Table II. Biosynthesis of labeled ergosterol from [2-^{14}C]-acetate in *S. cerevisiae* SK1 using CoA-enrichment and anaerobic-aerobic procedure. Anaerobic Growth in Coenzyme-A deficient medium[a, b]. Aeration suspension[c] (0.1% glucose, 0.1 *M* [2-^{14}C]acetate, 0.1 *M* phosphate, pH 7.0, 1.0% YNB containing 25 mg CoA[d]

Cell concentration (g wet cells/100 ml aeration medium)	Ergosterol recovered[e] (mg)	Incorporation efficiency[f]	Overall incorporation[g]
20	4.8	4.5	0.08

[a] 1% Wickerham's Yeast Nitrogen Base (YNB) without pantothenic acid, 10% dextrose, and 17 mg/ml Tween 80 and 20 µg/ml tetracycline. [b] Same as (b) in Table I. [c] Same as (c) in Table I. [d] Grade II-A from Sigma. [e] Same as (d) in Table I. [f] Same as (e) in Table I. [g] Same as (f) in Table I.

Table III. Biosynthesis of labeled ergosterol from [2-^{14}C]-acetate in *S. cerevisiae* SK1 during sporulation.
Sporulation Conditions for A and B: aerobic, 1% labeled acetate, 27°C, vigorous mechanical stirring, 4×10^7 cells/ml (1 l total volume, containing 5.5 g wet cells in 6 l flask)

Sporulation yield	(A) 99% asci in 3 days	(B) 90% asci in 5 days
Ergosterol recovered[b] (mg)	4.4	7.9
Incorporation efficiency[c]	71	76
Overall incorporation[d]	0.11	0.21

Presporulation growth conditions: (A) aerobic, YPD[a], 30°C, 2 days 1 l from loop, shaking at 200 rpm. (B) anaerobic, YPD, 30°C, 2 days 1 l from loop, shaking at 200 rpm. [a] Same as (a) in Table I. [b] Same as (d) in Table I. [c] Same as (e) in Table I. [d] Same as (f) in Table I.

As yeast sporulation in a liquid medium is carried out at approximately 4×10^7 cells/ml, a liter of 1% acetate is required to obtain the ergosterol yields we report. However, since the sporulation medium contains only acetate, the recovery of unincorporated label can be accomplished by a simple extraction.

Data of labeled substrate incorporation into a metabolite can be stated in various ways. We have reported firstly the overall incorporation as an indication of the metabolic magnitude of ergosterol biosynthesis from acetate. Secondly, the incorporation efficiency expresses the percentage of ergosterol carbons derived from acetate. The factor of 1/15 (cf. Table I, footnote e) in the calculation of incorporation efficiency allows for the biosynthesis of 1 mole of ergosterol from 15 moles of acetate. We have assumed that acetate contributes to all ergosterol carbons except C_{28} which arises from single carbon donors (e.g. formate) by transmethylation via methionine[15,16].

The data in Table III clearly indicates that the sporulation technique gives a more efficient incorporation than the 2 earlier methods described. Further work on biosynthesis in yeast using ^{13}C-labeled substrates is in progress in our laboratory.

Zusammenfassung. Es werden drei Methoden für die Durchführung von Versuchen mit ^{13}C-markierten Substraten (Einbau markierter Acetate in Hefeergosterol) verglichen: 1. Anaerobisches Wachstum mit nachfolgender Belüftung und Markierung. 2. Anaerobisches Wachstum in Nährlösung mit ungenügendem CoA-Gehalt mit nachfolgender Belüftung und Markierung in einer mit CoA angereicherten Lösung. 3. Sporenbildung mit markierten Acetaten.

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A New Method for Recording Migratory Restlessness in Caged Birds

Many birds that are normally day-active migrate at night. If kept in a cage, they develop nocturnal activity during the migratory seasons which manifests itself in an intense hopping off and onto perches, a fluttering about in the cage or a vigorous beating of the wings ('whirring'). This migratory restlessness or 'Zugunruhe' has often been taken for an expression of the migratory drive in birds. Quantitatively, however, it was either only the hopping or general locomotor activity that was measured so far[1–4], but never the whirring itself which might be a more specific expression of the migratory drive. Moreover, in recording perch hopping, both day- and night-activity of a

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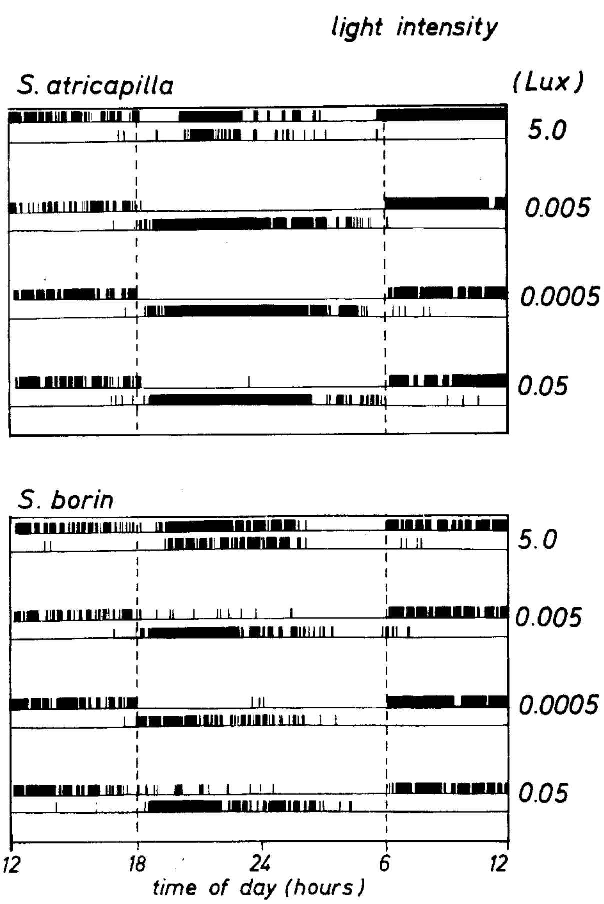
bird is recorded indiscriminately. Since migratory restlessness occurs only during the dark phase of a light-dark cycle, the only way to differentiate between the 2 activities is to know the exact time of onset and offset of the dark period. This differentiation, however, is often difficult and inexact, as for example when day- and night-activities merge during the main migration period.

Average duration of teh nightly hopping and whirring activity of 6 garden warblers (*S. borin*) and 2 blackcaps (*S. atricapilla*), kept in a 12:12-h light- and dark cycle (LD 12:12, 500:0.0 lux)

	Hopping	Whirring
S. borin	0.80 ± 0.47 (*n* 30)	6.42 ± 2.05 (*n* 26)
S. atricapilla	0.0 (*n* 9)	0.0 (*n* 8)

For each night, the total number of 15-min intervals was determined during which the birds showed activity. Average values (with standard error) are given. *n*, number of test nights.



Perch hopping and whirring activity of a blackcap (*S. atricapilla*) and a garden warbler (*S. borin*) kept for 4 consecutive days in a 12:12-h day-night cycle (night from 18.00 to 06.00 h). Night- light intensities were as shown on the right hand margin. In each double-strip the upper part shows hopping- the lower part whirring activity.

These considerations made it desirable to develop a method for selectively recording the whirring. As experimental animals, garden warblers (*Sylvia borin*) and blackcaps (*S. atricapilla*) were used, 2 species known to exhibit intense whirring during nights of migratory restlessness[5]. Whilst whirring, the birds trip sideways to and fro on the perch with the axis of the body inclined at an angle of about 45° to the plane, beating vigorously with their wings.

On the basis of these observations, a switch was constructed which responds to air movements. In a vertical brass tube (length 6 cm, diameter 1 cm) a brass rod, loosely suspended from the centre of the upper aperture, protrudes for about 1.5 cm below the lower rim, with a paper vane (about 2 × 3 cm) attached to it. One contact of the switch is provided by the rod, the other by the lower rim of the tube. Air movements press the rod against the rim and thereby close the circuit. Each stroke of the rod is converted into an electrical impulse which operates a marker on an event recorder. The sensitivity of the switch can be varied by changing the diameter of the tube or the size of the vane. One such switch was fixed on the outside of each cage at the same level and at equal distance from the 2 perches, so that whirring both from the back and the front perch was equally recorded. The sensitivity of the switch was adjusted so that it only very rarely made contact during daytime-activity. Simultaneously the hopping activity of each bird was registered by arranging a microswitch under each end of one of the perches. Air movement caused by a bird hopping from perch to perch was not sufficient to activate the switch.

The comparison of whirring activity with the simultaneously recorded hopping activity (Figure) shows considerable differences in the duration of these 2 types of nocturnal activity under various night-light intensities. During nights with a night-light of 5.0 lux, both species exhibit more hopping and less whirring than in darker nights (Figure). Under very weak night-light intensities (0.0005 lux), blackcaps (*S. atricapilla*) often showed no hopping at all but whirred throughout the night (Figure). In complete darkness, garden warblers (*S. borin*) still kept on whirring with very little hopping, whereas blackcaps did not do either (Table), suggesting species differences in the response of migratory restlessness to total darkness.

Zusammenfassung. Es wurde eine neue Methode zur Registrierung von Zugunruhe bei Gartengrasmücken (*Sylvia borin*) und Mönchsgrasmücken (*S. atricapilla*) entwickelt. Auf Luftbewegungen reagierende Schalter, die aussen am Vogelkäfig angebracht waren, ermöglichten es, das nächtliche Schwirren zugunruhiger Vögel auf Zeitmarkenschreibern aufzuzeichnen.

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