only a few hours, may play a significant role in the movement of disseminules from upland to aquatic sites. Transfer of resistant seeds from one bird to another, that is, from a "commuter species" to a "transoceanic express" might reasonably be assumed to occur anywhere shorebirds mingle in mixed flocks during spring and autumn migration. We have observed birds reingest seeds cast up by regurgitation.

The third objection—that migratory birds empty the digestive tract before extended flight—may be significant, but at present there is little information either to support or to counter this theory. White-crowned sparrows and several other granivorous birds apparently empty the digestive tract before migration (*12*). However, food normally passes through these birds within 2 to 4 hours. As yet, it is not known whether shorebirds, particularly those that do not regurgitate pellets, are unable to voluntarily empty the gizzard.

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6. Shorebirds were confined in compartments 40 by 60 by 50 cm. All other birds were in wire-mesh cages approximately twice this size, except for the geese which were in still larger cages. All birds were able to move about freely during the course of a retention trial. Separate trials were conducted for each seed species, and at least 24 hours elapsed between successive runs with the same birds. Food, water, and grit were available to birds before and throughout all experiments.
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8. All disseminules are here referred to as seeds, though by strict definition many were fruits. Fleshy or dry outer coverings, for example in *Celtis, Rhus, Desmodium,* and *Malva,* were removed before the seeds were presented to the birds. Viability of recovered seeds was determined either by germination or the use of tetrazolium (*5*).
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10. Most shorebirds will regurgitate in 1 to 3 hours if given a gelatin capsule containing small glass beads. Gizzard contents can be sampled in this manner without killing the birds.
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DNA (Cell Number) and Protein in Neonatal Brain: Alteration by Maternal Dietary Protein Restriction

Abstract. *Female rats were maintained on 8 or 27 percent protein diet by a pair-feeding schedule for 1 month before mating and throughout gestation. The brains of newborn rats from females on the 8 percent protein diet contained significantly less DNA and protein compared to the progeny of the females on the 27 percent diet. The data on DNA indicate that there are fewer cells; the protein content per cell was also lower. If, at birth, the brain cells are predominantly neurons, and their number becomes final at that time, then such dietary restriction may result in some permanent brain-neuron deficiency. This quantitative alteration in number as well as the qualitative one (protein per cell) may constitute a basis for the frequently reported impaired behavior of the offspring from protein-deprived mothers.*

The effects of malnutrition on development have been extensively studied. For brain, such studies were concerned mainly with the effects on weight or size (*1, 2*), which, however, depend on factors (such as lipids, water content) that do not reflect the number of brain cells. Winick and Noble (*3*) and Dickerson *et al.* (*4*) investigated the effect of malnutrition after birth on the DNA content of the brain. If the malnutrition occurred from birth to weaning, the animals (rats, pigs) exhibited a permanent brain DNA deficiency. The influence of malnutrition on learning behavior of rats has also been studied (*5, 6*). Many investigators have implied that protein deprivation before and after birth results in mental impairment in children (for reviews see *7*).

For the understanding of this influence of malnutrition on behavior, the study of changes in the number of brain cells is of interest. Whereas, in the rat the number of glial cells and the total number of brain cells increases for some time after birth (*8, 9*), the number of neurons does not increase (*8, 10, 11*), with the possible exception of short-axoned neurons (*11*). Thus, we studied the effect of maternal malnutrition before and during gestation, on the amount of brain DNA (brain cell number) in newborn animals.

Our report is a continuation of previous studies (*12, 13*) of factors influencing the amount of DNA in the brain, which reflects the number of brain cells because the DNA content of a diploid cell of a given species is constant; our eventual purpose is the elucidation of the relation between alterations in brain cell number and behavior.

We used albino rats derived from the Sprague-Dawley strain; these rats have been bred in our laboratory for at least ten generations; the females were virgin, 3 months old, and weighed 200 to 260 g. The animals were maintained (i) on powdered diets containing either 8 percent or 27 percent protein (*14*) by a pair-feeding schedule (intake 16 g/day); or (ii) another group was maintained on pelleted diet (*15*) as desired (16 g/day). The protein was casein. Both protein diets contained the same amounts of fats (10 percent) and salts (4 percent). In addition, the 8 percent protein diet contained 78 percent starch, and the 27 percent protein diet contained 59 percent starch. To both diets, 2.2 percent of Vitamin Diet Fortification Mixture in

Table 1. The effect of restriction of maternal dietary protein on weight and content of brain of newborns. Diet A, full pellet; B, full diet, containing 27 percent protein; C, restricted, containing 8 percent protein.

Diet	Number of animals		Offspring weights (g)		Brain content of offspring*	
	Mothers	Offspring	Body	Brain*	DNA (μg)	Protein (mg)
A	5	41	5.7 ± 0.4	0.159 ± 0.071	544 ± 20	
B	4	32	6.38 ± .4	.181 ± .014	546 ± 22	9.29 ± 0.43
C	4	31	4.46 ± .22	.139 ± .081	491 ± 29	7.45 ± .57
			Decrease† (%)			
			30	23	10	19.8
			Probability			
			$P<.001$	$P<.001$	$P<.001$	$P<.001$

* Cerebral hemispheres, without cerebellum and olfactory lobes. † Difference between 27 percent and 8 percent protein groups.

Dextrose (*14*) was added to a week's supply. The females were kept on these diets for 1 month before mating and throughout gestation. The restriction was such as to still permit full-term gestation (*16*) and normal number in litter.

The newborns were weighed, and then killed by decapitation, within 6 hours of delivery. The brains (cerebral hemispheres) were immediately removed without cerebellum and olfactory lobes (*13*) and weighed; they were then frozen and subsequently used for the analysis. DNA was determined by a modification of diphenylamine colorimetric method (*12*, *17*), and protein was determined by a modification of Folin colorimetric method (*18*).

The results (Table 1) show first that the rats on two different full diets exhibited differences in body and brain weights, but the total amount of DNA [and therefore total brain cell number (*19*)] was the same. Thus, cell number is a more constant indicator; the brain weight cannot be used as a measure of brain cell number.

As expected (*2*, *6*), dietary protein restriction of the mother resulted in considerably (30 percent) lower body weights of the newborn offspring; however, in contrast to previous experiments (*2*), in which the dietary restriction was during gestation only, in our experiments in which the restriction was also imposed 1 month before mating, the brain weights were also considerably (23 percent) lower. This decrease is reflected in comparable percentage decrease in total protein content. All these changes are statistically significant.

The restriction also resulted in a significantly lower (10 percent) DNA content, that is, significantly lower total brain cell number. However, this difference is less pronounced than the difference in brain weight which again indicates that the latter cannot be used as a measure of the former.

Since at birth the brain cells are reported to be predominantly neurons (*8*), it is likely that the decrease has indeed affected the number of neurons. Since, as discussed above, the neurons essentially do not divide any more after birth, any neuron deficiency at birth may persist throughout the life of the animal. Such deficiency may contribute to the impaired behavior of the offspring of protein-deficient mothers that has been reported in the literature.

The change in protein content, twice as large as that in DNA, indicates that not only the number of cells was altered but also the cells are qualitatively different. Whether these qualitative changes are irreversible or whether they merely represent a delay in maturation is still not known. However, when evaluated at 3 months of age, the experimental animals manifested abnormalities of gait and response to environmental stimuli.

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Tritiated Digoxin Binding to ($Na^+ + K^+$)-Activated Adenosine Triphosphatase: Possible Allosteric Site

Abstract. *Tritiated H^3-digoxin specifically binds to a cardiac ($Na^+ + K^+$)-activated adenosine triphosphatase. In the presence of adenosine triphosphate and other nucleoside di- and triphosphates, binding is stimulated by sodium ion, the apparent rate constant being similar to that reported for phosphorus-32 incorporation from adenosine triphosphate and for adenosine triphosphatase activity. In the presence of magnesium, manganese, inorganic phosphate, or other ions, sodium ion inhibits binding. The data support an allosteric type of sodium-potassium ion pump.*

A particulate Na^+,K^+-adenosine triphosphatase system apparently forms the enzymatic basis for active coupled-electrolyte transport in a variety of tissues (*1*). One of the important characteristics of the enzyme complex is the specific inhibition of activity produced by active cardiac glycosides, ascribed to a possible binding at a K^+-dependent site (*1*, *2*). However, the involvement of both Na^+ and K^+ in glycoside-induced inhibition and the fact that temperature causes alterations of the enzyme's sensitivity to such drugs indicate the complexity of the digitalis-enzyme interaction (*3*). Isotopically labeled digoxin and ouabain were used to determine the mechanism of action of the glycoside-induced inhibition. Initial studies indicated that the drug was actively bound to, and that it probably "stabilized" the phosphorylated enzyme (*4*). This finding would be of importance in view of the accumulated evidence in favor of a functional phosphorylated intermediate in the mechanism of action of the $Na^+ + K^+$)-activated adenosine triphosphatase (*5*). The data are interpreted as involving a Na^+-stimulated phosphorylation of the enzyme system by adenosine triphosphate (ATP) (*5*) followed by a K^+-dependent phosphate reaction (*6*).

The reaction sequence has been conveniently abbreviated as follows:

$$E + ATP \overset{Na^+}{\rightleftharpoons} E\text{-}P + ADP \quad (1)$$

$$E\text{-}P \overset{K^+}{\rightarrow} E + P_i \quad (2)$$

The compound E-P may be one of a number of phosphorylated intermediates, existing perhaps at different energy levels (*1*, *5*). Cardiac glycosides