north by selecting the smaller one. In all our experimental magnetic fields the birds took the direction in which the smaller angle was formed by the field lines and the gravity vector for "north."

In test arrangement 5 the magnetic field had no vertical component and, correspondingly, no inclination (Fig. 3). In this field the two angles γ and γ' were equal, so that the birds were unable to decide which end of the north-south axis of the magnetic field lines was "north." They were disoriented, and we could no longer find a significant directional preference.

A compass system that uses the axial direction of the magnetic field lines and their inclination will fail at the magnetic equator, where the field lines are horizontal; it will lead the birds in a wrong direction after they have crossed the equator and are flying in the southern hemisphere, where the smaller angle between the field lines and the gravity vector lies in the south. Our experimental species, the European robin, has all his wintering areas north of the magnetic equator and will never encounter these situations, but most bird migrants cross the magnetic equator twice a year. Experiments with long-distance migrants will have to show whether they have a similar compass system and, if they do, what other orientation aids they use to find their way across the magnetic equator.

On the whole this magnetic compass represents a highly flexible direction-finding system. Its ability to adjust to a varying intensity range makes it independent of any secular variation in total intensity, and the fact that it does not use the polarity of the magnetic field means that it is not affected by the reversals of the polarity of the earth's magnetic field that have taken place several times since the phylogenetic origin of birds (*7*).

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Angiotensin I: Metabolism by Plasma Membrane of Lung

Abstract. *(8-L-[^{14}C]phenylalanine)angiotensin I is metabolized in one passage through blood-free lungs. Approximately 20 percent of the radioactivity emerges as angiotensin II, the remainder as lower homologs. Radioactivity is not retained by the lungs but has the same volume of distribution and mean transit time as blue dextran, a compound unlikely to leave the intravascular space. Plasma membrane fractions of lung are capable of converting angiotensin I to angiotensin II. These data, taken together, indicate the circulating angiotensin I is metabolized by enzymes of the luminal surface of pulmonary endothelial cells.*

We have postulated that circulating angiotensin I and bradykinin, like the adenine nucleotides, are metabolized by enzymes located on the luminal surface of pulmonary endothelial cells (*1–3*). Our hypothesis is based on the findings that angiotensin I, bradykinin, and the adenine nucleotides disappear during a single passage through the lungs (*4*). Disappearance is accounted for by enzymatic degradation, and not by tissue uptake nor transfer to extravascular spaces. The metabolites of these compounds are recovered in quantitative yields in the pulmonary venous effluent, neither the parent compounds nor the metabolites being retained by the lung. Furthermore, the metabolism does not depend on enzymes of blood nor secretion of soluble hydrolase enzymes (*1–3*).

In the case of adenosine 5′-monophosphate (5′-AMP), we have shown that the relevant metabolic enzymes are located within endothelial caveolae intracellulares open to the vascular lumen (*3*). The close parallels of the disappearance of angiotensin I, bradykinin, and 5′-AMP suggest that they are metabolized by similar mechanisms (*1, 3*). In the present investigation we have extended studies of the kinetics of disappearance of (8-L-[^{14}C]phenylalanine)angiotensin I during circulation through the lungs and have examined plasma membrane and attached caveolae of lung for their ability to metabolize angiotensin I. The fate of angiotensin I, and therefore the subcellular localization of its metabolic enzymes, in the pulmonary circulation may be of physiologic significance as one of the metabolites is angiotensin II, the most potent hypertensive substance known (*1, 2*).

The first series of experiments were performed using Sprague-Dawley rats (0.2 to 0.3 kg). The rats were anesthetized with intraperitoneal injections of chloral hydrate (300 mg/kg). The trachea was cannulated and the lungs were ventilated with a Harvard (model 680) respirator. The aorta was ligated just above the aortic valve, and the lungs and heart were removed as a unit. The lungs were perfused via the pulmonary artery with Krebs-Henseleit solution aerated with O_2 and CO_2 (95 : 5) and heated at 37°C. The perfusion solution was pumped at 6 ml/min, at pressures of less than 25 mm-Hg. The venous effluent was collected from a cannula placed in the left atrium.

Our perfusion method is efficient in removing blood elements, and it does not cause damage detectable by electron microscopy (*5*). In particular, those structures most sensitive to mechanical damage and variations in *p*H, electrolytes, and nutrients, such as the mitochondria, cell membranes, and cell junctions, are well preserved. Furthermore, we have found no evidence by gravimetric or electron microscopic techniques of interstitial edema.

When the venous effluent became free of blood (2 to 3 minutes), blue dextran and (8-L-[^{14}C]phenylalanine)angiotensin I (100 μc/μmole) were added to the pump line for constant infusion. Blue dextran (molecular weight > 2 million) was used as a compound unlikely to leave the vascular space during a single circulation, and therefore provided a basis for estimating apparent mean transit times and intravascular volumes (*6*). The venous effluent was collected, dropwise, into scintillation

vials (five drops per vial). Scintisol-Complete (Isolab) was added to each vial. The radioactivities of the vials were measured by liquid scintillation counting.

Figure 1 shows results obtained by constant infusion of [^{14}C]angiotensin I and blue dextran. The times of appearance and then disappearance of radioactivity and blue dextran were identical. The recoveries of blue dextran and radioactivity were 90 and 92 percent, respectively. Less than 0.5 percent of the radioactivity and blue dextran were retained by the lungs. The remainder was not found and may have been lost by adsorption on tubing or during the rapid collection of samples (2.6 seconds per sample). In these experiments, because of its relatively low specific activity (100 μc/μmole), angiotensin I was used in a final concentration (16 ng/ml) about 50 to 100 times that found in normal man (*7*). The concentration used here is approximately one-fifteenth that used in a previous study by Barrett and Sambhi (*8*).

Our results indicate that the volume of distribution of the radioactivity of (8-L-[^{14}C]phenylalanine)angiotensin I within the lungs is no greater than that of blue dextran and probably does not exceed the volume of the intravascular space (*6*). However, in agreement with our previous studies (*1*, *2*), none of the radioactivity emerging in the venous effluent remained in the form of angiotensin I. In two experiments, effluent fractions were collected into test tubes (18 by 150 mm), each containing 1.0 ml of absolute ethanol. Radioactive peptides were purified and identified (*2*). Approximately 18 percent of the radioactivity was identified as angiotensin II, the remainder of the radioactivity being present as tetrapeptides (36 percent), tripeptides (22 percent), prolyl-phenylalanine (15 percent), and free phenylalanine (7 percent).

In another four experiments we measured the apparent mean transit times and volumes of distribution of blue dextran and radioactivity (*6*). A 100-μl portion of blue dextran (0.5 mg) and (8-L-[^{14}C]phenylalanine)-angiotensin I (65 ng, 10,000 count/min) was injected as a bolus into the pulmonary artery. The apparent mean transit times of radioactivity and blue dextran were identical (11.7 seconds, range 10.4 to 13.0 seconds). The variation was largely accounted for by the time required to collect each fraction (2.6 seconds), the peak activities occurring either late in the fourth fraction or early in the fifth fraction collected after injection. The volumes of distribution averaged 1.17 ml (range 1.04 to 1.30 ml). These values are not corrected for any dilution that might have occurred in the venous outflow tract.

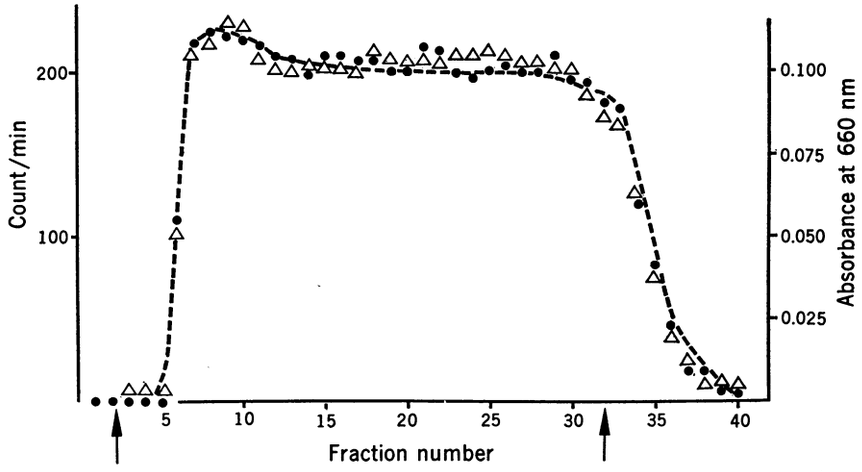

Fig. 1. Steady-state perfusion of blue dextran and (8-L-[^{14}C]phenylalanine)-angiotensin I through lungs. When the venous effluent was free of blood, blue dextran (△) and (8-L-[^{14}C]phenylalanine)-angiotensin I (●) were added to the perfusion line (first arrow). After collecting 30 fractions (2.6 seconds per fraction), blue dextran and angiotensin I were removed from the perfusion line (second arrow). Fractions were assayed for blue color (A_{660nm}) and radioactivity (count/min). Angiotensin I was in a final concentration of 16 ng/ml. Blue dextran was in a final concentration of 0.48 mg/ml.

Our kinetic data indicate that circulating angiotensin I is metabolized (~ 20 percent being converted to angiotensin II) by enzymes on or close to the luminal surface of pulmonary endothelial cells. However, evidence is not direct. Recently, Sander and Huggins (*9*) showed that a particulate fraction of rabbit lung metabolizes angiotensin I yielding histidyl-leucine, a product to be expected from the conversion of angiotensin I to angiotensin II. In terms of the "marker" enzyme, 5′-nucleotidase (E.C. 3.1.3.5.), their subcellular fraction appeared to contain plasma membrane. However, it also contained relatively large quantities of RNA, acid phosphatase, and β-glucuronidase, indicating the presence of ribosomal and lysosomal material. By electron microscopy, the subcellular fraction was found to contain cilia, and must, therefore, have been contaminated with bronchial epithelium or mesothelium of the pleura.

In this study we used a new, highly efficient method (*10*) of preparing plasma membrane to reinvestigate the question of the subcellular localization of enzymes capable of metabolizing circulating angiotensin I. In our method, plasma membrane was separated from cytoplasmic elements by reacting the 5′-nucleotidase of its caveolae intracellulares with 5′AMP in the presence of lead nitrate. As inorganic phosphate was released, it was precipitated as insoluble lead phosphate at or near the site of the 5′-nucleotidase enzymes. The lead-laden plasma membrane–caveolae fraction was then collected as a pellet by low-speed centrifugation leaving internal membrane systems suspended in the supernatant. Cilia and

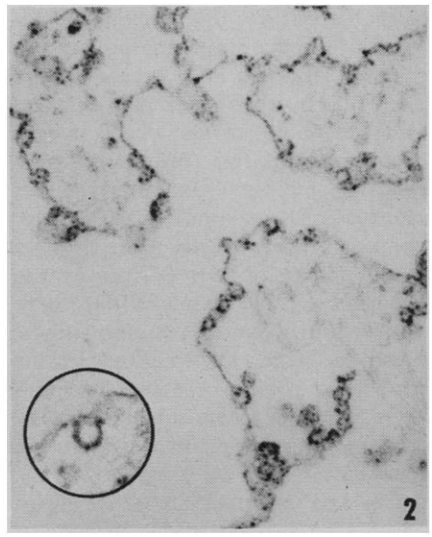

Fig. 2. Electron micrograph of the plasma membrane–caveolae fraction of lung, showing characteristic "clouds" of plasma membrane and associated caveolae intracellulares (pinocytotic vesicles) containing discrete lead phosphate deposits (× 52,000). The inset shows that the caveola retains its main morphologic features, including the delicate diaphragm covering its stoma (× 75,000).

other bronchial epithelial elements were eliminated or reduced by selecting peripheral areas of lung for homogenization. Electron micrographs of the pellet are shown in Fig. 2. The pellet was composed of "clouds" of plasma membrane and caveolae, many of which retain their characteristic morphology.

The plasma membrane–caveolae fractions were incubated at 37°C with (8-L-[^{14}C]phenylalanine)-angiotensin I in 2.0 ml of 50 m*M* tris-(hydroxymethyl)aminomethane-maleate buffer, *p*H 7.4, containing 100 m*M* KCl, 5 m*M* $MgCl_2$ and 100 m*M* Na_2HPO_4. (8-L-[^{14}C]phenylalanine)-angiotensin I (100 μc/μmole) was added in concentrations ranging from 0.4 to 4.0 μc/ml. Each reaction mixture contained plasma membrane (370 to 500 μg of protein per milliliter), derived from one-eighth to one-seventh of a lung. Reactions were stopped after 0.25, 5, 10, 15, 30, and 60 minutes of incubation by heating in a boiling water bath. Radioactive reaction products were identified by a combination of gel chromatography and paper electrophoresis (*1, 2*).

At any of the concentrations of angiotensin I used, conversion to angiotensin II was essentially complete (> 90 percent) within 15 seconds. Identification of angiotensin II was confirmed by bioassays with the rat colon and with mean arterial blood pressure preparations (*11*). The highest rate of conversion observed was 0.4 μmole per milligram of plasma membrane protein per minute. This rate is in excess of that likely to be required of lung in vivo, as angiotensin I probably occurs in concentrations of less than 50 pg/ml (~ 0.04 pmole/ml) in mixed venous blood (*7*). Angiotensin II, once formed, was not degraded in incubations of up to 60 minutes. Lower homologs were not observed. The reason for the failure of membrane fractions to form lower homologs is not known. Possibly, the peptidase enzymes capable of degrading angiotensin II are inhibited by the lead used for precipitation of inorganic phosphate.

Our data show that radioactive angiotensin I is metabolized during a single passage through the lungs. The volume of distribution of radioactivity does not exceed that of the intravascular space, and the mean transit time of radioactivity is no greater than that of blue dextran, a compound unlikely to leave the vascular lumen. Preparations of plasma membrane of lung are capable of converting angiotensin I to angiotensin II at rates sufficiently rapid to account for the conversion of angiotensin I to angiotensin II by intact lung. These points, taken together, are presented as strong support of the hypothesis that circulating angiotensin I is metabolized by enzymes of the luminal surface of pulmonary endothelial cells.

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Cochlear Microphonic Audiograms in the "Pure Tone" Bat Chilonycteris parnellii parnellii

Abstract. *Audiograms are sharply tuned to a frequency close to the second harmonic of the pulse. The tuning, which is much sharper than previously reported for any vertebrate receptor, provides a mechanism whereby the bat can effectively perceive echoes even during periods of pulse-echo overlap.*

Bats of the suborder Microchiroptera find their way about their environment and capture prey by echolocation (*1*). A number of species, often designated "CF" or "pure tone" bats, emit cries characterized by a long constant frequency (CF) component and brief beginning and terminal FM sweeps (*1–6*). It has been suggested that these two components have different functions, the FM sweeps being utilized for localization and ranging while the CF component is used for determining the relative velocity of the bat and its target (*4; 7–9*). Many species emit only brief FM pulses and they shorten their pulses as they approach a target to prevent pulse-echo overlap. However, in CF bats the pulse duration is so long that pulse-echo overlap is inevitable (*10*). How these bats extract information or even perceive echoes during periods of overlap has been a subject of considerable speculation (*3–5; 7–9*).

In the course of developing a technique for recording cochlear microphonic potentials from unanesthetized bats we obtained audiograms from nine *Chilonycteris parnellii parnellii* (Gray). These audiograms show the existence of highly specialized physiological properties in the cochlea. When coupled with detailed information on pulse design they offer a clear explanation as to how CF bats can effectively perceive echoes during periods of pulse-echo overlap.

Recording of cochlear microphonic potentials from unanesthetized animals was accomplished by intracranial implantation of tungsten electrodes near the cochlear aqueduct (*11*). With this technique high-amplitude potentials could be recorded with no interference to peripheral or central auditory structures. Audiograms were recorded from both anesthetized and awake animals (*12*) for periods of up to 3 weeks. During all experiments the bats were comfortably restrained with their heads held rigidly by a clamp attached to a keel of plastic cemented to the skull. The head was suspended in a free-field away from any objects, the wings were held away from the body and ears, and all surfaces behind the bats were lined with cotton to eliminate standing waves. The loudspeaker (*13*) was located 50 to 57 cm from the bat's ear and its orientation in the horizontal plane was adjusted