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Effect of Atrial Natriuretic Peptide on Vascular Permeation in the Ovine Fetus

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ABSTRACT. To study the effect of atrial natriuretic peptide (ANP) on vascular permeation of albumin in the fetus, ANP (167–600 ng/min) was infused into eight ovine fetuses and saline vehicle was infused into eight twin controls (gestational age 127 ± 3 d) over a 50-min period. Using two different radiolabeled albumin markers, we determined the tissue to blood isotope ratio (TBIR), an index of albumin permeation, and the albumin clearance. Although ANP had no hemodynamic effect, a marked increase in the hematocrit was observed in ANP-infused fetuses compared with initial values (0.37 ± 0.04 *vs* 0.42 ± 0.04, $p < 0.005$) but was unchanged in the twin fetuses receiving saline vehicle (0.35 ± 0.03 *versus* 0.35 ± 0.02). TBIR and albumin permeation were increased in combined tissues of ANP-infused fetuses compared with saline controls (TBIR: 1.49 ± 0.58 *versus* 1.29 ± 0.3, $p < 0.001$; albumin clearance: 1091 ± 1279 *versus* 827 ± 1464 nL/g/min, $p < 0.01$). In individual tissues, TBIR was significantly increased in skin (2.88 ± 0.67 *versus* 1.55 ± 0.35, $p < 0.02$), muscle (1.6 ± 0.27 *versus* 1.24 ± 0.26, $p < 0.02$), adrenal (1.33 ± 0.10 *versus* 1.13 ± 0.15, $p < 0.02$), bone (1.67 ± 0.45 *versus* 1.20 ± 0.40, $p < 0.02$), kidney (1.52 ± 0.25 *versus* 1.24 ± 0.26, $p < 0.03$), and gut (1.69 ± 0.20 *versus* 1.39 ± 0.34, $p < 0.03$). Albumin clearance was higher in most tissues but reached statistical significance only in skin (2135 ± 944 *versus* 775 ± 847 nL/g/min, $p < 0.05$) and bone (1012 ± 1107 *versus* 428 ± 482 nL/g/min, $p < 0.05$). We conclude that overall vascular filtration is higher in the fetus than the adult. Infusion of ANP causes fetal hemoconcentration, decreases blood volume, and enhances vascular permeation of albumin in most tissues, particularly fetal skin. We speculate that the cardiac atria, by secreting ANP, participate in blood volume regulation by maintaining a critical balance between the intravascular and extravascular fluid compartments. Dysregulation of the ANP system might result in fetal hydrops. (*Pediatr Res* 35: 555–559, 1994)

Abbreviations

ANP, α-human atrial natriuretic polypeptide
TBIR, tissue-to-blood-isotope ratio
I_p, tissue-permeating isotope
I_{bv}, blood volume–marking isotope

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In the adult, the primary action of ANP is to decrease blood volume by increasing urine flow (1). ANP infusion can also lower blood volume in anephric experimental animals (2–4), suggesting that it causes extravasation of plasma in the adult. Other recent work has implicated ANP in fetal blood volume regulation. ANP infusion into the fetus increases hematocrit and decreases blood volume, even though fetal urine output is only minimally increased (5). Consistent with this finding is the observation by Robillard *et al.* (6) that ANP causes hemoconcentration even though responsiveness of the fetal kidney to ANP is decreased compared with that of the neonatal and adult kidney in animals. These data raise the possibility that, in the presence of the functionally immature fetal kidney, the blood volume lowering effect of ANP in the fetus may be mediated primarily through vascular permeation, as initially suggested by Clark (7). Furthermore, the pathophysiologic implications of elevated circulating ANP concentration (8–10) in the disorder of fetal fluid balance known as fetal hydrops warrants investigation of the role of this hormone in the transport of fluid and protein from the vascular compartment into other tissues. In the present report, we describe the effect of ANP on regional albumin permeation in the chronically instrumented, unanesthetized, late gestation fetal lamb.

MATERIALS AND METHODS

Surgical preparation. All care and procedures were approved by the Oregon Health Sciences University Animal Care and Use Committee. Sterile surgeries were performed on eight ewes carrying twin fetuses at 122 ± 3 d gestation (term = 146 d). General anesthesia was induced using i.v. diazepam and ketamine; the ewe was intubated and anesthesia was maintained with halothane and 50% N_2O-50% O_2. After the uterus was exposed through a midline abdominal incision, a separate incision was made for each twin in a cotyledon-free uterine area to permit access to the fetal head and neck or hind limbs. In each twin, polyvinyl catheters (1.0 mm inner diameter) were inserted into the jugular vein or femoral vein and were positioned so that their tips were in the right atrium or abdominal vena cava for the infusion of ANP or vehicle, measurement of central venous pressure, and infusion of radiolabeled tracers. Catheters were inserted into the carotid artery or the femoral artery for measurement of arterial blood pressure and withdrawal of blood, and a catheter was positioned in the amniotic space for reference pressure measurements. At the end of surgery, 1 million U of penicillin G and 250 mg of streptomycin were administered directly into the amniotic fluid. All catheters were exteriorized through an s.c. tunnel and placed in a cloth pouch on the ewe's flank. After recovery from anesthesia, the ewes were kept in a restricted area and fed a standard diet. On the first and second postoperative days, the ewes received daily intramuscular injections of streptomycin (1 g) and ampicillin (800 mg).

Experimental protocol. Baseline data were obtained after a recovery period of 4–5 d (gestational age 127 ± 3 d). Arterial blood was collected anaerobically in heparinized plastic syringes, and pH, P_{CO_2}, and P_{O_2} were measured at 39°C (IL 1312 blood

gas analyzer, Instrument Laboratories, Lexington, MA). The hematocrit was determined in duplicate. Mean arterial and central venous pressure were measured with Statham p-23 Db strain gauge transducers, recorded on a six-channel polygraph recorder (Gould Instruments, Cleveland, OH), and referenced to amniotic fluid pressure. Percent change in blood volume was calculated from the change in hematocrit. Either ANP [167 ng/min (n = 2) or 600 ng/min (n = 6) α-atrial natriuretic polypeptide 1–28, Peninsula Laboratories Inc., Belmont, CA] or saline vehicle (n = 8) at a flow rate of 0.1 mL/min was infused for a total of 50 min (Fig. 1). Thus, eight fetuses received ANP and eight matched twin controls received saline vehicle. A bolus of either ^{131}I-BSA or ^{125}I-BSA (Iso-Tex Diagnostics, Friendswood, TX), injected i.v. into both fetuses 20 min after the beginning of the ANP or vehicle infusions, served as the I_p. Two minutes before the end of the experiment (48 min after initiating ANP or vehicle) either ^{131}I-BSA or ^{125}I-BSA (the I_{bv}) was injected simultaneously into both fetuses. For each experiment, if ^{131}I-BSA was I_p, then ^{125}I-BSA served as I_{bv} and *vice versa*. Four arterial blood samples (2 mL per sample, total blood withdrawn less than 2% of estimated blood volume) were obtained at time 0, 30, 40, and 50 min to determine hematocrit and to quantify tracer radioactivity. At the end of the ANP or vehicle infusion (50 min), the fetuses were killed with an i.v. injection of euthanasia solution. Close attention was paid to achieving simultaneous fetal death. The ewe was then killed and the fetuses were rapidly delivered, towel dried, and weighed. The fetal chests were opened, the hearts were excised, and various tissues were removed for quantification of tracers by gamma spectrometry. Before counting, each tissue was rinsed briefly in saline to remove any blood contaminating tissue surfaces and was then weighed.

The TBIR, an index of albumin permeation, has been described by Kilzer *et al.* (11). In the present investigation, radiolabeled albumin was used for the blood volume marker rather than radiolabeled red blood cells, a modification suggested by Williamson *et al.* (2). The TBIR was determined according to the following equation:

$$\text{TBIR} = [I_p/I_{bv}]t \div [I_p/I_{bv}]b$$

where the quotient of the I_p radioactivity and the I_{bv} radioactivity in each tissue (t) is divided by the quotient of the same isotopes in the arterial blood sample (b) drawn just before the conclusion of the experiment. A ratio greater than 1 indicates that the volume of distribution of I_p in the tissues is greater than in the blood and therefore indicates permeation of the vasculature by albumin into the extravascular space. Plasma albumin clearance was expressed as nL/g/min and was calculated according to method described by Williamson *et al.* (2):

$$\text{Albumin clearance (mL/g/min)} = \frac{I_{p_t}[(I_p/I_{bv})_t - (I_p/I_{bv})_b]}{\text{time average mean } I_p \text{ counts in blood}} \div 30 \text{ min}$$

where the tissue radioactivity of the I_p per gram (Ip_t) was corrected for the intravascular content of this tracer on the basis of the difference in the ratio of the radioactivity of the I_p and the I_{bv} in the tissue $(I_p/I_{bv})_t$ and the ratio of the I_p and the I_{bv} in the terminally drawn blood sample $(I_p/I_{bv})_b$. The tissue content of the I_p in excess of the vascular content of the I_p represents the amount of the I_p that has traveled into the tissue space. This radioactivity was divided by the mean plasma concentration of I_p (derived from the blood samples taken at 10, 20, and 30 min after injection of the tracer, assuming a linear decrease of I_p from plasma to tissue with time). It was then divided by the duration of time the tracer was permitted to travel into tissues (30 min) to obtain the albumin clearance.

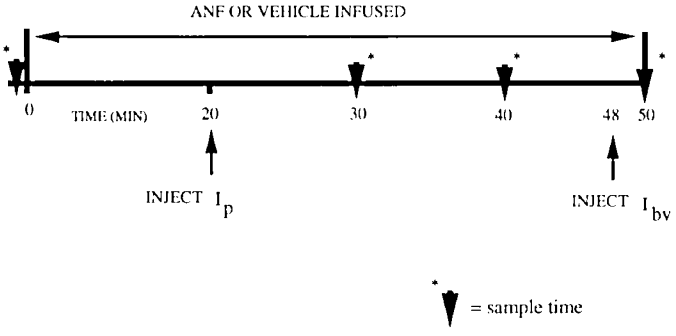

Fig. 1. Experimental protocol.

TBIR data are presented for 14 fetuses (seven twin pairs). In one of the eight twin pairs, an error in isotope administration prevented calculation of the TBIR. Albumin clearance was completed in 10 fetuses (five twin pairs). In seven twin pairs, fetal death at the end of the experiment occurred simultaneously. In one set of twins, death occurred 10 min later in the twin receiving ANP, thus allowing a longer I_p circulation time and potentially increasing albumin permeation. For this fetus, TBIR was calculated in two different ways: *1*) by assuming circulation time was the same in both fetuses, and *2*) by assuming that permeation continued during the additional time before fetal death and extrapolating the tissue counts back to the 30-min post–albumin injection time point. The statistical analysis of TBIR was not different with or without the extrapolation for this single fetus; therefore, these data were not excluded.

Data tabulation and statistical analysis. Quantification of radiolabeled tracers in tissues and blood was performed in a gamma spectrometer (Micrad, Knoxville, TN). The data were corrected for background and spillover before determination of TBIR and clearance. At least 10 000 counts were obtained for each isotope. Data were analyzed using the MacSS (Statsoft, Tulsa, OK) on a Macintosh SE computer. All data are expressed as mean ± SD. The effects of ANP or saline vehicle on blood volume through time were explored with analysis for repeated measures. When analysis of variance demonstrated significant differences, paired contrasts were performed to compare the baseline (time 0) values with subsequent time points (within-group comparison). Hemodynamic and albumin permeation data were explored using the Wilcoxon matched pairs test. Statistical significance was presumed in all cases at $p < 0.05$.

RESULTS

At the beginning of the experiment before any infusion was begun, arterial blood gases were similar when the twins were compared (Table 1). Body weights between the two groups were not different at autopsy (2.98 ± 0.45 kg *versus* 2.92 ± 0.3 kg). The hematocrit was significantly increased in ANP-infused fetuses compared with initial values (0.37 ± 0.04 *versus* 0.42 ± 0.04; $p < 0.005$), whereas the hematocrit remained statistically unchanged in the twin fetuses who simultaneously received saline vehicle for 50 min (0.35 ± 0.03 *versus* 0.35 ± 0.02). Furthermore, although the hematocrit differences between ANP fetuses and saline twins were initially not different, after 50 min a significant increase in hematocrit occurred in the ANP twins ($p < 0.005$). Other than its hemoconcentrating effect, the ANP infusion had no other significant hemodynamic influence.

Over time, ANP significantly reduced blood volume by a maximum of 11.1 ± 5.3% after 40 min ($p < 0.02$) compared with an increase of 1.4 ± 3.7% in the control twins (Fig. 2). Beyond 40 min, blood volume in the ANP group did not change. However, a minimal, nonsignificant increase in blood volume for the group receiving saline vehicle continued to be observed until the end of the experiment.

The effect of ANP on vascular permeation of radiolabeled albumin was estimated by calculating the TBIR and the albumin clearance (Fig. 3). For both TBIR and albumin clearance, ANP significantly increased permeation when tissues were grouped together and compared with saline vehicle [TBIR (n = 77): 1.49

Table 1. *Arterial blood gases and hemodynamic data during the control state and after 50 min of infusion of ANP or saline vehicle**

	Saline vehicle		ANP	
	0 Min	50 Min	0 Min	50 Min
pH	7.36 ± 0.03		7.36 ± 0.01	
P_{CO_2} (kPa)	6.5 ± 0.8		6.5 ± 0.4	
P_{O_2} (kPa)	2.7 ± 0.4		2.9 ± 0.4	
Heart rate (beats/min)	169 ± 12	169 ± 16	164 ± 21	184 ± 30
Central venous pressure (mm Hg)	3.4 ± 1.3	3.6 ± 1.5	3.1 ± 0.9	2.6 ± 1.1
Mean arterial pressure (mm Hg)	46.9 ± 6.6	47 ± 4.9	45.8 ± 6.5	43.3 ± 1.9
Hematocrit	0.35 ± 0.03	0.35 ± 0.02	0.37 ± 0.04	0.42 ± 0.04†

* Values are mean ± SD. For central venous pressure, $n = 5$ saline fetuses and 5 ANP fetuses (twins); for mean arterial pressure, $n = 6$ saline fetuses and 6 ANP fetuses (twins); for all other measurements, $n = 8$ saline fetuses and 8 ANP fetuses (twins).

† $p < 0.005$ compared with ANP fetus at 0 min and to saline fetus (twin) at 50 min.

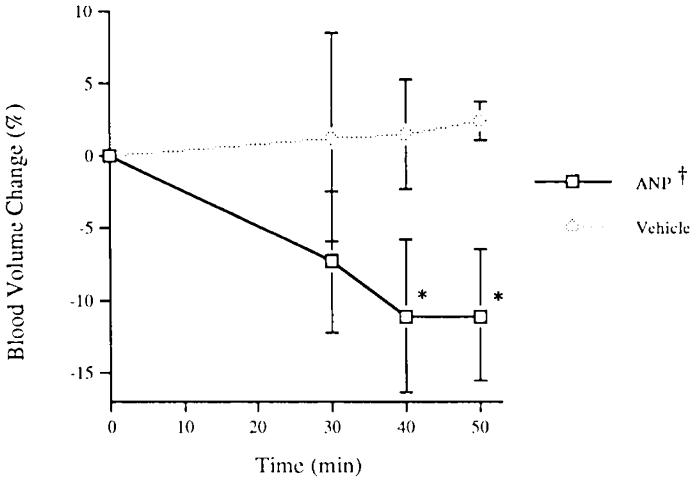

Fig. 2. Time course of the change of blood volume in 16 fetuses receiving either ANP or saline vehicle. Data are expressed as mean ± SD; $n = 8$ matched twin controls and 8 ANP fetuses. †, ANP group time course is different from twin controls as determined by repeated measures analysis of variance ($p < 0.001$); *, $p < 0.03$ compared with time 0 min (within-group paired contrast).

± 0.58 *versus* 1.29 ± 0.30, respectively, $p < 0.001$; albumin clearance ($n = 55$): 1091 ± 1279 *versus* 827 ± 1464, respectively, $p < 0.01$)]. Specific tissue types were then matched to determine whether any regional vascular bed contributed to the overall ANP-induced increase in vascular albumin permeation (Fig. 3). TBIR was increased in most fetal tissues types after ANP, although a statistically significant difference could only be demonstrated in skin (all values ANP *versus* saline vehicle: 2.88 ± 0.67 *versus* 1.55 ± 0.35, $p < 0.02$), muscle (1.60 ± 0.27 *versus* 1.24 ± 0.26, $p < 0.02$), adrenal (1.33 ± 0.10 *versus* 1.13 ± 0.15, $p < 0.02$), bone (1.67 ± 0.45 *versus* 1.20 ± 0.40, $p < 0.02$), kidney (1.52 ± 0.25 *versus* 1.24 ± 0.26, $p < 0.03$), and gut (1.69 ± 0.20 *versus* 1.39 ± 0.34, $p < 0.03$). Albumin clearance was higher (ANP *versus* saline vehicle) in most tissues but reached statistical significance only in skin (2135 ± 944 *versus* 775 ± 847 nL/g/min, $p < 0.05$) and bone (1012 ± 1107 *versus* 428 ± 482 nL/g/min, $p < 0.05$).

DISCUSSION

The principal finding of this study is that ANP enhances vascular permeation of albumin in many fetal tissues. The amount of ANP administered had no hemodynamic effect but did have a marked influence on the hematocrit. Inasmuch as ANP does not change red blood cell volume (2), it seems to lower plasma volume partly through an effect on the microcirculation that is independent of urine flow (5, 6). The precise mechanism for this action cannot be ascertained from this investigation. The TBIR, an index of albumin permeation, may be influenced by various factors including tissue vascularity, rate of blood flow, blood pressure, and vascular porosity (2, 11). Hence, the mechanism for ANP-enhanced albumin permeation in fetal skin and other tissues might be through recruitment of vascular channels, increased capillary surface area, or increased regional blood flow. Alternatively, ANP might regulate protein transport itself by modulating either capillary pore size or lymphatic flow. Hydrostatic forces do not seem to play a role, inasmuch as neither venous nor arterial blood pressure was affected by the doses of ANP infused in this study.

ANP causes significant increases in albumin permeation in adult models (2, 12). However, the absolute values of fetal albumin clearance we observed in the control state were 5- to 20-fold higher than those that Williamson *et al.* (2) described in the adult rat. This finding is consistent with the observation that the capillary filtration coefficient in the fetal sheep is 5–10 times greater than in the adult (13). Thus, compared with the adult, the impact of ANP on fluid and protein movement in the fetus may be several orders of magnitude greater. Yet the fetal vascular compartment seems to be more stable than the adult vascular compartment. Fetal vascular compliance (14) is one half that of adult values, and the fetus may maintain blood volume within even closer limits than the adult when subjected to sudden volume expansion or contraction. After acute saline volume expansion, intravascular volume increased only 6–7% compared with 40% in adults receiving the same volume load (15). Thus, it would seem that decreased vascular compliance compared with the adult and the capacity to readily move fluid out of the fetal vascular compartment contribute to the stability of the fetal intravascular volume.

The observation by Brace and Gold (13) that the capillary filtration coefficient of the ovine fetus is greater after saline volume expansion compared with fetal hemorrhage supports the idea that the fetus can modulate capillary surface area. We speculate that ANP may be a mediator of this regulatory ability. When increasing blood volume increases cardiac filling, atrial myocyte stretch stimulates ANP secretion (16). Higher circulating ANP in turn reduces blood volume by increasing vascular fluid and protein permeation. By controlling the balance of the intravascular and extravascular fluid volume, ANP may serve to maintain the most advantageous relationship between cardiac filling and cardiac output, thereby optimizing cardiac function in the face of the normal rapid growth of the fetal blood volume.

Furthermore, alteration in albumin permeation through increased circulating ANP may contribute to the tissue edema seen in fetuses with severe anemia (8) and supraventricular tachycardia (10). Brace *et al.* (17) infused amounts of ANP similar to those used in the present study into fetal lambs and achieved circulating concentrations that ranged from 220 to 970 pmol/mL (700 to 3000 pg/mL), values that are higher than the physiologic range (17) but similar to values routinely found in clinical (8, 9) and experimental (10) hydrops fetalis. It is interesting that we found ANP has its greatest effect on vascular permeation of skin, a tissue that is profoundly affected in hydrops fetalis and serves to define the syndrome itself (18). In the newborn lamb,

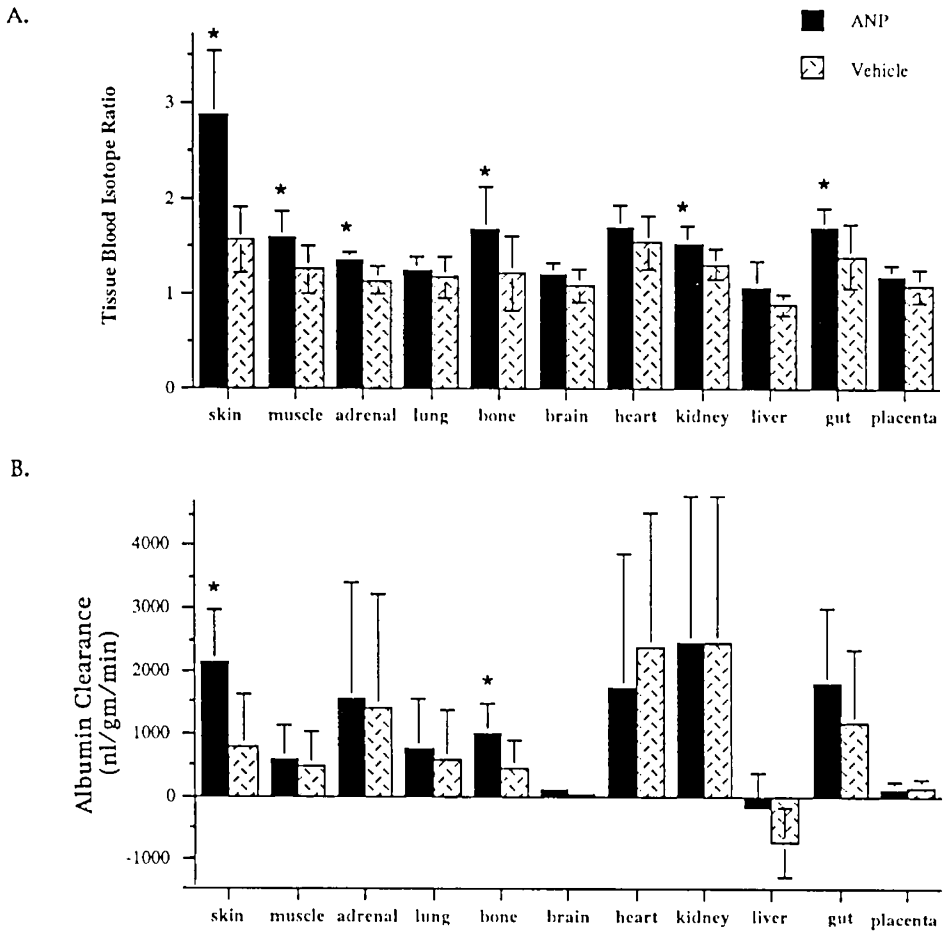

Fig. 3. Comparison of albumin permeation between fetuses receiving ANP and twins receiving saline vehicle. *A*, TBIR in seven ANP fetuses and seven saline fetuses. *B*, Albumin clearance in five ANP fetuses and five saline fetuses. *, $p < 0.05$ compared with twin control (values are mean ± SD).

skin weight accounts for a significant portion of the total body weight, and in the chronically anemic newborn lambs, only skin weight was found to be increased (19). Thus, skin may be an important site of ANP-induced edema formation in the anemic newborn.

There are some statistical and methodologic limitations to our study. The fact that TBIR calculation is independent of tissue weight, a potential source of error in measurement, may partly account for the relatively smaller statistical variability compared with the albumin clearance data. In any case, given the small number of experiments and the overall variability within the data, few conclusions can be drawn from negative results. Tissues in which the null hypothesis would seem to be acceptable might actually reveal ANP effect given sufficient statistical power. For example, to be able to state with certainty (power = 80%) that ANP had no effect on albumin permeation in brain or placenta, 47 and 182 experiments, respectively, would be required. Thus, the principle conclusions of the present study rely on the observation that ANP increases albumin permeation in some tissues, and no reliable conclusions can be derived where a difference could not be demonstrated. A methodologic aspect of our study also limits any conclusions on the basis of negative results. In tissues with a high degree of permeability, the lack of difference between ANP and saline fetuses may mean that I_{bv} had equilibrated with the tissue during its 2-min circulation time, thus eliminating tissue *versus* blood differences. Only in tissues where differences were observed can valid conclusion be drawn. In this regard, it is reasonable that certain regional capillary beds that have open vascular fenestrations, such as the liver, might equilibrate rapidly with the blood, precluding the observation of a difference. It is interesting that consistent ANP effects (both TBIR and albumin clearance) were observed in bone, a tissue that in the adult has high permeability but in the fetus may, along with the skin, serve as a capacitance organ for the vascular compartment. It is unclear why albumin clearance in the liver was a negative value. This may be partly caused by error in measurement. Alternatively, it is possible that $(I_p/I_{bv})_t$ was lower than $(I_p/I_{bv})_b$. This situation could occur if I_p moved very slowly into the tissue, if the liver transported I_p back to the blood, or if I_p was sequestered in the lymphatic circulation.

In conclusion, overall vascular filtration is higher in the fetus than in the adult. Infusion of ANP causes fetal hemoconcentration, a decrease in blood volume, and enhancement of vascular permeation of albumin in most tissues, particularly fetal skin. These data support the hypothesis that the cardiac atria, by secreting ANP, may participate in blood volume regulation by maintaining a critical balance between the intravascular and extravascular fluid compartments. Dysregulation of the ANP secretory mechanism might culminate in the edema-forming syndrome known as fetal hydrops.

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