then in 0.25*M* sucrose in deionized water made to 0.0018*M* with $CaCl_2$ (Ca-sucrose solution). The ovaries were allowed to stand for 20 min in 5 volumes of the Ca-sucrose, drained and homogenized in a Waring Blendor at slow speed (35 v) for 1 min in 4 volumes of Ca-sucrose. Four drops of caprylic alcohol were added to break the very stiff foam. (ii) The homogenate was filtered through two, and then four layers of coarse cheesecloth (20 by 30 mesh). The filters were washed with Ca-sucrose, and the washings were added to the filtrate to make 700 ml. The filtrate was centrifuged for 5 min at 150 *g* in 100-ml Lusteroid centrifuge tubes. (iii) The supernatant was discarded or saved for the isolation of cytoplasmic fractions, and the precipitate was resuspended in Ca-sucrose and filtered through eight layers of 40- by 40-mesh cheesecloth. The filtrate and washings were made up to 400-ml volume and centrifuged for 5 min at 150 *g*. (iv) The supernatant was discarded, and the precipitate was resuspended in 400 ml of 0.34*M* sucrose in ion-free water and centrifuged for 5 min at 150 *g*. The supernatant was discarded, and the precipitates were combined, made up to 100-ml volume with 0.34*M* sucrose solution, and centrifuged for 5 min at 150 *g*. (v) The supernatant was discarded, and the precipitate made up to 15-ml volume with 0.34*M* sucrose, then the nucleoli were concentrated, and the final contaminants were removed as described by Vincent (*6*). For nucleic acid and phosphorus studies, the sucrose was removed from the final pellet by two washes each in 70 percent, 95 percent, and absolute alcohol. The washed nucleoli were then vacuum dried and stored at – 20°C until used. All procedures were carried out at 2° to 4°C. The average time for isolation was 2 hours. The average yield has been about 1 mg of dry nucleoli from 10 g (wet weight) of ovaries.

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8. In the experiments described here, the RNA_1 fraction averaged 4.9 percent of the total RNA of the nucleolus.
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10. These studies were supported in part by AEC grant AT(30-1)1343.

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Table 1. Homing performance of displaced bats.

Distance (mi)	Number released	Percentage that returned at speed (mi/hr) indicated*					
		> 15	10–15	7–10	5–7	< 5	lost†
				Experiment A, 20–21 Oct.			
5	59			3.4	6.8	33.9	55.9
10	58			1.7	13.8	25.9	58.6
15	57			3.5	17.5	7	71.9
20	60		1.7	6.7	8.3		83.3
				Experiment B, 23–24 Oct.			
30	50	4	2	12	10	8	64
40	50		8	8	6		78
50	50			4	4		92
60	50	2	2	2			94
				Experiment C, 23–24 Oct.			
Blindfolded	25‡		7.2	21.6	21.6	21.6	28.1
Controls	25		12	32	8	12	36

* The figures in the last two columns of experiment A and in the last three columns of experiment B were variously influenced by inequalities in the time that was available for returning before the termination of the experiment. Therefore, they should not be used in comparing the performances of the various distance-groups.

† Netting was terminated at dawn on the first night. Any animals that did not return on the first night were designated lost.

‡ Of the 25 blindfolded bats that were released, 18 were recovered at the mine entrance, and, of these, eight had scratched the covering from one or both eyes. Figures in the table are based on the ten bats which were still blindfolded on their return, multiplied by a factor of 25/18 to render the figures directly comparable with the return percentages of the other experiments.

Homing in Bats

Homing ability has been demonstrated in a variety of terrestrial vertebrates. Of the many theories advanced for explaining the sensory basis of this phenomenon, only sun compass orientation has survived critical experimentation (*1*). The navigational problems of homing generally involve more than compass orientation, however (*2*). Matthews (*3*) has proposed a complete celestial navigation theory, based on vision, for certain birds, but others have questioned his interpretations (*2, 4*). Lindenlaub (*5*) has recently demonstrated definite homing tendencies in mice in an apparatus which eliminated any possibility of visual or olfactory clues.

A survey of the literature on homing in bats is given by Cockrum (*6*). Past work has consisted largely of displacing bats from a cave and then attempting to find them in the intricacies of the cave some days, or even months, later. This technique has yielded little information on the homing speeds or the percentage of bats that returned. In 1954 Cockrum released 68 bats (*Myotis velifer*) 28 mi from a cave in Arizona and recaptured two of these as they left the cave the following evening. On the assumption that these bats did not fly during the daytime, it appears that they must have traveled these 28 mi in something less than 4 hours.

During the fall of 1956 we performed a few simple homing experiments with bats which revealed a remarkably accurate navigational ability, even in blindfolded individuals (7). A total of 484 bats were removed from their hibernating roosts in an inactive lead mine in southwestern Wisconsin during the early hours of darkness on the nights of 20–21 and 23–24 October and transported to the east to points at distances that varied between 5 and 60 mi, for release. Distinctive markings were painted on the wings of the animals for each release point, and the animals were grouped in carrying cages for transport; all had fully recovered from the torpor of hibernation before release. Recaptures were made in a modified Japanese mist net as the bats entered the mine, and the exact time of each arrival was noted.

Homing performance was measured in terms of (i) the percentage of bats that returned to the mine during the same night, and (ii) the over-all homing speed of these returning animals, from the time of release to the time of entering the mine. Return records were obtained for all releases and percentages ranged from 6 percent in the 60-mi group to 72 percent in one of the 5-mi groups (Table 1). Homing speeds ranged up to 19 mi/hr and exceeded 10 mi/hr in 14 cases. Light tail-winds favored most of these animals (*8*), but the performance is regarded as indicating a rapid orientation and a direct homeward course, since flight speeds for bats of this genus have been measured as only 10 to 11 mi/hr (*9*).

Although vision is poorly developed in bats (*10*), its demonstrated importance for distance orientation in other animals indicated that a blindfolding experiment should be performed. Accordingly, 25 bats, blindfolded with eye caps of lampblack in collodion, were released, together with 25 controls, at a point 5 mi east of the mine. Although some of the blindfolded animals had scatched away all or part of their masks before reaching home, others retained theirs, and the fact that they reached home as rapidly as the controls did demonstrated that obstruction of vision had no gross effect on homing performance (Table 1C).

Since bats are able to detect obstacles in their flight path by echolocation (*10*), it is interesting to speculate on the possibility that they are aided by auditory mechanisms in distance orientation. Bats are also capable of learning landmarks and complex spatial patterns within a room by auditory means (*11*), and it is possible that whole landscapes may be memorized. However, evidence presented by Griffin (*12*) and Möhres (*13*) suggests that the maximum effective distance of echolocation for bats of this genus is less than 10 m, and it is difficult

to believe that an animal with these sensory restrictions could attain an "auditory familiarity" with an area of 50 or 60 miles' radius.

Our preliminary experiments thus indicate that bats possess a well-developed ability to orient and to home over long distances by sensory means other than vision. Audition has not been eliminated as a possible mechanism, but it appears to hold little promise of providing a complete answer. Further studies are in progress.

H. C. MUELLER
J. T. EMLEN, JR.
Department of Zoology,
University of Wisconsin, Madison

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Ethylenediaminetetraacetate and Mitochondrial Adenosine Triphosphatase Activity

The adenosine triphosphatase activity of isolated mitochondria is variable and is influenced by many factors (*1, 2*). We have observed, as is shown in Table 1, (*3*), that the inclusion of ethylenediaminetetraacetate in the sucrose used for tissue homogenization results in marked changes in the adenosine triphosphatase activity of mitochondria isolated from these homogenates.

Mitochondria were isolated from 0.25*M* sucrose homogenates with and without ethylenediaminetetraacetate, 0.01*M*, *p*H 7.4 (*4*). After 10 minutes' centrifugation at 600*g*, the supernatant solution was centrifuged for 10 minutes at 8500*g* to sediment the mitochondria. The particles were then resuspended in sucrose of the same composition as that used for homogenization and, after resedimenting, were suspended in sucrose without ethylenediaminetetraacetate. All operations were performed at 2°C. Adenosine triphosphatase activity was measured using a 10-minute incubation at room temperature in a volume of 1 ml containing, in micromoles, the following: adenosine triphosphate (Pabst), 8; $MgCl_2$, 4; tris(hydroxymethyl)aminomethane hydrochloride (*p*H 7.4), 20; and sucrose, 176. Reactions were initiated by addition of mitochondria and were terminated by addition of 0.02 ml of 70-percent perchloric acid. These solutions were immediately cooled to 0°C. Inorganic phosphate was determined by the Fiske-Subbarow procedure (*5*) on an aliquot of the supernatant solution.

Table 1. Adenosine triphosphatase activity of mitochondria isloated from sucrose and sucrose-ethylenediaminetetraacetate (EDTA) homogenates. Adenosine triphosphatase was measured at two enzyme concentrations ranging from 40 to 200 μg of protein N. Activities are expressed as micromoles of phosphate hydrolyzed per 10 minutes, per milligram of mitochondrial nitrogen at room temperature. Average activity values are reported except for two cases where wide variation in the ratio, activity/enzyme concentration, occurred. This may result from inhibition of the adenosine triphosphatase by accumulated adenosine diphosphate, for addition of the latter to the incubation mixture yields a marked inhibition of the adenosine triphosphatase (*8*).

Source of mitochondria	EDTA added	Δ Inorganic phosphate (μmole)			
		Fresh mitochondria		Aged mitochondria (30 min, 38°C)	
		No addition	Dinitrophenol added ($2.5 \times 10^{-4}M$)	No addition	Dinitrophenol added ($2.5 \times 10^{-4}M$)
Rat liver					
1	–	0.6	9.6	6.3	9.0
1	+	1.3	6.6	1.4	1.6
2	–	0.3	12.2	6.9	
3	+	3.4	3.7	2.4*	
Rabbit heart					
1	–	10.1	21.9	5.2	6.3
1	+	15.3	(33.0, 23.4)†	18.4	19.1
2	–	2.7	8.3	1.7	2.1
3	+	8.7	(18.8, 10.3)†	7.7	11.5

* Aged 2 hours at room temperature. † Values were not averaged because of large variation in activity with variation in the amount of mitochondria added.

When ethylenediaminetetraacetate is used, the supernatant solution from the mitochondrial pellet is redder and the final mitochondrial suspension is more cream-colored than when sucrose alone is used for homogenization. Differences in the adenosine triphosphatase activity are clearly evident even though the final mitochondrial pellet isolated by both procedures is suspended in sucrose without ethylenediaminetetraacetate.

Rat liver mitochondria isolated from sucrose-ethylenediaminetetraacetate homogenates have a higher initial adenosine triphosphatase activity and a lower "latent adenosine triphosphatase" (*1*) activity than do mitochondria isolated from sucrose alone.

The adenosine triphosphatase of rabbit heart mitochondria isolated from sucrose decreases on aging, while that of heart mitochondria isolated from sucrose-ethylenediaminetetraacetate does not. Although the activity of aged preparations may be increased slightly by addition of dinitrophenol, the amount of phosphate released from adenosine triphosphate is below that obtained with fresh preparations in the presence of dinitrophenol.

These data show that isolation of mitochondria in sucrose containing 0.01*M* ethylenediaminetetraacetate alters the adenosine triphosphatase activity pattern from that observed with mitochondria isolated from sucrose alone (*6*). On the other hand, we find that ethylenediaminetetraacetate yields heart muscle mitochondrial suspensions which have a uniformly more dependable oxidative capacity. This is in conformity with reports of other investigators (*4, 7*), who have noted an increased stability of mitochondria that have been isolated in the presence of ethylenediaminetetraacetate.

The data presented here show the marked variation of an enzymic activity that can result from a modification commonly employed in the isolation of mitochondria. While it is well known that the source of mitochondria and the nature of the isolation procedure may modify the enzymatic spectrum of the particles, the effect of a small change in procedure may pass unrecognized in many instances. When the rate of adenosine triphosphate turnover is an important factor, these changes may assume a primary significance.

R. W. VON KORFF*
Department of Pediatrics,
Variety Club Heart Hospital,
University of Minnesota, Minneapolis