A Double Isotope Technique to Determine Regional Albumin Permeability: Effects of Anesthesia (43127)

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Abstract. **The transvascular leakage of albumin in various organs and tissues was studied with a double isotope technique in rats anesthetized with sodium pentobarbital, given intraperitoneally or intravenously, and in unanesthetized (conscious) rats. ^{125}I-labeled albumin and ^{131}I-labeled albumin were injected into the tail vein 1 hr apart. The albumin permeability index in tissues and organs is indicated by the local ratio $(X_a/Y_a)/(X_b/Y_b)$, where (X_a/Y_a) is the ratio of ^{125}I/^{131}I-albumin activities per g of tissue and (X_b/Y_b) is the ratio of ^{125}I/^{131}I-albumin activities per g of blood. If there is no passage of albumin across the capillary membrane over the 1-hr period of study, the permeability index will be equal to one. In unanesthetized rats, the liver, lung, kidney, femoral muscle, and femoral skin were regions with a high albumin permeability index (above 2). In these organs, intraperitoneal and intravenous anesthesia caused a decrease or no significant change of the albumin permeability index. There was no significant albumin leakage over 1-hr period (index not significantly different from 1) in the mesentery, abdominal muscle, abdominal skin, cremaster, heart, and brain of unanesthetized rats. Intraperitoneal anesthesia caused the albumin permeability index to increase to approximately 4 in the mesentery, abdominal muscle, and the abdominal skin, but not in the cremaster, heart, or brain. These results demonstrate that pentobarbital anesthesia when given into the peritoneal cavity causes a significant increase in albumin leakage in the abdominal region.** [P.S.E.B.M. 1990, Vol 195]

Leakage of macromolecules from the circulation to the interstitium is determined by physiochemical properties of the macromolecules (including the size) and the membrane, by hemodynamic parameters, and by local tissue activity (e.g., skeletal muscles during exercise). Microvascular permeability has been estimated from the fractional rate of removal of ^{131}I-labeled albumin injected into the interstitial space by external monitoring of radioactivity (1–3). This method can only be used to study permeability in a single site, and the injection procedure may alter the local permeability. Albumin permeability has also been estimated from the tissue concentration of plasma albumin labeled with a single marker, e.g., a radioisotope (4–6), Evans Blue dye (7), or fluorescent fluorescein isothiocyanate (8) following their intravenous injection. In this method, the effect of an experimental procedure on albumin permeability is determined by comparing the concentration of the labeled albumin in various organs of the experimental group with that of control animals. Interanimal variations reduce the sensitivity of the between-group comparison when using only one tracer. It is also to be noted that extravascular and intravascular albumin cannot be distinguished by using only a single label.

The present study was undertaken (i) to study albumin permeability with the use of a double isotope technique and (ii) to determine the effects of pentobarbital anesthesia on albumin permeability.

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Materials and Methods

Procedures. The experiments were performed on 22 male Sprague-Dawley rats (Charles River Breeders, Wilmington, MA) weighing 350–400 g. The rats were allowed free access to tap water and rat chow. They were divided into three groups: (A) unanesthetized rats

(six rats), (B) rats given intraperitoneal (ip) anesthesia with sodium pentobarbital (35 mg/kg in nine rats), and (C) rats given intravenous (iv) anesthesia with sodium pentobarbital (35 mg/kg in seven rats). In all rats, a polyethylene catheter (PE 50) was inserted into the tail vein for the injection of labeled albumin.

Permeability Study. At zero time, 3 μCi of ^{125}I-labeled albumin (radioiodinated ^{125}I human serum albumin; Mallinckrodt, Inc., St. Louis, MO), dissolved in normal saline, was injected into the tail vein. Fifty-five minutes later, 3 μCi of ^{131}I-labeled albumin (radioiodinated ^{131}I human serum albumin; Amersham, Chicago, IL), dissolved in normal saline, was injected into the same tail vein. The free ^{125}I or ^{131}I was less than 1% in each batch of radioiodinated albumin and less than 2% in blood and homogenized tissue as determined by trichloroacetic acid precipitation (Table I) and less than 1.5% in plasma by using a Sephadex G25 Column PD-10. With the use of the Sephadex column, we also found that the albumin existed as monomer in the radioiodinated albumin. At the end of the experiment (5 min after the ^{131}I albumin injection), a blood sample was withdrawn by cardiac puncture, and the rat was euthanized by injecting a saturated KCl solution into the left ventricle. Tissues were immediately removed. Femoral muscle and skin samples were obtained from the leg contralateral to the cannulated artery. Free ^{125}I and ^{131}I in tissues were also determined by counting homogenized tissue samples before and after trichloroacetic acid precipitation. The ^{125}I and ^{131}I activities were measured with a gamma counter (Packard 5130, Auto-Gamma System; Packard Instrument Co., Downers Grove, IL) connected to a multichannel analyzer (Tracor Northern Co., Middletown, WI).

Calculation of Albumin Permeability. The following equation was used to calculate the albumin permeability index:

$$(Xa/X_b)/(Y_a/Y_b)$$

where X_a = ^{125}I-albumin activity/g tissue (60 min after injection); X_b = ^{125}I-albumin activity/g blood (60 min after injection); Y_a = ^{131}I-albumin activity/g tissue (5 min after injection); and Y_b = ^{131}I-albumin activity/g blood (5 min after injection).

Thus, the 60 min to 5 min ratio of radioiodinated albumin counts in tissue, corrected for the corresponding counts in blood, was used as an index to represent albumin permeability. If there is no passage of albumin across the capillary membrane over the 55 min between the two injections, the permeability index should be equal to unity.

The statistical significance of changes was evaluated by analysis of variance, followed by Student-Newman-Keuls test for multiple comparisons.

Table I. Percentage of Free ^{125}I or ^{131}I[a] in Each Batch of Radioiodinated Albumin, Blood, and Tissue (Trichloroacetic Acid Precipitation)

Sample	^{125}I	^{131}I
Injectate albumin	0.7	0.9
Blood	1.1	1.0
Liver	1.9	1.7
Lung	1.2	1.5
Kidney	0.9	1.3
Femoral muscle	1.4	1.2
Femoral skin	1.3	1.1
Diaphragm	1.1	0.9
Salivary gland	1.2	1.0
Mesentery	0.8	1.2
Abdominal muscle	1.1	1.1
Abdominal skin	1.1	1.4
Heart	1.4	1.0
Cremaster	1.2	1.1
Brain	0.9	0.9

[a] % free ^{125}I or ^{131}I = $\frac{\text{cpm in trichloroacetic acid supernatant}}{\text{total cpm in sample}} \times 100$.

Results

In unanesthetized rats, the regions with a high albumin permeability index (above 1, $P < 0.05$) were the liver, lung, kidney, femoral muscle, femoral skin, diaphragm, and salivary gland (Fig. 1). Intraperitoneal and intravenous anesthesia caused a decrease or no significant change of the albumin permeability index in these organs (Fig. 1). In unanesthetized rats, there was no significant albumin leakage (index not significantly different from 1, $P > 0.1$) in the mesentery, abdominal muscle, abdominal skin, heart, cremaster, and brain over the 1-hr period of study (Fig. 2). In the mesentery, abdominal muscle, and abdominal skin, the albumin permeability index increased from approximately 1.4 in the unanesthetized rats to approximately 2 following intravenous anesthesia and approximately 4 following intraperitoneal anesthesia ($P < 0.05$, Fig. 2). There was no significant change in albumin permeability index in the cremaster muscle, heart, and brain after either intravenous or intraperitoneal anesthesia (Fig. 2). The results of statistical analyses are summarized in Table II.

To test the time-dependent behavior of albumin leakage, we measured the albumin permeability index at different times in organs with high permeability index in rats under intraperitoneal anesthesia (Fig. 3). The results demonstrate that the leakage is essentially linear over the time period of 5–60 min.

Discussion

The objectives of this study were (i) to use a double isotope technique to study regional permeability simultaneously in many organs and tissues in an intact animal, and (ii) to investigate the effect of pentobarbital

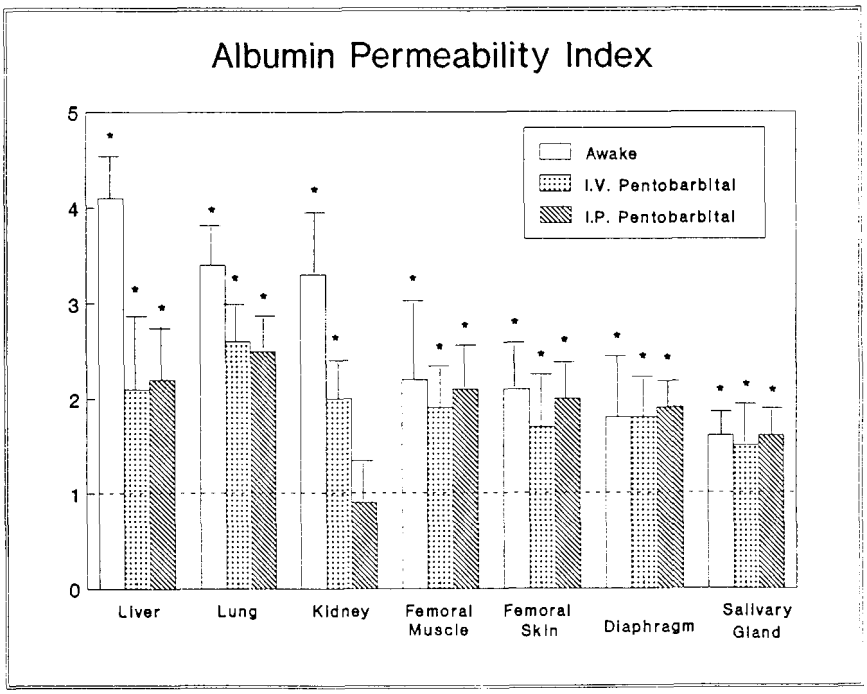

Figure 1. Effects of pentobarbital anesthesia on relative albumin permeability indices in the liver, lung, kidney, femoral muscle, femoral skin, diaphragm, and salivary gland (organs and tissues arranged in descending order of the index). Values greater than one indicate significant leakage of albumin in the region. In unanesthetized rats, all of these regions had albumin permeability index above 1 ($P < 0.05$). Intravenous and intraperitoneal pentobarbital anesthesia caused a decrease or no change in albumin permeability in these regions (Table II).

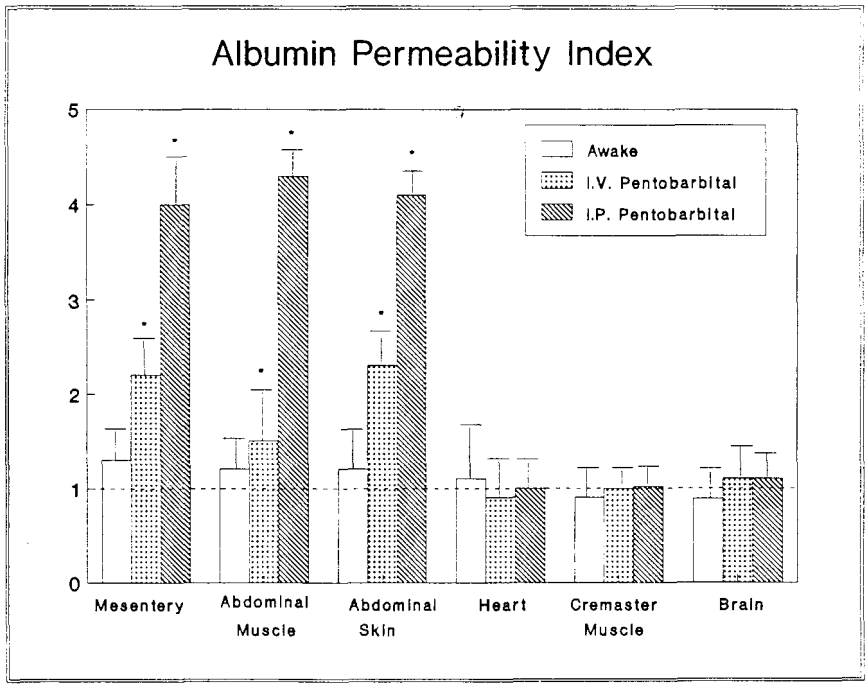

Figure 2. Effects of pentobarbital anesthesia on albumin permeability index in the mesentery, abdominal muscle, abdominal skin, heart, cremaster, and brain. There was no significant albumin leakage in all of these organs over the 1-hr period of study (index not significantly different from 1, $P > 0.1$) in the unanesthetized rat. Intravenous and especially intraperitoneal pentobarbital anesthesia selectively increased albumin permeability in the mesentery, abdominal muscle, and abdominal skin (index significantly greater than 1, $P < 0.05$).

Table II. Statistical Analysis of Effects of Anesthesia on Albumin Leakage in Various Organs (% changes)

Organ	Intravenous versus control	Intraperitoneal versus control	Intraperitoneal versus intravenous
Liver	−48.9[a]	−46.3[a]	+4.8
Lung	−23.5[a]	−26.5[a]	−3.8
Kidney	−39.4[a]	−72.7[a]	−55.0[a]
Femoral muscle	−13.6	−4.6	+10.5
Femoral skin	−19.0[a]	−4.8	+17.6[a]
Diaphragm	0	+5.6	+5.6
Salivary gland	−6.3	0	+6.7
Mesentery	+69.2[a]	+207.7[a]	+81.8[a]
Abdominal muscle	+18.0[a]	+258.3[a]	+186.7[a]
Abdominal skin	+91.7[a]	+141.7[a]	+26.1[a]
Heart	−14.7	−9.1	+7.7
Cremaster	+11.1	+12.9	+2.0
Brain	+13.6	+13.6	0

[a] Indicates difference being statistically significant ($P < 0.05$).

anesthesia on albumin leakage using this technique. The results show that this method can be used in unanesthetized animals to yield measurements of albumin leakage in different organs and tissues which agree with the known regional differences, e.g., the high permeability in the liver and the low permeability in the brain. The advantages of this method are that (i) it can be used to determine albumin permeability in unanesthetized animals, (ii) many organs can be studied simultaneously, and (iii) the use of two isotopes allows the normalization of data in each organ of each animal, thus eliminating the error due to individual variations. By counting both tissue and blood activities for the two isotopes labeled, this method allows the assessment of extravascular leakage in the presence of intravascular albumin.

Studies have shown that intravenous pentobarbital anesthesia causes marked alterations in neurohumoral control and hemodynamic functions (9), and these variations are associated with regional changes in albumin permeability (10). The effect of anesthetic agents on albumin leakage can be due to their actions on circulatory dynamics or on microvascular membrane (11). Pentobarbital anesthesia also has been found to cause vasodilation in the skin and an increase in blood flow (12–14). Couture *et al.* (7) have shown that intraperitoneal pentobarbital anesthesia causes an increase in the disappearance of Evans Blue dye-labeled albumin from plasma to the skin. Feinstein *et al.* (15) suggest, however, that the effect of anesthesia is mainly on the membrane permeability rather than on hemodynamic function.

The results of this study indicate that intraperitoneal pentobarbital anesthesia leads to a selective increase in permeability of abdominal structures, including the mesentery, abdominal muscle, and abdominal skin, which may reflect the direct action of intraperitoneal administration of pentobarbital on the membrane permeability of these abdominal structures to albumin. These results suggest that intraperitoneal pentobarbital anesthesia should be avoided if one is interested in studying the abdominal region.

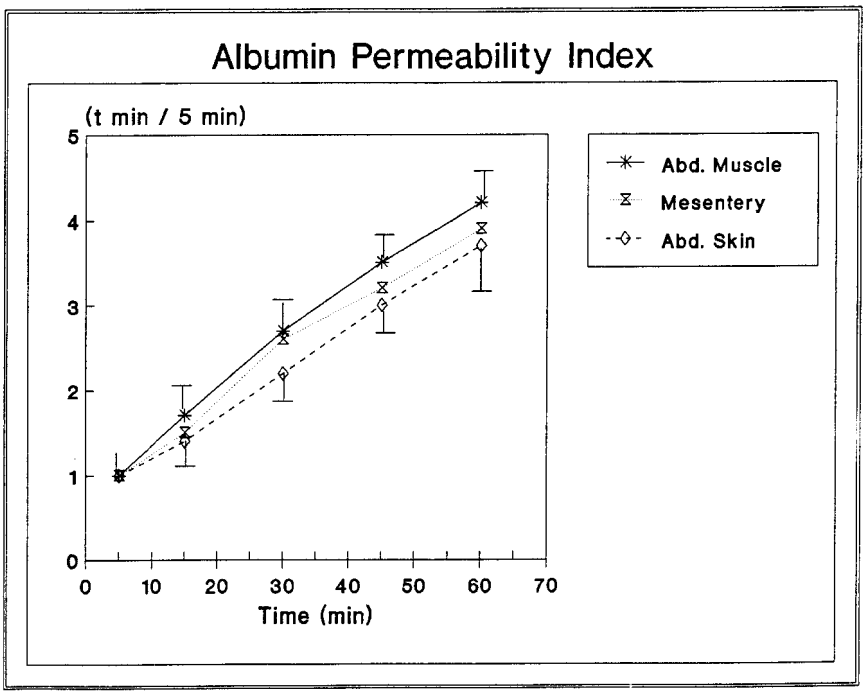

Figure 3. The time course of the relative albumin permeability indices in the abdominal muscle, mesentery, and abdominal skin in rats anesthetized with intraperitoneal pentobarbital.

The double isotope procedure used in the present study is a sensitive method, which can be employed to determine albumin permeability in different organs and to study the effects of various experimental procedures, such as effects of pharmacologic agents. Our findings stress the importance of considering the effects of anesthesia and the route of administration in studying microvascular permeability to macromolecules.

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