in Fig. 2. Six precipitin lines appear between the wells containing whole axoplasm (*WA*) and axoplasm antiserum (*A*); no lines appear at the wells containing squid blood (*SB*). Conversely, no precipitin lines appear between whole axoplasm (*WA*) and squid blood antiserum (*B*), but three lines appear with squid blood and its own antiserum.

When polyacrylamide disc electrophoresis is combined with immune diffusion on agar, a form of high-resolution immune electrophoresis is achieved, which takes advantage of the good electrophoretic separation obtained in polyacrylamide and the rapid diffusion occurring in agar. After the electrophoretic run the unfixed and unstained acrylamide gel is placed in a bed previously formed on an agar gel. Troughs for the antiserum are then cut in the agar, parallel to the acrylamide gel.

Figure 3 shows the result after separating electrophoretically on acrylamide the proteins of whole squid axoplasm (*WSA*) and allowing axoplasm antiserum (*AA*) and squid blood antiserum (*BA*) to diffuse from the troughs. By using this technique, seven antigenic proteins have been resolved thus far, none of which is present in a detectable amount in squid blood. No attempts to obtain quantitative immunological results have yet been made. The fractionation and identification of some of the axoplasm proteins will be reported later.

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Homeostasis of the Nonfat Components of Migrating Birds

Abstract. *Obesity in migratory birds appears to differ from obesity in man in that gains and losses in body weight do not involve changes in the tissue structure of the body; fat is added to and used from preexisting tissue spaces without appreciable change in the water content or the nonfat dry weight of the body as a whole. Evidence is presented which supports the hypothesis that the nonfat body is essentially homeostatic during migration despite very large scale changes in total body weight.*

Many species of small land birds, even though relatively slow and weak fliers, migrate between eastern North America and Central or South America by crossing the long stretches of open water of the Gulf of Mexico or the Caribbean. Just prior to long flights, individuals of these species build up huge temporary stores of body fat equal to two or three times the weight of the total nonfat dry weight of the body (*1*). These stores apparently are used as the sole energy source for nonstop flights which may last for many hours and cover distances of 1000 kilometers or more (*2*). Our studies of specimens accidentally killed by tall television towers during nocturnal migration revealed that migratory fat deposition is quite different from obesity in man, laboratory rats, or other nonmigratory species. In man, for example, gains and losses of body weight in obesity usually include gains and losses of water and nonfat tissue components as well as lipids. Thus, a gain or loss of a gram of body weight has been variously estimated to include a gain or loss of 5.8 to 7.8 kcal, depending on the nitrogen balance and the amount of water present (*3*). Pitts (*4*) summarizes a recent study on guinea pigs by stating that "changes in fatness are followed by changes in the same direction of fat-free adipose tissue and adipose-free body." In contrast, gains and losses of weight in migrating birds do not include addition or subtraction of water or nonfat tissue components, but merely gains and losses of dry lipids which have an energy value of at least 9 kcal/g. In other words, components of the nonfat body remain essentially homeostatic despite very large and rapid changes in total body weight. The migratory bird is thus analogous to the airplane in that "high octane" fuel (that is, fat) is added to and used from preexisting "tanks" (tissue spaces) without appreciable change in the tissue structure of the body as a whole. The purpose of this report is to outline briefly the five different lines of evidence we have to support this hypothesis.

Frozen specimens of birds killed by television broadcasting towers during nocturnal migration provided the principal material for our studies. Total lipid, water, and nonfat dry weights were determined by vacuum-drying chopped-up whole specimens which were then extracted in alcohol-ether. For studies on the adipose tissue itself, samples were removed from frozen specimens and were either prepared for histological sectioning, or were extracted with chloroform after the tissue had been freeze-dried and homogenized.

At the tissue level of organization, we would expect the percentage of water and nonfat residue in adipose tissue to decrease as the bird became fat—if water, blood vessels, connective tissue, and other nonfat components are not added while the fat is being deposited prior to migration. Table 1 shows that this is indeed the case. Adipose tissue of birds at the peak of lipid deposition just prior to long flights con-

Table 1. The percentage of water, nonfat residue, and total nonfat material in samples of adipose tissue dissected from red-eyed vireos (*Vireo olivaceus*) killed by a television tower during nocturnal migration. The nine birds are arranged in a series from lean to very fat condition. Percentages are means of samples from several body locations totaling an estimated 10 to 20 percent of "depot" fat tissue in each individual. Data from Walker (*8*).

Water (%)	Nonfat dry residue (%)	Total nonfat (%)
*Lean spring (April) migrant (fat index = 0.2)**		
40.2	24.0	64.2
Moderately fat early fall (Aug.–Sept.) migrants (fat index 0.5–1.5)		
23.7	15.2	39.9
18.7	10.0	28.7
12.4	5.5	17.9
7.5	4.1	11.6
*Very fat late fall (Oct.) migrants (fat index > 2.0)**		
6.5	3.1	9.6
6.0	3.5	9.5
6.0	2.3	8.3
4.9	2.2	7.1

* Fat index = grams of fat per gram of non-fat dry weight.

tains only about 7 percent water and nonfat residue (and is, thus, 93 percent dry fat) as compared with up to 65 percent nonfat material in very thin birds. In another series of experiments in which five different species were used, the adipose tissue of six lean individuals with a mean fat index of 0.25 averaged 46 percent nonfat as compared with 8 percent nonfat in 13 birds with an average fat index of 2.5. If a gram of adipose tissue of a lean bird with a fat index of 0.25 contains 0.46 g of nonfat, and the amount of fat is increased tenfold (fat index 2.5) without change in nonfat, then the percentage of nonfat should be $0.46/0.54 \times 10 = 8.5$ percent in the fat bird—an estimate that is very close to the actual value.

The effect of fat on nonfat at the whole-body level of organization has been investigated by means of a computer program based on the method of partial correlation in which the standard F-test procedure was used. The total weight of the fat and water and the total nonfat dry weight were individually related to wing length, a standard measurement of basic body size that does not vary in the individual, so as to eliminate the body size variable in the analysis. Specimens of several species of trans-Gulf migrant warblers (family Parulidae) collected at a television tower on the Gulf coast of Florida during both fall and spring migrations were used (*5*). The series contained individuals in all stages of fat deposition and utilization. Since age and sex proved to have a negligible effect on body composition in three of these species, all specimens could be used in the analysis of the effect of fat on the nonfat components of the body. The number of individuals available was 75, 30, and 71, respectively. Confidence percentages for relationships between fat and nonfat in the three species were: fat versus nonfat dry weight, 70, 50, and 50; and fat versus water content, 50, 70, and 50. If the 95 percent confidence level is accepted as the minimum one for significance, then it is evident that the level of fat does not influence the nonfat dry weight and total body water content to a statistically significant degree.

In another analysis, fat specimens, each with a fat index greater than 2, were paired with lean specimens of the same wing length, age, sex, and species, but each with a fat index less than 0.5. There was no statistically significant difference in mean water content or in nonfat dry weight between the two series (*5*).

In Fig. 1, the fat index of a series of parula warblers killed at the same location is plotted against the water index. The fall birds had not yet undertaken long southward overwater flights, while the spring birds had presumably completed similar northward flights. Evidently, the ratio of water to nonfat is not affected by the fat level within either the fall or spring series. However, the ratio is significantly higher in the spring series than in the fall series. Since there is no food intake during long flights, and since the digestive tract of birds killed during actual migratory flight is invariably empty, we would expect at least a slight loss of weight to result from the catabolism of protein during the period of negative nitrogen balance. On the other hand, water lost in respiration or temperature regulation can be replaced by water produced during metabolism of the fat. Consequently, the slightly higher ratio between water and nonfat in postmigrant spring birds seems reasonable, even though the fat level did not significantly affect the quantities of water or nonfat when they were related to wing-length. What is so remarkable is that changes in both the water and nonfat body components are so small as to be difficult to demonstrate statistically.

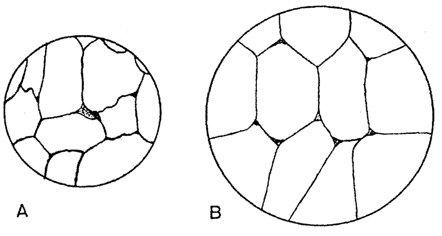

Fig. 2. Adipose tissue of two autumn migratory wood thrushes (*Hylocichla mustelina*) drawn to the same scale (×970) but with the field of view showing approximately the same number of cells. *A*, Bird in early stages of fat deposition prior to migration (fat index 0.76). Note irregular shape and small size of many cells. *B*, Bird in a late stage of fat deposition (fat index 1.58).

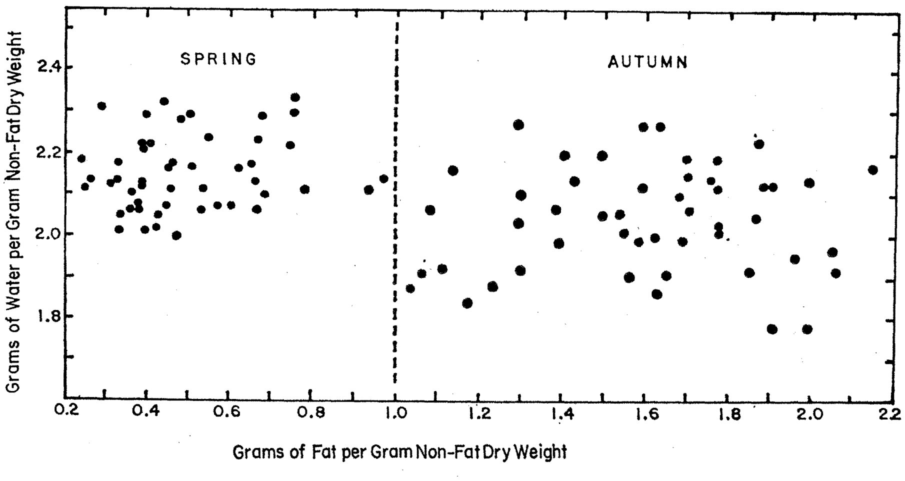

Fig. 1. Water index (grams of water per gram of nonfat dry weight) plotted against the fat index (grams of fat per gram of nonfat dry weight) of 53 spring and 48 autumn parula warblers killed during nocturnal migration at a television tower on the Florida coast of the Gulf of Mexico. Means ± one standard error are as follows. Water index: spring 2.15 ± 0.1; autumn 2.04 ± .02. Fat index: spring 0.49 ± .02; autumn 1.59 ± .04.

Finally, the gross histological picture is in accord with the "tank" theory that we are proposing. In Fig. 2, sketches of sections of adipose tissue of a thin and moderately fat migratory bird are drawn at the same magnification but showing the same number of cells within the field of view. The diagrams illustrate how the filling of the preexisting cell spaces with lipids can result in a great enlargement of the fat bodies without an increase in the number of cells. While an increase in cell size would be expected to occur in lipogenesis, the remarkable feature is the tremendous capacity of bird cells to accommodate anhydrous lipids that remain as sources of rapidly available energy.

As would normally be expected in homeostasis, there are limits beyond which the organism may be unable to regulate. What seems to happen is that the nonfat portion of the body remains remarkably homeostatic as the fat is catabolized during long flights until a fat index of 0.3 to 0.2 is reached; below this level a decline in nonfat dry weight is observed suggesting that at least 0.2g of fat per gram of nonfat is not storage or "depot" fat, and can be used only at the expense of burning some of the nonfat tissue as well. The great majority of spring birds which had made the critical northward flight from Central or South America were in excellent condition. For example, most of the spring parula warblers still had a fat index well above 0.3 (Fig. 1). Ornithological evidence, in fact, strongly suggests that under favorable weather conditions spring migrants do not stop at the first landfall but continue inland for as much as 300 kilometers (*6*). We have seen specimens (which presumably had encountered unfavorable winds) that had exhausted the depot fat and had been forced to burn an appreciable amount of nonfat tissue along with the remaining fat. The water index of such specimens was not reduced, but was sometimes higher, possibly due to the loss of nonfat weight. Contrary to the suggestion of Yapp (*7*), we believe that fuel, not dehydration, is the limiting factor in long migrations as long as weather conditions remain favorable for sustained flight.

It is evident that the lipid bioenergetic system, as evolved in migratory birds, has several advantages over the usual glycogen energy system. The available energy is greater per unit of weight, the storage capacity is greater, and water balance is facilitated, because fat, unlike protein or carbohydrate, can be stored "dry" yet yields water on combustion.

Preliminary studies of the fatty-acid composition of migratory fat in our laboratory indicate that there is nothing particularly unusual about the nature of the fat itself (*8*). As might apply to the depot fat of animals in general, unsaturated fatty acids C_{18-1}, C_{18-2}, and C_{18-3} make up more than two-thirds of the stored lipids while the saturated acids C_{14}, C_{16}, and C_{18} comprise most of the rest. Differences seem to be more related to species and diet than to migratory status, although the subject requires more study. The arrangement of enzyme systems, however, must be in some way unique to account for the rapid storage and utilization of lipid without change in tissue structure. One of the purposes of this report is to point out how studies at the ecological levels of organization can pinpoint relationships which would not be evident at the molecular level, but which must also be studied at this level if the mechanisms responsible for the relationships are to be understood.

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Gonadal Dosages in Investigative Radiography

Abstract. *Using radiation-sparing and radiation-limiting techniques, gonadal (skin) dosages in the course of investigative radiography averaged under 0.3 milliroentgen for a set of radiographs, a value less than the irreducible background dosage for 1 day.*

Although there have been numerous reports on gonadal radiation dosages in the course of diagnostic radiography (*1*), no data have been given for purely investigative studies where optimal radiation-sparing and radiation-limiting techniques were in constant use.

To remedy this, we have accumulated new data on skin dosages in the gonadal region from 244 subject determinations made in the course of radiographic studies of bone growth, physical development, and body composition. Fast film (duPont type No. 508), forced development, par-speed intensifying screens, and 3 mm aluminum-equivalent filtration were used to spare radiation dosages, and a variety of lead-rubber gonadal shields (*2*) and a rectangular Picker collimator cone were used to limit the area of irradiation.

Radiation measurements were made on a model 687 Victoreen transistorized minometer II, exceeding the manufacturer's recommended warm-up period by 15 minutes. A set of three pocket-sized dosimeters was used in each subject determination to minimize the root-mean-square response error. Within the limits of the radiographic techniques actually used, there was no need to correct the dosimeter readings for reduced efficiency at short exposures, or for saturation at high milliroentgen-per-second rates. This was verified by repeated calibration studies made at stepwise intervals from 1/60 of a second to 1 second (*3*).

Table 1. Gonadal (skin) dosage from investigative radiography.

Age (yr)	Subjects (No.)	Radiographs (No.)*	Average dosage† (mr)
3–6	30	2.5	0.13
6–9	39	3.8	0.15
9–12	47	3.4	0.21
12–15	27	2.9	0.17
15–18	24	3.3	0.24
8–10	25	Chest	0.20
21–22	17	Chest	0.15
41–75	35	Hand	0.16

* Numerical values are averages of radiographs of the subject's hand, head, forearm, knee, ankle, or chest. † From three dosimeter readings per set of films per subject.

Since the skin dosages to the gonadal area for individual radiographs fell well below 0.1 mr under the conditions of our studies, we were obliged to group different sets of radiographs on the same individual, reading out immediately upon insertion of each of the three dosimeter chambers. As shown in Table 1, these showed an increase in gonadal dosages from infancy through adulthood, paralleling the increase in peak kilovoltage and milliampere-seconds that is necessary in larger and in radiographically denser subjects. Radiographs involving the thoracic region tended to result in higher gonadal dosages than those involving the extremities and the head or the head alone. No single gonadal dosage was as high as 0.3 milliroentgen.

In general, gonadal (skin) dosages for a set of investigative radiographs of the same individual taken under the conditions described averaged between 0.15 and 0.30 milliroentgen per subject. Taking the irreducible background radiation as 100 to 300 mr/year, depending upon altitude (*4*), these values are equivalent to less than the radiation load of 1 day of normal living.

In these studies, the equivalent of 3 mm of aluminum filtration was in-