the induced flow, the average velocity, V, of the wing can be estimated. In the case of the dragonfly, even with conservative estimates of the stroke-plane angle and the portion of the stroke producing lift, C_L values calculated for the wing exceeded those measured from similarly shaped flat plates under steady-state conditions at equivalent Reynolds numbers.
9. L. E. Chadwick, *Bull. Brooklyn Entomol. Soc.* **35**, 109 (1940); J. W. S. Pringle, *Insect Flight* (Cambridge Univ. Press, Cambridge, 1957), p. 22.
10. M. W. Luttges, C. Somps, M. Kliss, M. Robinson, in *Proceedings of Workshop on Unsteady Separated Flow*, M. Francis and M. Luttges, Eds. (Colorado Associated University Press, Boulder, 1984), pp. 127–136.
11. Because of the mechanical low-pass filtering characteristics of the force balance used in these studies, lift variations with frequencies greater than 100 Hz were heavily damped. A newly designed three-dimensional force balance responsive to high frequencies indicates that high-frequency events may be superimposed on the dominant 25 to 30 Hz lift peaks reported here.
12. S. B. Savage, B. G. Newman, T.-M. Wong, *J. Exp. Biol.* **83**, 59 (1979).
13. Because of the relatively short duration of flight episodes (2 to 3 seconds) of varying wing-beat frequency (25 to 30 Hz), it is difficult to obtain detailed information about the local flow fields produced by self-initiated flight in the tethered dragonfly. To circumvent these problems, we presently use a modeling strategy in which electrical stimulation delivered to specific neuromuscular control centers of a tethered dragonfly produces stereotyped wing kinematics similar to those in self-initiated flight behavior. Visualization of the associated flow reveals an energetic, repeatable, unsteady vortex field interacting directly with the dragonfly wings.
14. On the basis of a typical wing-beat frequency of 28 Hz and a typical chord length of 1 cm, the nondimensional reduced frequency K ($\omega c/2V$, where ω is angular frequency) ranges from 0.3 to 3.0 for typical free-flight velocities of 3 to 0.3 m sec^{-1}, respectively.
15. W. J. McCroskey, *Annu. Rev. Fluid Mech.* **14**, 285 (1982); M. C. Robinson and M. W. Luttges, in *Proceedings of Workshop on Unsteady Separated Flow*, M. Francis and M. Luttges, Eds. (Colorado Associated University Press, Boulder, 1984), pp. 117–126; J. E. Lamar and J. F. Campbell, *Aerosp. Am.* **22**, 95 (1984).
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Monitoring the Time Course of Cerebral Deoxyglucose Metabolism by ^{31}P Nuclear Magnetic Resonance Spectroscopy

Abstract. *The phosphorylation of 2-deoxyglucose by the mammalian brain is used as an index of the brain's energy metabolism. The results of phosphorus-31 nuclear magnetic resonance (^{31}P NMR) monitoring of conscious animals in vivo showed rapid phosphorylation of 2-deoxyglucose by brain tissue. The rate of phosphorylation as determined by ^{31}P NMR was consistent with results achieved by tracer methods using carbon-14–labeled 2-deoxyglucose. However, the disappearance of 2-deoxyglucose-6-phosphate was shown to be faster than that reported by tracer studies and occurred without alterations of intracellular pH and energy homeostasis. These results were confirmed by gas chromatography and mass spectroscopy. It is postulated that 2-deoxyglucose may be metabolized in several ways, including dephosphorylation by a hexose phosphatase.*

Brain neuronal activity requires large amounts of energy. Regions within the brain selectively increase or decrease energy utilization in a manner commensurate with behavioral changes, as is revealed by electroencephalography, evoked potentials, cerebral blood flow (*1*), and methods that employ metabolic substrates such as oxygen and glucose (*2*). The use of glucose analogs, including ^{14}C-labeled 2-deoxyglucose (2-dGlc) (*3*), has enhanced the applicability of techniques such as positron emission tomography and autoradiography. All the methods provide mutually confirming results. However, the basic assumptions of the analog methods have been challenged (*4*).

Phosphorus-31 nuclear magnetic resonance (^{31}P NMR) spectroscopy can monitor high-energy phosphorus metabolites—such as adenosine triphosphate (ATP), phosphocreatine (PCr), inorganic phosphate (P_i), and various phosphomonoesters (PME) including 2-deoxyglucose-6-phosphate (2-dGlc-6-P)—in living animals (*5*) (Fig. 1). The surface-coil radio frequency antenna, a small transmitter-receiver that can be placed over the intact skin of the anatomic region of interest, has allowed long-term, spatially localized observation of ongoing normal metabolic events (*6*). To study the accumulation of intracerebral 2-dGlc-6-P after a single intravenous injection of 2-dGlc in the conscious animal, we used surface-coil ^{31}P NMR to observe PME resonances before and after injection.

The subjects were 12 Sprague-Dawley rats (250 g in body weight), housed in group cages with free access to food, that had adapted to standing upright in an NMR probe while fully awake. For the experiments, the rats were deprived of food for 16 hours, after which their blood glucose content was measured. If the content for a particular rat was less than 100 mg/dl (5.6 m*M*), the animal was anesthetized with halothane and a catheter was inserted in the tail vein. After the rat awakened and could stand steadily on all four paws, pentobarbital (20 mg/kg) was delivered through the catheter, which was considered patent if the animal collapsed immediately. The rat was supported and restrained in an NMR probe. An oval, single-turn surface coil (~1.75 by 1 cm) was placed 1 mm above the cranium, and the rat was ascertained to be responsive by an active corneal reflex. The probe was inserted into an 8.5-T spectrometer (Bruker model WH 360) operating at a frequency of 145.8 MHz. Spectra for ^{31}P were collected in 5-minute blocks for 20 minutes before and 240 minutes after each rat received a bolus injection (0.25 cm^3; dose, 500 mg/kg) of either 2-dGlc (DG-NMR group, n = 4) or dextrose (DEX-NMR group, n = 4) in sterile water. A third group was not injected (NIC-NMR group, n = 4).

Statistical differences between relative concentrations of metabolite (as determined from peak heights) among groups over time were determined with an analysis of variance for repeated measures; the t-test was used to evaluate differences between two specific time blocks (*7*). In the DG-NMR group, the broad PME resonance rose after the injection, with maximum intensity at 40 minutes (Fig. 2A). A rapid decline then ensued, with the PME peak reaching half-maximum intensity 120 minutes af-

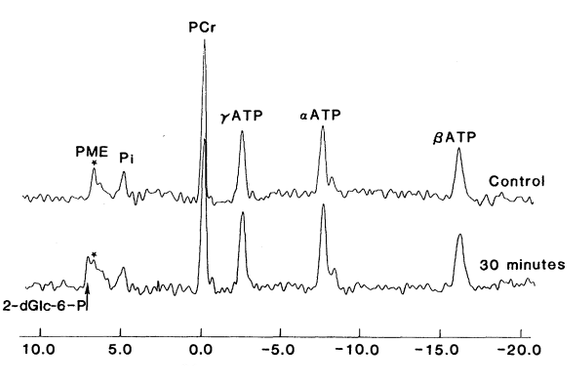

Fig. 1. Surface-coil ^{31}P NMR spectra from a rat treated with 2-dGlc. The normally intense baseline bone phosphate contribution was removed by convolution difference, and resolution-enhancement was carried out (*7*). In the control (upper) spectrum, resonances are identified as phosphomonoesters (PME, *), inorganic phosphate (P_i), phosphocreatine (PCr), and the γ-, α-, and β-phosphates of adenosine triphosphate (γATP, αATP, and βATP). In the lower spectrum [30 minutes after a bolus injection of 2-dGlc (500 mg/kg)], a new peak (↑) appeared at 7.11 parts per million, which is the chemical shift of 2-dGlc-6-P (*12*). Acquisition parameters: pulse width, 40 μsec at 100 W; spectral width, 10 KHz; 1024 data points; pulse repetition rate, 1 sec^{-1}; accumulation time, 10 minutes.

ter the injection and returning to the range of control by 4 hours after the injection (Fig. 2A). The maximum height (40 minutes) was significantly different from that observed before injection ($P = 0.0001$), at 100 minutes after injection ($P = 0.004$), and at later times. The values before injection and at 240 minutes (final value) did not differ significantly ($P = 0.51$) (*8*). In the DEX-NMR and NIC-NMR groups, the PME resonance showed no systematic changes over time but was significantly different ($P < 0.008$) from the DG-NMR group at 20 through 80 minutes after injection.

Our finding of maximum concentration of 2-dGlc-6-P by 40 minutes is in agreement with results of others (*3*) who used tracer amounts of ^{14}C-labeled 2-dGlc in the conscious rat; however, in our experiment, the concentration of 2-dGlc-6-P declined more rapidly than predicted by the ^{14}C tracer method. Although our resolution-enhanced spectra made it possible to distinguish the 2-dGlc-6-P resonance from that of the other PME (see Fig. 1), we carried out a separate study to identify 2-dGlc-6-P by its molecular weight and to measure its concentration by means of both gas chromatography (GC) and GC–mass spectroscopy (GC-MS) (Fig. 2B). Rats were treated as for NMR except that a group of four was decapitated at each interval (30, 60, 90, 150, 210, and 270 minutes) after injection of 2-dGlc (plus one group before injection), with collection of the head directly in liquid nitrogen. The cerebral cortex was dissected at −20°C and analyzed as described (*8*). Data from both NMR and GC studies showed that 2-dGlc-6-P reached its highest concentration in the brain within 30 to 50 minutes after an intravenous bolus injection of 2-dGlc and then declined rapidly. The slope of the NMR disappearance curve for 2-dGlc-6-P indicated a half-life of 90 minutes; the half-life as measured by GC was 122 minutes. Statistical comparison of these slopes showed them not to differ ($P = 0.08$, one-tailed t-test) (*9*).

An assumption of the 2-dGlc analog method for measuring glucose metabolism in the brain has been that 2-dGlc-6-P is not metabolized further and remains trapped within cells (*4*). It has been suggested (*10*) that the methods would be invalidated by the proof of a hexose phosphatase that could free 2-dGlc from brain tissue (*4, 11*). Our data directly address this point of controversy from a new perspective. First, the results from both our NMR and GC studies demonstrate that the concentration of 2-dGlc-6-P in the brain is maximum at 30 to 50 minutes after injection, which corresponds to the recommended timing of ^{14}C tracer experiments (*3*). Second, the data show a rapid, significant decrease in the concentration of 2-dGlc-6-P, which indicates a mechanism for rapid alteration of large quantities of 2-dGlc-6-P in the brain.

The major question raised by these data concerns the mechanism of 2-dGlc-6-P loss. There were negligible changes in the concentrations of P_i, PCr, and ATP in all three NMR groups, and intracellular pH remained stable throughout the experiments (*12*). For rats analyzed by GC, blood glucose concentrations were measured at each sample point [mean of values: 80 mg/dl (4.4 m*M*) before injection; 300 mg/dl (16.6 m*M*) at 2.5 hours after injection (peak); and 120 mg/dl (6.7 m*M*) at 4.5 hours after injection (final)]; these concentrations were unlikely to alter glucose utilization constants (*13*). Cerebral cellular integrity was maintained, as evaluated by light microscopy of thionin-stained sections (*14*). We thus conclude that removal of 2-dGlc-6-P occurs under conditions of physiologic intracellular pH and high-energy phosphate concentrations.

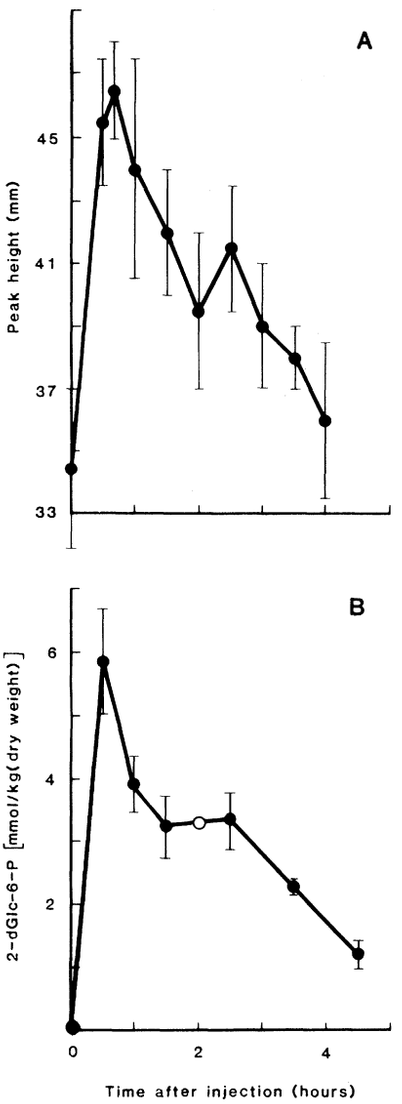

Fig. 2. (A) Phosphorus-31 NMR peak heights for PME (determined in the absence of resolution enhancement) at designated time intervals after an intravenous bolus injection of 2-dGlc (500 mg/kg). Four rats were monitored 20 minutes before injection and for 4 hours after injection. Means and standard error of the mean are plotted (*7*). (B) Gas chromatographic analysis of 2-dGlc-6-P in the cerebral cortex of a rat after an intravenous bolus injection of 2-dGlc (500 mg/kg). Tissue samples were analyzed by means of a phosphorus-specific detector (*8*). Time points with error bars are groups of four rats. The open circle at 2 hours represents a single animal monitored by NMR (data not shown in Fig. 2A) before it was killed for analysis by GC-MS. Means and standard error of the mean are plotted.

Several mechanisms may take part in the loss of 2-dGlc-6-P. Some could result in 2-dGlc remaining in the cell (*11, 15*), and others, including dephosphorylation by a hexose phosphatase, could lead to loss of 2-dGlc from the brain (*4, 16*). Most studies of 2-dGlc metabolism used tracer amounts of ^{14}C-labeled 2-dGlc, with resultant concentrations of ^{14}C-labeled 2-dGlc-6-P that were so low that the enzymatic processes responsible for removal of 2-dGlc-6-P in our study may not have been engaged. Therefore, in comparing studies, large differences in the concentration of 2-dGlc-6-P in the brain produced by different methods must be considered. Direct identification of the mechanisms that affect the concentrations of 2-dGlc-6-P in the brain should resolve this issue.

RUTHMARY K. DEUEL
Departments of Pediatrics and *Neurology* and *McDonnell Center for Study of Higher Brain Function, Washington University School of Medicine, St. Louis, Missouri 63178*
GENEVIEVE M. YUE
Department of Internal Medicine, Washington University School of Medicine
WILLIAM R. SHERMAN
Departments of Psychiatry and *Biological Chemistry, Washington University School of Medicine*
DAVID J. SCHICKNER
JOSEPH J. H. ACKERMAN
Department of Chemistry, Washington University

References and Notes

1. H. G. Vaughn, *Ann. N.Y. Acad. Sci.* **388**, 125 (1982); J. Astrup, G. Blennow, B. Nilsson, *Brain Res.* **177**, 115 (1981); F. Plum, A. Gjedde, F. E. Samson, *Neurosci. Res. Prog. Bull.* **14**, 457 (1976); Y. Nishizawa *et al.*, *J. Neurophysiol.* **48**, 458 (1982); W. G. Lennox, F. A. Gibbs, E. L. Gibbs, *Arch. Neurol. Psychiatr.* **34**, 1001 (1935).

2. M. E. Raichle, *Brain Res. Rev.* **1**, 47 (1979); M. E. Raichle *et al.*, *Am. J. Physiol.* **228**, 1936 (1975); R. A. Hawkins, W. K. Hass, J. Rausahoff, *Stroke* **10**, 690 (1979).
3. L. Sokoloff *et al.*, *J. Neurochem.* **28**, 897 (1977); L. Sokoloff, *Brain* **102**, 653 (1979); R. C. Collins, J. B. Posner, F. Plum, *Am. J. Physiol.* **218**, 943 (1970); S. C. Huang *et al.*, *ibid.* **238**, 269 (1980).
4. R. A. Hawkins and A. L. Miller, *Neuroscience* **3**, 251 (1978); W. C. Sacks, S. Sacks, A. Fleischer, *Neurochem. Res.* **8**, 661 (1983); W. M. Pardridge, *Physiol. Rev.* **63**, 1481 (1983).
5. R. S. Balaban, *Am. J. Physiol.* **246**, C10 (1984).
6. J. J. H. Ackerman *et al.*, *Nature (London)* **283**, 167 (1980); J. L. Evelhoch, M. C. Crowley, J. J. H. Ackerman, *J. Magn. Res.* **56**, 110 (1984); R. K. Deuel, *Pediatrics* **70**, 650 (1982).
7. Data analysis was carried out after combining the 5-minute collections into 10-minute blocks. Relative concentrations were determined (i) by integrating the area under the peak after convolution difference and resolution enhancement (see Fig. 1); (ii) by measuring the PME peak height (by computer) after removal of baseline artifact by Chebyshev polynomial fit and subtraction [J. J. H. Ackerman *et al.*, *J. Magn. Res.* **56**, 318 (1984)]; and (iii) by measuring the PME peak height (manually) relative to the bone phosphorus–derived baseline (Fig. 2A) with β-ATP concentration used as a covariable for each individual animal to compensate statistically for possible minor differences in brain volume observed by the surface coil in different rats. All three methods yielded similar results.
8. Brains were dissected at −20°C, and 50 mg of cerebral cortex was lyophilized, weighed, and treated with trimethylsilylating reagent [A. L. Leavitt and W. R. Sherman. *Methods Enzymol.* **89**, 9 (1982)]. Aliquots were separated by gas chromatography on a 4-foot by 0.25-inch column packed with 3 percent OV-17. The effluent was measured with a flame photometric detector operated in the phosphorus-selective mode. Control tissue samples contained small amounts of an uncharacterized phosphorus-containing substance that eluted with the first of the two trimethylsilyl 2-dGlc-6-P peaks; therefore, in all analyses only the second of the 2-dGlc-6-P peaks was used for quantification. In validating the GC analyses, GC-MS was carried out on the same column with ammonia chemical ionization. The substance from the cerebral cortex of rats treated with 2-dGlc had the same protonated molecular ion (m/z, 605) as authentic pentatrimethylsilyl 2-dGlc-6-P; also, like the authentic material, it eluted from the chromatography column as two peaks, which presumably represent the α and β anomers of 2-dGlc-6-P. Quantitative measurements by GC-MS of three samples at 1.5, 2.5, and 3.5 hours gave results identical with the corresponding data from GC at the flame photometric detector.
9. J. H. Zar, *Biostatistical Analysis* (Prentice-Hall, New York, 1974), p. 228.
10. J. L. Fox, *Science* **224**, 143 (1984).
11. T. Nelson, E. E. Kaufman, L. Sokoloff, *J. Neurochem.* **43**, 949 (1984).
12. Chemical shifts for both P_i and 2-dGlc-6-P were used independently to measure *p*H after resolution enhancement by means of curves plotting chemical shift against *p*H derived in our laboratory [I. A. Bailey *et al.*, *Biochem. J.* **196**, 171 (1981)]; both measurements gave the same results.
13. A. Gjedde, *Brain Res. Rev.* **4**, 237 (1982); P. D. Crane *et al.*, *J. Neurochem.* **36**, 1601 (1981).
14. Rats used in brain histology experiments were managed in the same way as those used for NMR and GC studies except that they were killed at 4, 48, and 92 hours after the bolus injection of 2-dGlc and perfused with saline and formaldehyde. Brains were then cut in 30-μm sections and stained with thionin.
15. Because studies performed in vitro have shown *myo*-inositol-1-phosphate synthase to transform 2-dGlc-6-P to 5-deoxy-*myo*-inositol 1-phosphate and have also shown this substance to be hydrolyzed to 5-deoxy-*myo*-inositol by *myo*-inositol 1-phosphatase (Y.-H. H. Wong and W. R. Sherman, *J. Biol. Chem.*, in press), cerebral cortex was examined for these deoxy-inositols by GC-MS. A lower limit of detectability for the substances under the conditions employed is about 0.1 mmol per kilogram of tissue (wet weight). However, none was found, so this pathway cannot make a significant contribution to the disappearance of 2-dGlc-6-P.
16. J. M. Anchors and M. L. Karnovsky, *J. Biol. Chem.* **250**, 6408 (1975); M. Huang and R. L. Veech, *ibid.* **257**, 11358 (1982); H. R. Stephens and E. B. Sandborn, *Brain Res.* **113**, 127 (1976); M. L. Karnovsky, B. L. Burrows, M. A. Zoccoli in *Cerebral Metabolism and Neural Function*, J. V. Passoneau, R. A. Hawkins, W. D. Lust, F. A. Walsh, Eds. (Williams and Wilkins, Baltimore, 1980), pp. 359–366; R. W. Horton, B. S. Meldron, H. S. Bachelard, *J. Neurochem.* **21**, 507 (1973).
17. Supported by NIH grant GM-30331, NSF grant CHE-8100211, and funding from the McDonnell Center for Study of Higher Brain Functions and the Washington University Mass Spectrometry Facility (RR-00954, AM-20579). We thank S. Sondgeroth and S. Goodman for adapting the rats; L. Munsell and R. Berger for the GC and GC-MS analyses, respectively; and G. Levy for providing NMR software (National Institutes of Health Biotechnology Research Resource grant RR-01317).

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Sonar Tracking of Horizontally Moving Targets by the Big Brown Bat *Eptesicus fuscus*

Abstract. *When following a moving target, echolocating bats (*Eptesicus fuscus*) keep their heads aimed at the target's position. This tracking behavior seems not to involve predicting the target's trajectory, but is achieved by the bat's pointing its head at the target's last known position. The bat obtains frequent position updates by emitting sonar signals at a high rate. After the lag between head and target positions and the nonunity tracking gain were corrected for, bats' tracking accuracy in the horizontal plane was ±1.6°.*

Most species of echolocating bats are insectivorous and use their sonar system to locate, identify and capture flying insects (*1*). During pursuit the bat keeps its head aimed at the target as it follows and finally intercepts it. The accuracy of head-aim tracking as measured from stroboscopic photographs of bats maneuvering to catch prey is about ±5° (*2*). A bat's capture success depends on knowing the prey's location throughout the interception process. Head-aim tracking keeps prey in front of the bat, where angular acoustic resolution is best, on the order of ±1.5° as measured with stationary targets (*3*). In addition, by keeping prey in the middle of the echolocation sound beam, head tracking functions as one of several gain control mechanisms that reduce variation in the perceived echo strength as the bat closes in on its prey (*4*).

Bats hunting by sonar do not receive continuous information about target location, but, rather, the echo from each sonar emission provides an acoustic "snapshot" from which the bat updates its current perception of range and position. Through the use of these snapshots, a bat could keep track of prey by two general techniques. It could simply keep its head pointed at the target's last known position (a nonpredictive tracking strategy), or it could attempt to predict the target's trajectory on the basis of the history of target parameters such as position, velocity, and acceleration (a predictive strategy). Since photographic techniques are not sufficiently accurate to distinguish between these two types of

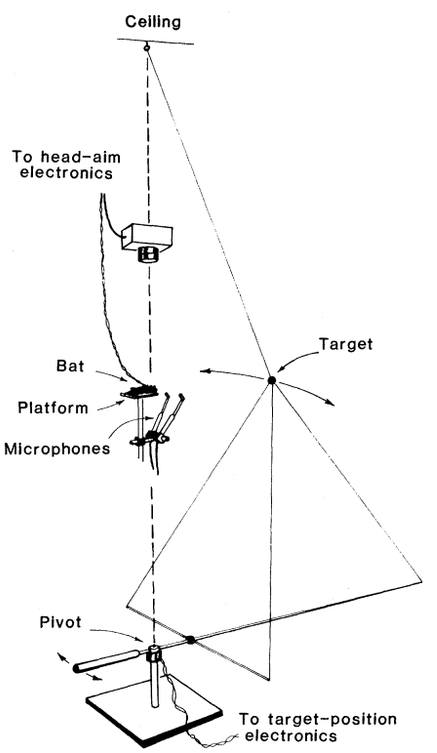

Fig. 1. Apparatus and procedure for studying target tracking by bats. The bat was trained to sit on the platform and keep its head aimed at a target (a black styrofoam ball 3.6 cm in diameter) suspended in space by four nylon monofilament lines (diameter 0.18 mm, and acoustically invisible to the bat) arranged tetragonally to keep the ball from swinging relative to the frame. These lines were attached to a *t*-shaped frame 1 m below the platform that could be moved by the experimenter to control the ball's position. To monitor the bat's head aim, light from two light-emitting diodes attached to the head was focused by a camera lens 60 cm above the bat onto a position-sensing diode (*8*). The lens and platform were mounted coaxially with the attachment point on the ceiling and the pivot of the *t* frame. Two microphones (*9*) placed slightly in front of and below the platform were used to record the bat's sonar emissions. A detector circuit converted the sounds to pulses, which were recorded on the tape recorder.