All gas volumes were reduced to standard pressure and temperature conditions. Energy production was calculated as J/g·h from gaseous metabolism and the conversion factor of 1 ml $O_2 \triangleq 20$ Joule.

Measuring instruments: Hartmann & Braun Caldos 4T (CO_2-analyzer: 0–5%) and Magnos 2T (oxygen-analyzer: 0–2%). Respiration frequency was recorded simultaneously in undisturbed birds during day and night by a highly sensitive (0–35 mbar) pressure transducer (Bell & Howell, type BHL 4104) which was inserted into the respiratory chamber. This allowed the monitoring of pressure oscillations caused by lung ventilation under standardized conditions without handling the bird. These data allowed the determination of O_2-consumption per breathing and the calculation of tidal volume (based on the assumption of a mean O_2-extraction rate of about 5% and an air-oxygen concentration of 21%).

Results and discussion. All data are summarized in the table. *Metabolic rate:* The mean metabolism-weight regression line of the day-time values follows the equation $M = 266 \cdot W^{0.862}$ ($r = +0.79$; M = metabolism in J/h and W = b.wt in g); that of the night-time values is $M = 244 \cdot W^{0.456}$ ($r = +0.55$). These correlations are not based on measurements in the thermoneutral zone (TNZ) of the birds. Corresponding values at an ambient temperature of 30 °C (TNZ) result in the following regression line: $M = 102 \cdot W^{0.716}$ (night-time) (Lübben and Prinzinger in prep.); the resulting values are in the same range as those reported for the mean basal metabolism of most passerine and nonpasserine birds: $(127 \pm 34.4) \cdot W^{0.723 \pm 0.006}$ (combined data from previous measurements by Dawson and Hudson[1], Aschoff and Pohl[2], Lasiewski and Dawson[3], Prinzinger and Hänssler[4]). Hummingbirds, which are physiologically analogous to sunbirds, show night-time values of energy metabolism which are approximately 100% (and more) above these levels (Prinzinger, Krüger and Schuchmann[5]). Diurnal increases in metabolism in sunbirds during periods of activity are between 58–69% (mean values, range: 30–77%). These are relatively large differences between day- and night-time levels (in comparison to other birds: mean difference 25%, Aschoff and Pohl[2]), indicating an energy-saving strategy through a severe reduction in metabolism during the resting phase.

Breathing frequencies (F, min^{-1}) during activity (day-time) correlate inversely with b.wt (g) using the equation $F = 314 \cdot W^{-0.38}$; during the night $F = 457 \cdot W^{-0.88}$. The resulting values clearly correspond more closely with the empirical equations for mammals (higher frequencies) than those normally obtained for birds (see Calder[6], Adolph[7]). Our results on unrestrained birds therefore do not support the opinion that birds breathe at lower frequencies than do mammals of similar body mass. It seems possible that this observed difference may result in part from differences in the conditions under which the data were collected (only very few data are from unrestrained animals).

The day/night differences in the exponents of the allometric equations are based on the fact that heavier sunbirds show a greater reduction of breathing frequency (and metabolism) during resting time than lighter species do. Breathing frequency correlates directly ($\sim M^1$) with gaseous metabolism. The equations (linear regression) are $F = 93 + 0.21 \cdot M$ for the day-time and $F = 0.97 + 0.87 \cdot M$ for the night-time (F in min^{-1}, M in J/g·h). These linear correlations lead to the assumption that the O_2-extraction rate is the same for all birds tested (that is, independent of body weight, or proportional to W^0) and that tidal volumes are directly related to b.wt (proportional to W^1). Nevertheless the lack of detailed experiments on O_2-extraction rates in birds and the paucity and variability of data on tidal volumes in birds which has been directly determined and not derived from other parameters prohibits a useful regression analysis. Therefore, the statement that the tidal volumes of birds are more than twice those of mammals of the same weight range must be viewed with a certain scepticism because of the fact that many other breathing parameters are closely correlated in these two homoeothermic groups.

Regarding the mean O_2-consumption per breathing act we could not find great differences between day and night. Consequently the diurnal cycle of O_2-consumption (high activity levels, low resting levels) seems to be primarily regulated by the observed modulations in breathing frequencies while the other respiratory parameters (O_2-consumption per breathing act, oxygen-extraction rate and tidal volume) remain relatively constant.

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Acquired protein appetite in rats: Dependence on a protein-specific need state[1]

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Summary. Rats are shown to acquire a preference for protein-predictive olfactory cues which depends on a state of mild deficit in protein intake – i. e. a learned protein-specific appetite.

Key words. Protein appetite; nutrient self-selection; need cue learning; protein-conditioned preference; odour conditioning.

When faced with diets varying in nutrient content, animals can choose sufficient protein to sustain growth [2–5]. Rats adapt their dietary selection patterns successfully in the face of changing protein requirements and availability[6–9].

Yet, no reliable natural cues to the protein content of a diet are known that might allow protein selection via innate preferences or aversions. Therefore, protein-specific dietary selection behaviour must depend on learning which dietary cues predict protein content, by associating those sensory characteristics with the protein-specific after-effects of eating[10–12]. However, such learned preferences for protein-predictive dietary cues would only produce selection behaviour sufficient to satisfy metabolic demands if the preferences were activated by incipient or current protein need. Protein-conditioned preferences have been demonstrated in mildly hungry rats, but there was no evidence that the preferences depended on a protein-specific deficit rather than, e. g., an energy deficit[13]. The experiment reported here provides the first evidence for an acquired protein appetite – that is, a preference for protein-predictive cues that depends on current lack of protein intake.

The rats were associatively conditioned to prefer a flavour by pairing it with the effects of ingested protein on mild food depri-

vation. The dependence of this conditioned preference for a protein-predictive flavour on a protein-specific deficit was determined by testing the effects on the flavour preference of prior administration of either protein or carbohydrate. If the flavour preference were indeed a learned appetite specifically for protein, then the preference should be reduced by pre-infusion of protein to a greater extent than by pre-infusion of isocaloric carbohydrate.

Methods and materials. Eight naive adult male hooded rats, 282 ± 23 g b. wt, were housed singly in suspended stainless steel cages, and maintained on a 12/12 h light-dark cycle (light phase: 05.00–17.00 h), with temperature kept at 21 ± 2 °C. They were fed standard laboratory chow pellets (Diet 41B, Pilsbury, Birmingham) ad libitum on all non-experimental days. Water was available at all times. All training and testing was carried out in the home cage.

First, for two days, the rats were accustomed to drinking 0.02% saccharin (g/100 ml of solution in distilled water) from a 10-ml calibrated glass tube clipped to the cage-front. This was followed by a series of six training days, on each of which the rats were given a single distinctively flavoured protein meal from the tube for 35 min at 15.00 h, after food-deprivation from 10.00 h. The protein meal consisted of 10% calcium caseinate ('Casilan', Farley Health Products, Plymouth) dissolved in 0.02% saccharin solution, flavoured with 1 ml of almond essence (Rayner, London) per 100 ml of solution. Maintenance diet was withheld for a further 2.5 h after the end of the protein meal. On training days, the rats were also adapted to gavage without mandibular restraint, using a wetted, round-tipped feeding tube (French gauge 6, Portex, Hythe). For the first three training days, this adaptation was carried out once per day, at 12.00 h. For the remaining days, gavage was carried out at 2 h, at 0.5 h, and immediately before the training meal.

After training, the rats were tested for baseline intake levels of almond-flavoured solution containing no protein, as an 'extinction' measure of conditioned preference for protein-predictive flavour. Conditions were unchanged from those during training, except that the protein solution was replaced by a non-nutritive mixture having similar sensory characteristics: this consisted of 0.3% sodium carboxymethylcellulose (a thickener) dissolved in 0.02% saccharin, with 0.3% calcium carbonate suspended and the same level of almond flavouring. On this first test day, at 2 h, at 0.5 h, and immediately before the test meal, the rats were sham-intubated, i.e. gavage without nutrient preloads.

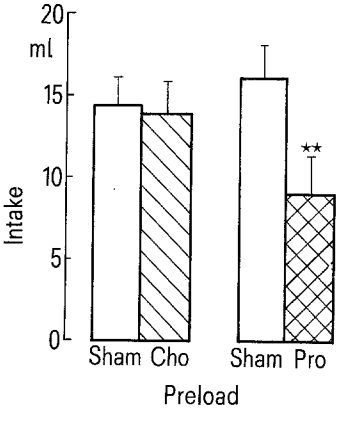
On the second test day, the rats received the same test meal of non-nutritive almond-flavoured solution, but were gastrically intubated with 2.5 ml of a nutritive solution at 2 h, at 0.5 h, and again just before test. Half the rats were given carbohydrate loads and half protein loads. Animals were selected so that the previous day's intakes did not differ significantly between the nutrient-load groups. The protein preloads were 10% casein hydrolysate (BDH Chemicals, Poole), and the carbohydrate preloads were equicaloric 10% maltodextrin (CPC, Manchester). The quantity of protein amino acids (0.75 g) was calculated to replenish amply the amount of protein normally absorbed over the 5-h daytime period for which these animals were deprived of chow[14].

Results. The relative effects of the protein and carbohydrate preloads on preference for the almond-flavoured non-nutritive solution could be determined by comparison of those day's intakes with the intakes following sham-intubation on the preceding test day.

The protein preloads greatly reduced intake of the protein-paired flavour, whereas the carbohydrate preloads had no effect at all (fig.). Thus, the amount consumed of non-nutritive diet having a flavour previously paired with protein is independent of the current state of energy supply. Rather, this measure of protein-conditioned flavour preference is attenuated specifically by protein repletion.

Discussion. The data demonstrate an acquired protein-specific appetite in rats – a protein-conditioned flavour preference that is augmented by a protein-specific need state. The question now arises what are the physiological mechanisms by which protein consumption, or lack of it, gives rise to internal stimuli capable of inhibiting or eliciting nutrient-selective behaviour. The deficit signals could arise in the gut, the liver or the brain, or all three, or elsewhere[13, 15–21].

While the existence of a precise regulatory system controlling protein intake seems unlikely[6, 8, 10, 13], there is a rough and ready system for avoiding gross excess or deficiency of essential amino acids by dietary choice[2–9]. The nutrient-specific learned control of feeding reported here is likely to be a sufficient mechanism to allow the degree of adaptive control of protein consumption that is evidenced.



Effect of preloads of carbohydrate (Cho), protein (Pro) or nothing (Sham), on intake (mean + SE) of non-nutritive fluid having an almond flavour previously paired with protein meals. Paired columns represent within-rat comparisons, over two test days (N = 4 per group). Analysis by two-way ANOVA (repeated on one factor) revealed a significant effect of nutrient preload versus sham gavage ($F(1,6) = 37.4$, $p < 0.001$), as well as a significant interaction (nutrient/sham vs Cho/Pro groups: $F(1,6) = 29.4$, $p < 0.01$). The only significant difference (**) between mean intakes under any condition was that between the Pro-load and Sham-load conditions (correlated t-test: $t(3) = 8.15$, $p < 0.005$, one-tailed).

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