rat red cells, although smaller than RIHSA volume, was increased following estrogen by the same percentage as was seen with the iodine-labeled proteins, and (e) the ratio of red cell space to protein space was unchanged, demonstrating that no change in permeability occurred at the time the measurement was made (Table I).

When estrogen was administered acutely to ovariectomized mice and rats, a specific increase in uterine blood volume, independent of weight, occurred. This was probably due to an increase in vascular capacitance rather than a vascular proliferation, as may occur with chronic estrogen treatment. The findings suggest that uterine blood volume can be used as a quantitative index of the changes in uterine circulation associated with estrogen-induced hyperemia. Other studies have indicated that uterine blood flow increases in rodents following estrogen administration (9, 13, 16). It is clear that uterine blood volume changes, although corresponding in time to measured changes in uterine blood flow, do not represent in a direct way changes in flow. However, the fact that estrogen-induced uterine hyperemia is associated with an increase in uterine blood volume allows this measurement to be useful as a conveniently obtained measure of the hyperemic state.

What explanation, at the vascular level, can be given for the demonstration that blood volume increases following estrogen administration? Any decrease in uterine vascular resistance large enough to produce a substantial increase in blood flow would undoubtedly be produced by arterial and/or arteriolar vasodilatation, since the

major fraction of vascular resistance is provided by precapillary segments of the vascular tree. In contrast, precapillary segments contribute a relatively small fraction of the total volume of an organ; the major fraction of volume being contained in the so-called capacitance or postcapillary portion of the vessels. The present measurements of increased volume suggest therefore that the capacitance vessels, *i.e.*, the uterine veins, have enlarged following estrogen administration. The significance of an increase in uterine capacitance is not clear. It may be postulated that such a change could reflect an effort to regulate uterine capillary pressure at a time when capillary permeability is increasing.

Summary. A method is described for the measurement of uterine blood volume using RIHSA as a tracer. Estrus induced by 2-day administration of estrogen to ovariectomized mice or normally occurring estrus in mice was associated with a parallel increase in uterine weight and uterine blood volume. This increase may be attributed to either increased vascularity or increased vascular capacitance. After acute administration of estrogen to ovariectomized mice and rats, uterine blood volume increased without a corresponding change in uterine weight. This change occurred when the uterus was visibly hyperemic and at a time which corresponds roughly to that when uterine blood flow has been shown to increase. Changes in the volume measured with protein were demonstrated to be valid and to not result from increased vascular permeability.

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