and tetraploid amounts of DNA were found only during early proestrus. 4. The paucity of a tetraploid nuclear population during estrogenic stimulation may indicate rapid cellular division once DNA synthesis has occurred.

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Bioenergetic Basis of Light-induced Fat Deposition in the White-crowned Sparrow.* (22755)

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It has been clearly established in at least 6 species of migratory birds that vernal premigratory deposition of fat in visceral and subcutaneous depots can be simulated by subjecting such birds to artificially prolonged daily photoperiods(1-6). That this phenomenon is the consequence of temporary hyperphagia and not the result of increased time for feeding, as daylength increases, appears evident from the experiments of Odum and Major(6) with white-throated sparrows (*Zonotrichia albicollis*) and Koch and de Bont(5) with a single chaffinch (*Fringilla coelebs*). These investigators, and Wallgren(7) also, have proposed that this response must involve the hypothalamus, a suggestion which is consistent with known effects of light on avian hypothalamo-hypophyseal system(8) and the role of hypothalamic subcenters in regulation of food intake in laboratory mammals(9-11). This investigation provides a more extensive and more quantitative basis for understanding this interesting phenomenon by *1.* definition of temporal course of fat deposition in wild populations of a typical migratory passerine bird (*Zonotrichia leucophrys gambelii*), *2.* measurement of energy intake during premigratory period in captive birds under natural conditions of light and temperature, and *3.* examination of bioenergetics of fat deposition obtained experimentally with prolonged daily photoperiods.

Material and methods. Animals: White-crowned sparrows (*Z. l. gambelii*) were captured from migrant and wintering populations in the vicinity of Pullman, Wash. Specimens for extraction of fat were weighed and killed immediately and kept frozen until extractions

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were made. Reserve stocks were maintained in large outdoor flight cages. These, as well as experimental birds, were fed a nutritionally adequate chick-starter mash having fat, protein, and carbohydrate components of 3.1%, 20.7% and 74.7% respectively, plus mineral and vitamin additives. The two batches had caloric contents of 4.439 and 4.552 kcal/g, respectively. *Fat determinations:* Entire carcasses were quantitatively extracted with diethyl ether in Soxhlet apparatus(15). Data are expressed as grams of ether-extractable lipid/100 g live body weight; this ratio is designated as *lipid index*. In all, 110 carcasses were extracted from collections in January to May, 1952, 1953, and 1954. The 1953 series of lipid indices, for the greater part, was obtained in conjunction with studies of morphology and composition of subcutaneous fat bodies(15,16). *Energy intake:* Birds were housed individually in Hendryx breeding cages ($22 \times 41 \times 26$ cm) from which floors had been removed. Each cage was placed in a close-fitting pan, 12.5 cm in depth, which permitted complete collection of excreta and spilled food. Food weighed to nearest 0.1 g was provided daily in hopper attached to side of cage; the unused feed from previous day was also weighed. Feed and water were continuously available. Moisture content of feed was determined weekly. Periodically (intervals of 4 to 7 days), pans were changed at a fixed hour. Spilled food and excreta were separated and dried to constant weight at 98° C. From these data the *gross energy* intake was calculated. Subtracting from this the total caloric value of excreta collected during the same period gives the *metabolizable energy*(14,17), which, except for energy potentially lost in specific dynamic action of food ingested and in bacterial fermentation, is the energy actually available to the bird. Calorimetric determinations of food and excreta were made with oxygen bomb calorimeter with experimental error of less than 0.5% between duplicate samples. Experimental error in de-

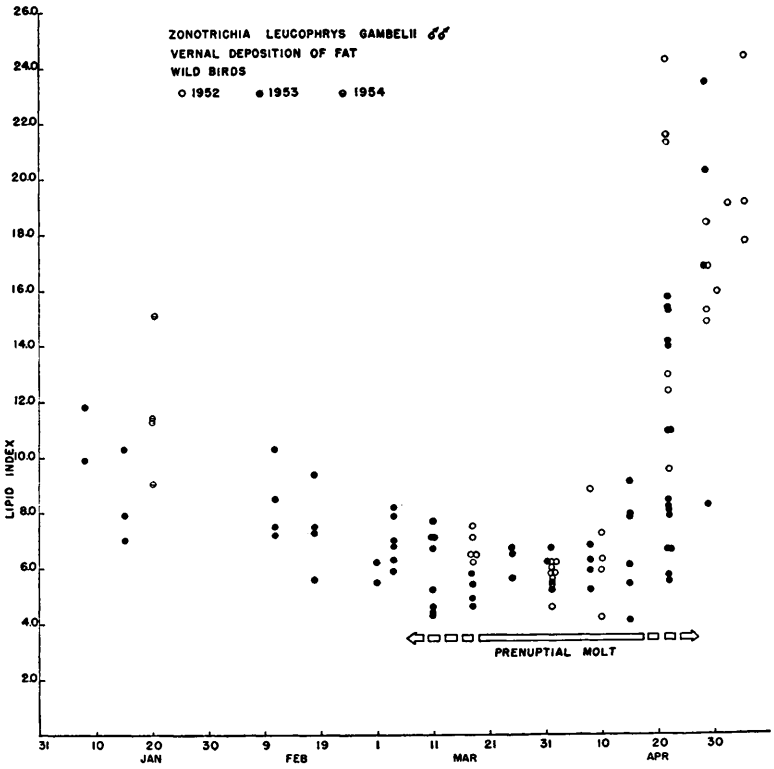

FIG. 1. Lipid indices in wild populations during late winter and spring. Migratory movement began during last 10 days of April.

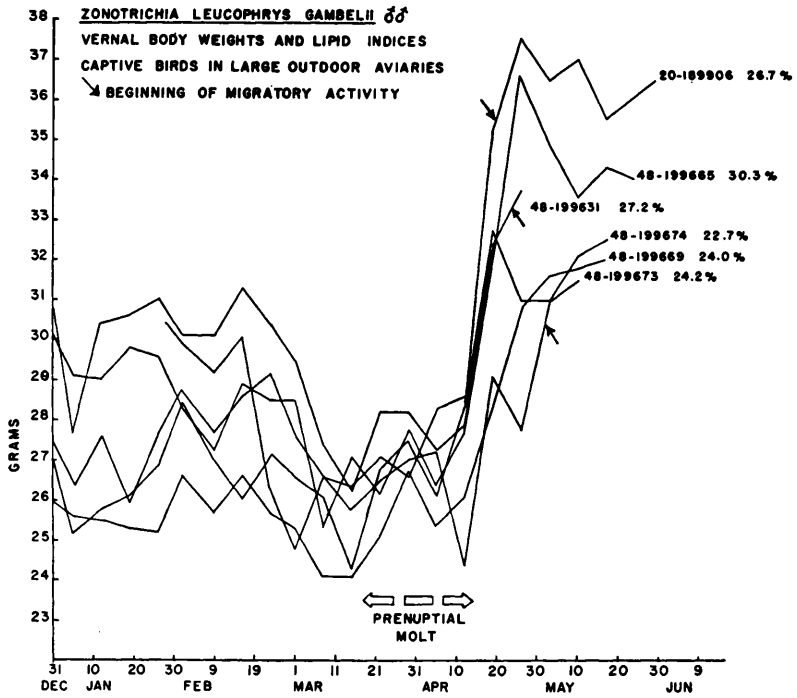

FIG. 2. Percentages at right are terminal lipid indices. Oblique arrows indicate onset of *Zugunruhe* in 3 birds in which activity was recorded.

termination of metabolizable energy is of the order of 3%. *Respiratory metabolism:* Oxygen consumption and carbon dioxide production were measured with apparatus similar to that of Schwabe and Griffeth(18). Attainment of *quasi*-basal conditions was guided by precautions suggested by Benedict(19) and Wallgren(7). *Activity recording:* Recording was by scheme similar to that described by Farner and Mewaldt(20) with the addition of impulse-counting circuits, which gave a numerical total for each hour for each cage. It must be emphasized that activity recording system of this type does not measure absolute or total activity but gives an *index of activity* as a fraction of total activity of a given subject. Since this fraction may differ among experimental animals, activity levels must be compared on a relative basis(21).

Results. Vernal Fat Deposition: Fig. 1 shows eruptive nature of the vernal premigratory fat deposition in nature. Migratory movement began during the last week of April in each of the 3 years. This pattern is similar to premigratory fat deposition for *Z. albicollis* in Georgia(22), except that the amount of fat deposited appears to be somewhat greater in *Z. l. gambelii.* This difference, however, might be only the result of differences in opportunity for collecting specimens during the first day or two of migratory movement. Odum and Connell(23) have recently shown that lipid indices of 25 to 30% are common in passerine birds captured in migration.

Fig. 2 demonstrates that the pattern of premigratory fat deposition, as indicated by a sample from the wild population, is substantially the same for individual birds held under natural conditions of temperature and photoperiod. Here it has been necessary to use body weight as an index of fat deposition. This is justifiable since our data and those of Odum and Perkinson(22) indicate an annual variation in fat-free weight of less than 10%. Therefore, variation in body weight of birds routinely weighed at fixed times of the day is due largely to variation in fat content. The terminal lipid indices in these captive birds are slightly higher than those of wild birds,

probably because of somewhat restricted activity and readily available food supply. However, the date of beginning and rate of deposition of premigratory fat as well as temporal relationship with prenuptial molt are essentially the same. *Experiment 1:* The intake of metabolizable energy, the daily activity, and the variation in body weight were measured over 7-day intervals in 6 males confined in outdoor cages during the late winter and spring of 1955. It was found that a marked increase in body weight (fat deposition) in the last half of April was accompanied by a definite increase in energy intake. In 4 birds for which these data are acceptable, the increase was 30 to 50% above the preceding minimum. Although the energy *utilization coefficient* (metabolizable energy/gross energy $\times$ 100) increased in all birds from an average of approximately 70 in March to 76 in early June, the change was continuous and gradual and could not logically explain the very abrupt increase in metabolizable energy income. Likewise, ambient temperature oscillated only slightly around an increasing mean, with no extreme minima in April which can be correlated with timing or magnitude of maximum energy intake. Daylength, and consequently time for feeding, increased 9% (14.4 to 15.7 hours) during the last 3 weeks of April. A normal prenuptial molt occurred. The daily activity of birds continuously increased during the experiment, as previously shown(21) with *Z. l. gambelii* under the same conditions. Pronounced nocturnal unrest, or *Zugunruhe* (the behavioral homologue of migratory movement in caged migrants during migratory season)(24) developed on 28 April. *Exp. 2:* In mid-January, well after annual period of refractoriness to photic stimulation (25,26), 8 males were placed in individual metabolism-activity cages in constant-condition room maintained at 12 $\pm$ 2°C and 35-40% relative humidity. For 29 days these birds received 9 hours light per day (0900-1800) from incandescent lamps so arranged that light intensity measured at floor of cages was 35-40 foot-candles. A photoperiod of this duration is essentially subliminal for stimulation of testicular development and fat deposition. On Feb. 13 the group was divided into 2 groups of 4 each. One group was retained as untreated control. The experimental group was transferred to another room maintained at same temperature, humidity, and light intensity. The photoperiod for this group was then increased by 2-hour daily increments to 20 hours per day (lights out 1900-2300). The increase in photoperiod evoked patterns of hyperphagia and concomitant increase in body weight (fat deposition) which were very similar to those observed under natural conditions. A temporary increase in energy income of 27 to 43% above preceding minima occurred. The mean utilization coefficients in both groups were relatively constant at about 71 throughout experiment. The mean terminal lipid indices for experimental and control birds were 23.5 ($\sigma = 3.05$) and 12.3 ($\sigma = 3.34$) respectively. The difference is statistically significant ($P = 0.03$). Standard metabolic rates of controls and experimentals, computed on basis of lean body weight, show a sharp divergence after beginning of the increase in photoperiod, the experimentals uniformly attaining a rate approximately double that of controls. Respiratory quotients in the 2 groups ranged between 0.72 and 0.82. While it is very probable that this *apparent* increase in standard metabolic rate is due in part to a decrease in depth of sleep, as Bergman(29) has shown to occur in several passerine species during development of *Zugunruhe,* it is of particular importance here that there was no *decrease* in standard metabolic rate which could contribute energy to fat deposition. This aspect of our investigations will be considered in detail in subsequent publication. Activity of the 2 groups shows that there is no *decrease* in muscular activity of the 20-hour experimentals which could spare energy for fat deposition. By the end of the experiment all of experimentals were definitely in *Zugunruhe.* A very light molt occurred only in the spinal tract after the change to the 20-hour photoperiod. No molt was observed in the 9-hour controls.

Discussion. These observations suggest very strongly that the premigratory fat depo-

sition in *Z. l. gambelii* is largely, if not entirely, the result of a photically induced hyperphagia. It is evident at least in the white-crowned sparrow, in the chaffinch(5), and the Ortolan bunting (*Emberiza hortulana*)(7) that premigratory adiposity cannot result from temporary decline in basal energy requirements as Groebbels(12) has suggested, nor from a combination of this and a moderate increase in food intake, as proposed by Merkel(13). Also it is apparent that there is no decrease in muscular activity nor increase in digestive efficiency of sufficient magnitude to account for the surplus of energy stored as fat. Our data apparently are inconsistent also with the hypothesis(14,24,27,28) that "productive" or surplus energy available for fat deposition is the result *primarily* of energy-sparing effects of increasing environmental temperature in spring, and the cessation of energy-demanding prenuptial molt plus increased energy intake made possible by more daylight for feeding. It seems unlikely that the eruptive nature and temporal precision of vernal fat deposition can be dependent to any substantial degree on such a fluctuating variable as environmental temperature, although it may have a secondary modifying role. With respect to the effect of increasing daylength, data from Exp. 1 show an average increase of 36% in rate of caloric intake (1.54 to 2.10 kcal bird/hr of daylight) during a period when daylength increases by only 9%. Clearly there is no simple, linear relationship. The data also indicate that neither elevated environmental temperatures nor cessation of molt are essential in development of vernal adiposity. The simultaneous occurrence of molt and fat deposition has been noted previously under experimental conditions by Farner and Mewaldt(26) and Odum and Major(6).

In view of the similarity between development of obesity in mammals with hypothalamic lesions(11) and of premigratory fat deposition in birds it appears probable, as Koch and de Bont(5) have suggested, that this photically induced hyperphagia in birds is mediated by hypothalamic feeding centers. Although this hypothesis awaits the demonstration of feeding centers in the avian hypothalamus, such a mechanism controlled by vernal increase in daily photoperiod is consistent with observed annual precision of premigratory fattening.

Summary. In *Zonotrichia leucophrys gambelii* there is a striking deposition of fat in subcutaneous and visceral depots following prenuptial molt and preceding beginning of vernal migration. During the last 2 weeks of April, in natural populations, mean lipid indices increase from about 5% to more than 20%. A similar premigratory fat deposition occurs in captive birds under natural conditions of photoperiod and temperature. Similarly in these captive birds this fat deposition is followed by development of migratory behavior (*Zugunruhe*). Measurements of metabolizable energy intake in these birds show that fat deposition is accompanied by a marked increase in energy intake to levels of 30 to 50% above the preceding minima. This temporary hyperphagia represents an altered physiologic state rather than simply the effect of more daylight for feeding since, during hyperphagia, the amount of daylight increases only 9%. The temporary hyperphagia is the principal factor responsible for positive energy balance required for premigratory fat deposition. The role of daily photoperiod in development of temporary hyperphagia was demonstrated experimentally by subjecting birds in mid-winter to 20 hours of light per day. These developed a hyperphagia and an accompanying fat deposition very similar to those observed in spring under natural conditions.

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Glycogen Deposition in Squamous Metaplastic Epithelium of the Rat Uterus.* (22756)

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The deposition of glycogen in the normal uterus of the rat has been studied by both chemical(1) and histochemical methods(2). It has been demonstrated by chemical analysis that the total glycogen content of the rat uterus varies with the stage of the estrous cycle, being maximal during proestrus and minimal during diestrus. Histochemically, it has recently been shown that the glycogen of the rat uterus is limited to the muscle cells of the myometrium; in the proestrous animal the material is abundant throughout the longitudinal layer but is irregularly distributed in the circular layer while in the diestrous uterus, the glycogen content of the longitudinal muscle layer is greatly reduced and that of the circular muscle layer has disappeared entirely.

The distribution of glycogen in abnormal uteri, particularly in epithelial metaplasia of the rat uterus has not been reported. One of the methods used to produce and study uterine metaplasia in the intact rat is by placing the animal on a vit. A deficient diet(3). Using this method to produce epithelial metaplasia, the present investigation was undertaken to study, by the periodic acid-leucofuchsin technic, the distribution of glycogen in the metaplastic uterine glands of the intact rat.

Materials and methods. Twenty-five rats of the Wistar strain were used in these experiments. The animals were 20 to 22 days of age and weighed approximately 35 to 40 g when placed on the vit. A free diet.† The rats were killed with chloroform during the 9th to 14th week of the deficiency and the uteri were fixed immediately. A portion of each uterus

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† Vitamin A Test Diet U.S.P., Nutritional Biochemicals Corp., Cleveland, O.