LASTING BIOLOGICAL EFFECTS OF EARLY ENVIRONMENTAL INFLUENCES*

I. Conditioning of Adult Size by Prenatal and Postnatal Nutrition

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As commonly used, the phrase "early influences" denotes the conditioning of behavior by the experiences of early life. Early experiences, however, do more than condition behavioral patterns; they also affect, profoundly and lastingly, other biological characteristics such as initial growth rate, efficiency in the utilization of food, anatomic structures, physiologic attributes, maximum adult size, resistance to infection, response to various forms of stimuli, in brief almost every phenotypic expression of the adult.

Epidemiologic evidence strongly suggests that some of the most important medical problems in underprivileged countries (and in prosperous ones as well) have their origin in environmental influences that affect human beings during the formative phases of their prenatal and neonatal development. Many effects of such early influences appear irreversible.

Among early influences, the most extensively studied so far are those associated with the nutritional state. Reviews of the literature on this topic will be found in references 1–8.

The lasting character of the biological effects of early nutritional influences is strikingly illustrated in the results of investigations carried out in England during the 1960's by McCance and associates (3–5). These investigators compared the growth rate of rats suckled in small litters (3 young per lactating female) with that of comparable animals suckled in large litters (18 young per lactating female). They found that the rats of the latter group (large litters) became much smaller adults than those of the former group, even though the animals of both groups were given the same diet after weaning. An obvious reason for this finding is that the animals raised in small litters enjoyed a nutritional advantage during the lactation period. As McCance and Widdowson pointed out, however, other factors may have played a role in the difference between

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the two groups. It is not unlikely, for example, that infectious and behavioral disturbances occurred when very large numbers of young were nursed by the lactating females. The experiments to be described in the present paper were designed to minimize the disturbing effect of such indirect, nonnutritional factors.

The study was carried out with so-called specific-pathogen-free (SPF)[1] mice in order to avoid as far as possible the disturbances resulting from the activation of latent pathogens by nutritional deficiencies. The composition of the animal's intestinal flora was checked by numerous bacteriological tests; there was no evidence of infectious disease in the experiments recorded.

In all cases, the litter size was reduced to eight young, and litters smaller than seven were discarded. The litter was removed from the experiment if the dam destroyed some of the young at any time after birth.

In several of the experiments, the animals were put on the experimental diets for 2 wk before mating so as to minimize or eliminate altogether the behavioral disturbances that commonly occur at the time of a dietary change.

In all experiments, the animals were fed exactly the same diet (D&G pellets) ad lib. after weaning; it is known from many earlier studies that this diet is readily accepted by all animals, and that it secures a rapid growth rate, large adult size, effective reproduction, and high resistance to infection.

Irrespective of the dietary regimens used during gestation and lactation, practically all animals survived throughout the period of observation. In the two experiments that were continued for the whole life span of the mice, deaths began to occur only at the end of the second year (see, for example, Text-fig. 8 and Table II). In view of these facts, it seems legitimate to assume that, in the tests now to be recorded, the nutritional factors that operated during prenatal and neonatal life played a dominant role in determining the growth rate and the adult size of the animals.

Materials and Methods

All experiments were carried out with animals from two colonies of specific-pathogen-free (SPF) albino mice, both of them derived from the so-called "Swiss" colony which has been maintained in our institution for more than 40 yr.

One of these two colonies, that we have designated Nelson Collins Swiss, or New Colony Swiss, or NCS, originated from nine animals obtained from two pregnant Swiss mice by

[1] The meaning of the expression "specific-pathogen-free" (SPF) is not clear. In order to determine whether SPF animals are really free of latent pathogens, it would be necessary to carry out a large number of complex and prolonged tests—for example, by an extensive use of varied cultural techniques, or of conditions likely to convert latency into activity. Such tests have not been carried out in the present study (or in any other of which the authors are aware). Nevertheless, the expression SPF will be used in this report, but for the sake of convenience only, and not to imply that the animals so designated have been proven to be completely free of potential pathogens.

cesarian section; the progeny of these nine animals has been subsequently bred in a protected environment. Details concerning the origin and characteristics of the NCS colony will be found in references 9–12.

NCS mice were bred either in the animal room of The Rockefeller University or, for special experiments, in our own laboratory. In the latter case, single pairs were housed for mating and breeding in so-called "Isocages" (Lab Cages, Inc., Kennett Square, Pa.) which were covered with an air filter, made of synthetic plastic material.

In more recent experiments, we have also used the SPF mice designed COBS (Caesarian-Obtained, Barrier-Sustained) produced by The Charles River Breeding Laboratories, Inc., Wilmington, Mass.; these animals were derived from the ICR colony (which is itself derived from the Swiss colony) and are random bred.

Whatever the origin of the mice, their dates of birth were noted so as to know the precise age of the animals. The number of young per litter was reduced to eight on the 2nd day of life; animals with litters smaller than seven were discarded as well as those which destroyed some of their young at any time after birth.

Sex was determined at 4 wk of age; males and females were separated at that time. Weights, either of groups (for the very young animals) or of individual animals (after weaning time) were determined in the morning; the time of weighing is important because, as reported elsewhere (11–12) mice usually stop eating and drinking after 8 a.m. and therefore lose weight during the rest of the day.

Most animals were observed from the time of birth throughout adulthood; some were kept through their whole life span. They were usually weighed at weekly intervals.

Unless otherwise stated in the description of the experiments, all adult animals were given water ad lib. and the pasteurized diet D&G (supplied by Dietrich and Gambrill, Frederick, Md.). This diet is processed so as to assure the absence of organisms known to be pathogenic to rats and mice. According to the manufacturer, it contains an adequate amount of the various vitamins and minerals and it has the following composition:

	%
Crude protein, minimum	24.0
Crude fat, minimum	5.0
Crude fiber, maximum	4.5
Ash, maximum	10.0
Carbohydrate	50.9
Calcium	0.9
Phosphorus	0.9

The other four experimental diets were prepared in our laboratory as follows:
Casein-starch diet: A 5 kg batch of this diet has the following composition:

	g
Casein	750
Dextrin	1830
Cornstarch	1750
Jones Foster salt mixture	125
L-cystine	6.5
Inositol	6.5
Vitamin diet fortification mixture	62.5
Peanut oil	157
Alphacel	310
Water to 5000 g	

Unless otherwise stated, the various components of the diet were obtained from Nutrition Biochemicals Corp., Cleveland, Ohio.

Casein-starch-magnesium diet: This has the same composition as the casein-starch diet, but is supplemented with 10 g of $MgSO_4$, 7 H_2O per 5 kg of diet.

Casein-starch-magnesium-corn steep liquor diet: This is the same as the casein-starch-magnesium diet, but supplemented with 250 g of corn steep liquor[2] per 5 kg of diet.

Wheat gluten diet: A batch of 5 kg of this diet was prepared as follows:

	g
Wheat gluten	1000
Dextrin	2930
Jones Foster salt mixture	200
L-cystine	10
Inositol	5
Vitamin fortification mixture	100
Peanut oil	250
Alphacel	500
Water to 5000 g	

The period at which these four experimental diets were given are indicated in the text for each particular experiment.

RESULTS

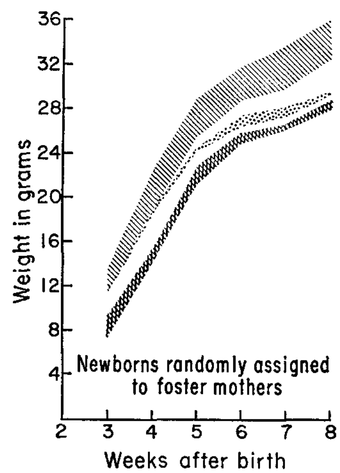
Foster Mothering, Weaning Weights, and Adult Size.—As described earlier, the NCS colony was derived a few years ago from the progeny obtained by cesarian section of two mice of a colony which had been inbred for some 50 yr (9, 10). Despite the fact that the colony has been maintained ever since by brother-sister matings, NCS animals bred in the same room and fed the same diet continue to exhibit marked individual differences in growth rate, weaning weights, and adult size. In contrast, the uniformity is much greater among the young of each particular mother. It was assumed that the diversity from litter to litter and the uniformity within each litter were determined by differences in the genetic endowment of the young; but the following observations show that this was not the case.

In several experiments, efforts were made to render the distribution of physical characteristics in the various experimental groups more uniform by pooling all the newborn animals and reallocating them randomly to foster mothers. Contrary to expectation, however, the growth characteristics of the young animals were found to be highly uniform for each foster mother, but to differ markedly from one foster mother to the other. In other words, the individual differences had their origin, not in the genetic endowment of the young animals, but in some characteristic of the foster mother.

[2] The phrase "corn steep liquor" designates an ill-defined water soluble extract of corn grain, widely used for penicillin production. This material was obtained through the courtesy of Dr. Stanley A. Watson, Corn Products Company, Argo, Illinois.

In one particular experiment, 240 mice born the same day were separated from their 30 mothers 2 days after birth, and pooled. They were then reallocated randomly to these mothers, each of the latter receiving eight young. All foster mothers accepted the eight young, which grew normally. All litters were maintained in the same room and fed the same diet ad lib. (pasteurized pellets and sterilized water).

At weaning time, the young were separated from their foster mothers, placed in individual cages, but kept on the same nutritional regimen. Their weights were followed for a period of several weeks. Text-fig. 1 presents the range of weights for the young of three foster mothers. The two extremes and an intermediate group have been selected for illustration; other groups exhibited a similar uniformity of weight distribution.



TEXT-FIG. 1. NCS mice pooled on second day of life, then immediately allocated randomly to foster mothers (eight per mother). All animals fed D&G pellets.

It will be noticed that the weaning weights of the young animals fell within a narrow range for each particular foster mother. Furthermore, the relative order of weights of individual mice persisted for several weeks after the animals had been separated from their foster mothers, and fed the same diet in the same room. More recent tests still being continued indicate that the differences persist for many months.

In another experiment, the newborn animals were randomly allocated to foster mothers approximately 18 hr after birth. Table I shows the individual weights at that time and for 2 wk thereafter.

It will be seen that differences in weight among the litters of the two foster mothers became obvious within a very few days and that the relative weight rank of the newborn animals was reflected in their adult weights.

The differences in weight curves among the various litters could not be due to the individual genetic endowment of the young mice since these had been allocated at random to various foster mothers; nor could they have had a prenatal origin, since the animals had been randomized after birth. The differences illustrated in Text-fig. 1 and Table I must therefore have been due to an influence exerted by the foster mothers during lactation. Quantity of milk, quality of milk, transmission of a microbial agent, or some behavioral attributes of the foster mother are among the factors that could be considered to account for the

TABLE I

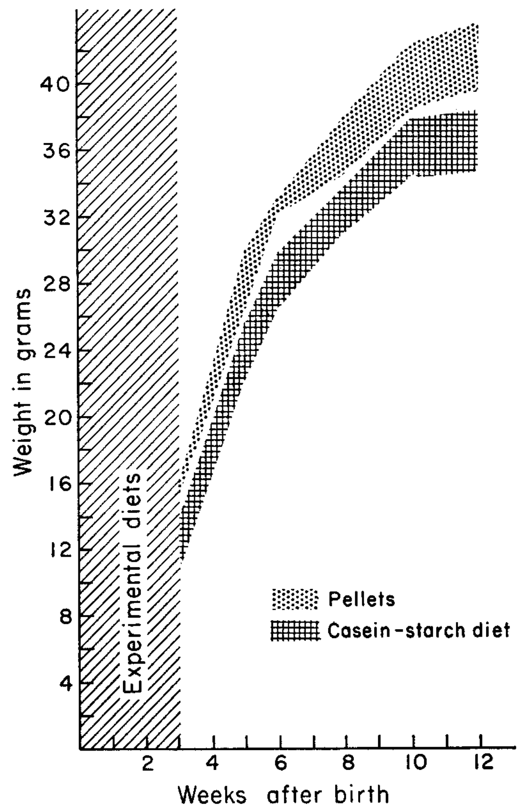
*Effect of Foster Mother on Neonatal Growth of NCS Mice**

Foster mother	Age of the animals (in days)			
	1	4	8	14
	Individual weights			
	g	g	g	g
A	1.4	2.8	5.0	7.4
	1.4	2.8	5.2	7.9
	1.5	2.8	5.2	8.0
	1.5	2.8	5.4	8.1
	1.5	2.9	5.4	8.1
	1.6	3.1	5.6	8.1
	1.8	3.2	5.7	8.2
	1.8	3.3	5.8	8.4
B	1.5	1.8	3.6	6.3
	1.5	1.9	3.6	6.3
	1.5	2.0	3.7	6.4
	1.7	2.0	3.9	6.6
	1.7	2.0	4.0	6.7
	1.8	2.2	4.1	6.7
	1.8	2.8	4.9	7.7
	1.8	2.9	5.0	7.7

* NCS mice, 7 wk of age, gave birth the same day. All newborns were pooled and reallocated randomly to these animals 1 day after birth, eight per foster mother. The table presents results for two foster mothers having received young which were comparable in initial weight.

differences in growth rates among the various groups of young. No attempt has been made to differentiate between these possibilities. What is certain, in any case, is that an early experience associated with the nursing mother had not only affected the development of the animals during lactation, but also had continued to condition their growth for long periods thereafter, and probably for their whole life span.

Nutrition of the Lactating Mother and Growth Rates of the Young.—The following experiment illustrates that the nutritional state of the mother during the lactation period can affect not only the initial growth rate of the young but also their weight as adults.

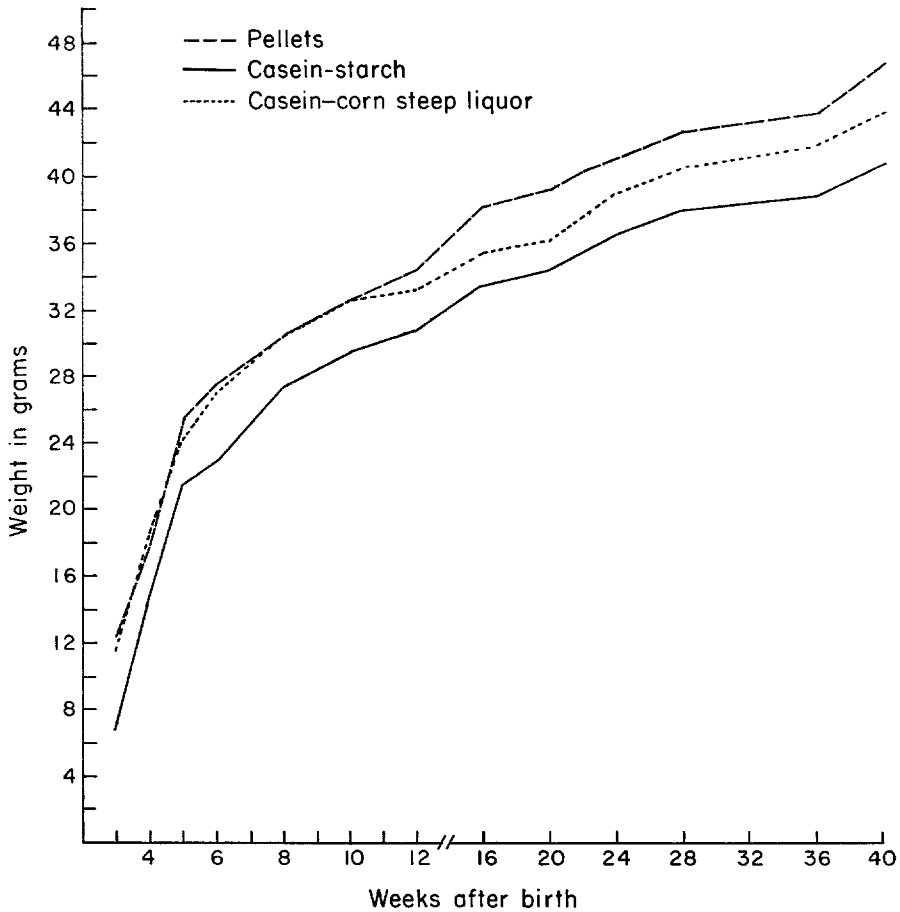


TEXT-FIG. 2. COBS mice fed D&G pellets during the period of gestation. Half of them were changed to casein-starch diet immediately after birth of the young and kept on this diet until weaning of the progeny at 3 wk of age. All animals were fed D&G pellets after weaning. The males were kept in the original cages; the females were discarded.

The graphs show the range of variability between averages of weights of males in each cage (six litters for each diet, with number of males ranging from two to six per cage).

COBS mice were maintained on D&G pellets throughout gestation. 12 animals that gave birth on the same day were selected for the experiment. Immediately after birth, half of them were changed to the casein-starch diet described under Materials and Methods. The other half continued receiving the D&G pellets. The young were weaned at 3 wk of age, and from then on all of them were fed D&G pellets ad lib. (Text fig. 2).

The casein-starch diet is adequate according to usual criteria since it allows rapid growth and healthy maintenance of normal adult mice. It is deficient for lactating females, however, as shown by the fact that a few of these died after 2–3 wk of lactation. Furthermore, all the young nursed by females receiving

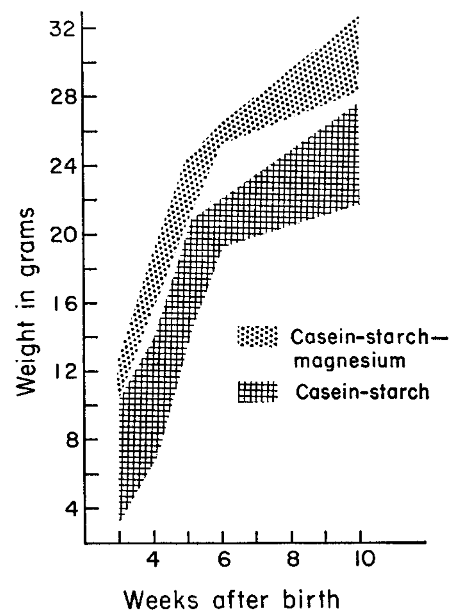


TEXT-FIG. 3. NCS mice fed D&G pellets during gestation, then either one of the two following diets from the time of delivery through lactation period: (*a*) casein-starch diet, (*b*) casein-starch diet supplemented with corn steep liquor. All animals were fed D&G pellets after weaning, at 3 wk of age.

Individual points on the curves represent averages for the males of each litter (maintained in original cages).

the casein-starch diet had a subnormal weight at weaning time, and they became small adults even though they were fed D&G pellets thereafter (Text-fig. 2).

A few attempts have been made to determine the period during which the nutritional deficiency of the lactating mother could exert a weight-depressing effect on the young. Some depression of growth occurred when the casein-starch diet was first given to the mother from the 2nd to the 5th day after birth of her young, but there was no significant effect when the beginning of the regimen was delayed beyond that time. The first 5 days of life, therefore, seem to constitute a critical period for the effect of lactation on the utimate physical development of the mouse.



TEXT-FIG. 4. NCS mice fed D&G pellets until 3 days before delivery. From then on, and throughout lactation, half of them were fed casein-starch diet, the other half the same diet supplemented with magnesium chloride. All animals transferred to pellets after weaning. The graphs show the weight range for each group (48 animals per group) until the 4th wk of life, then for only the males.

Partial Correction by Magnesium Chloride of the Weight-Depressing Effect of the Casein-Starch Diet.—The weight depression caused in the young by feeding the casein-starch diet to the lactating mother could be prevented by adding "corn steep liquor" to her diet during lactation. As seen in Text-fig. 3, the weaning and adult weights of the young were then almost as high as those of animals nursed by mothers fed D&G pellets. While it has not yet been possible to duplicate completely the growth promoting effect of the corn steep liquor with substances of known composition, a partial correction of the deficiency has been achieved by adding magnesium chloride to the casein-starch diet.

7-wk old NCS mice were fed D&G pellets during pregnancy until 3 days before delivery. Half of them were then changed to the casein-starch diet, and the other half to this same diet supplemented with magnesium chloride; they were maintained on the two experimental diets throughout the period of lactation. The initial number of pregnant animals was sufficiently large that six litters, each reduced to eight mice, survived in each diet group. All the young were transferred to D&G pellets after weaning. Their weaning weights and growth curves until the 10th wk of age are presented in Text-fig. 4.

The findings illustrated in Text-figs. 3 and 4 establish that mice nursed by mothers fed the casein-starch diet were much smaller at weaning time than the animals nursed by mothers fed D&G pellets. The results show, furthermore, that this difference could be completely eliminated by supplementing the casein-starch diet with corn steep liquor, but only partially with magnesium chloride.

In all cases, the differences among the various groups were detectable at 1 wk of age, and were quite marked at weaning time; furthermore, they persisted during the 50 wk that the animals were kept under observation, even though all of them were maintained under the same conditions after weaning.

Amino Acid Deficiency and Growth Depression.—A profound and lasting depression of the weight curve of the young mice could be consistently achieved by feeding to the mother a synthetic diet containing wheat gluten instead of casein during gestation and lactation. While the deficiency in lysine and threonine which resulted from this substitution decreased the size of the young, it did not cause any apparent pathological disorder in them, nor did it affect the health of the dam.

The experimental design was the same as in the preceding experiment. Only two diets were used, namely D&G pellets and the wheat gluten diet described under Materials and Methods (Text-fig. 5).

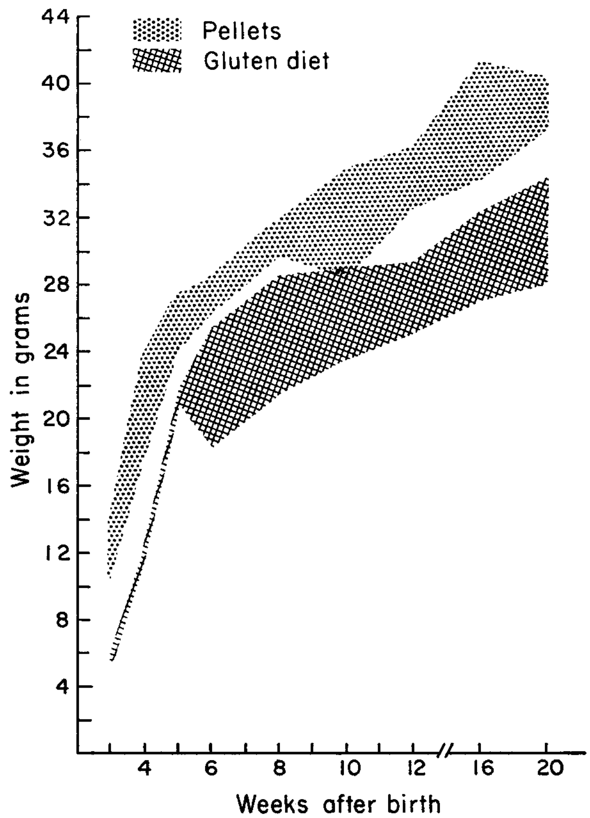
An additional feature of the experiment was that the animals issued from mothers which had been fed D&G pellets were shifted to the gluten diet for 2 wk when they were 5 months old; then D&G pellets were again fed to them (Text-fig. 6).

As seen in Text-fig. 5, the animals issued from mothers fed the gluten diet during gestation and lactation weighed on the average only 5 g at weaning time, as against 12 g for the D&G pellet group. Although the animals of the gluten group were fed pellets ad lib. after weaning, they remained abnormally small throughout the period of observation but appeared healthy.

Adult animals nursed by mothers which had been fed pellets continuously lost weight rapidly when they were transferred to the gluten diet at 5 months of age; furthermore, they failed to gain weight as long as they remained on this diet. In contrast, their growth rate was extremely rapid as soon as they were once more given pellets, so much so that the effect of the gluten diet was erased within 1 wk (Text-fig. 6).

These two complementary parts of the experiment confirm that the growth-depressing effect of dietary deficiency appears to be irreversible when it occurs very early in life, whereas it is rapidly reversible when it occurs during adult life, after development has been completed.

In both parts of the experiment, the weight depression resulting from con-



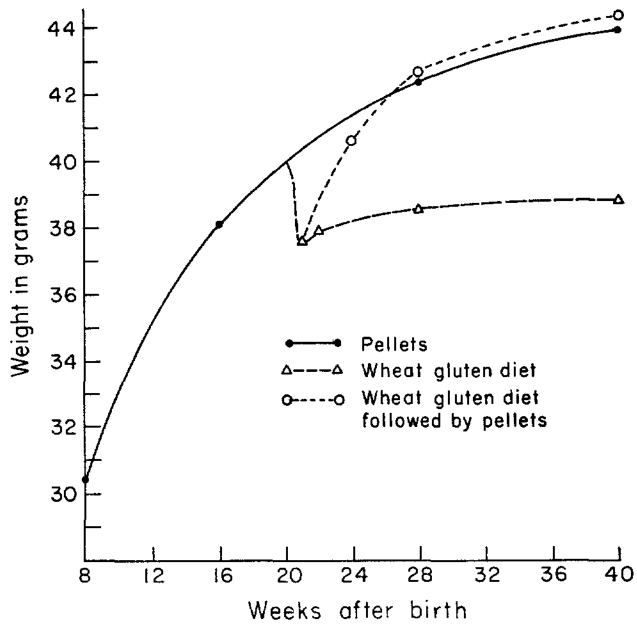
TEXT-FIG. 5. 4-wk old mice were fed either one of the two diets: (*a*) D&G pellets; (*b*) wheat gluten diet. They were mated 3 wk later and maintained on these same diets until weaning of their progeny. All animals were then placed on D&G pellets after weaning.

The figures give the average weights for each cage of the two groups of progeny (five cages of eight mice each per diet); until the 5th wk they included both males and females; after the 5th wk, only the males (i.e., approximately 20 for each diet).

sumption of the wheat gluten diet was due exclusively to the low concentration of lysine and threonine in this plant protein. This is shown by the fact, not to be documented here, that the weight depression could be abolished by supplementing the gluten diet with lysine and threonine (11–12).

Nutritional Deficiency, Depression of Growth, and Life Span.—The results of the preceding experiments show that the nutritional experiences of the dam during gestation and lactation, or during lactation alone, exert effects on her progeny that extend for several months after weaning. In only two experiments has it been possible so far to observe the animals for more than 2 yr after weaning.

Pregnant NCS mice were fed either pellets or the wheat gluten diet (described under Materials and Methods) for approximately 1 wk before delivery and were maintained on this



TEXT-FIG. 6. 60 NCS male mice were bred on D&G pellets. When 5 months old, they were divided into three groups: (*a*) 20 remained on the D&G diet; (*b*) 20 were shifted to the wheat gluten diet and kept on this diet for the duration of the experiment; (*c*) 20 were shifted to the wheat gluten diet and then 2 wk later fed D&G pellets again.

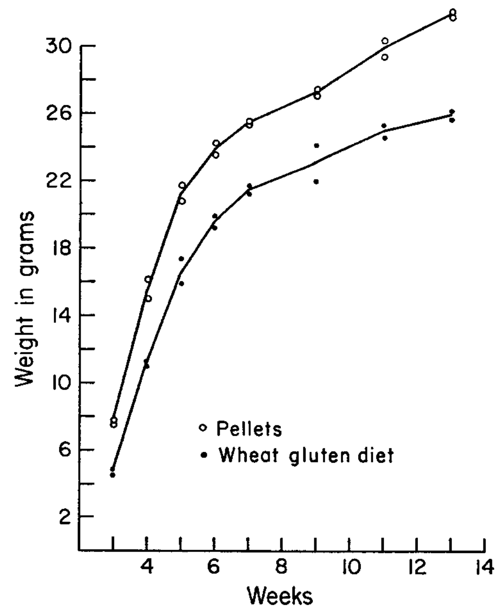
The three curves represent average weights for the 20 animals of each of the three groups.

same diet during the lactation period. After weaning, all animals were fed pellets ad lib. through the rest of their life-span. Only the males were kept beyond the 14th wk of life. At that time they were transferred to individual cages; their weights were determined at weekly intervals (Text-figs. 7 and 8).

As in the preceding experiment, the young produced and nursed by mice fed the wheat gluten diet weighed less at weaning time than those of the pellet group; furthermore, the difference between the two groups persisted from then on throughout the whole life span of the animals even though all of them were housed under exactly the same conditions and fed the same diet (pellets) from the time of weaning.

Two additional facts emerged from the experiment.

It will be noted that in Text-fig. 8 a marked loss of weight occurred in both groups of animals around the 15th wk of life; this was due to the fact that the male mice were transferred at that time from plastic cages with bedding of sawdust, in which they were in groups of 3–5, to individual stainless steel cages on wire grids. On several occasions thereafter, marked fluctuations occurred in the slopes of the weight curves; the cause of these fluctuations has not been identified. The point of interest, however, is that the two weight curves remained parallel throughout and that the weight losses exhibited by both groups—what-



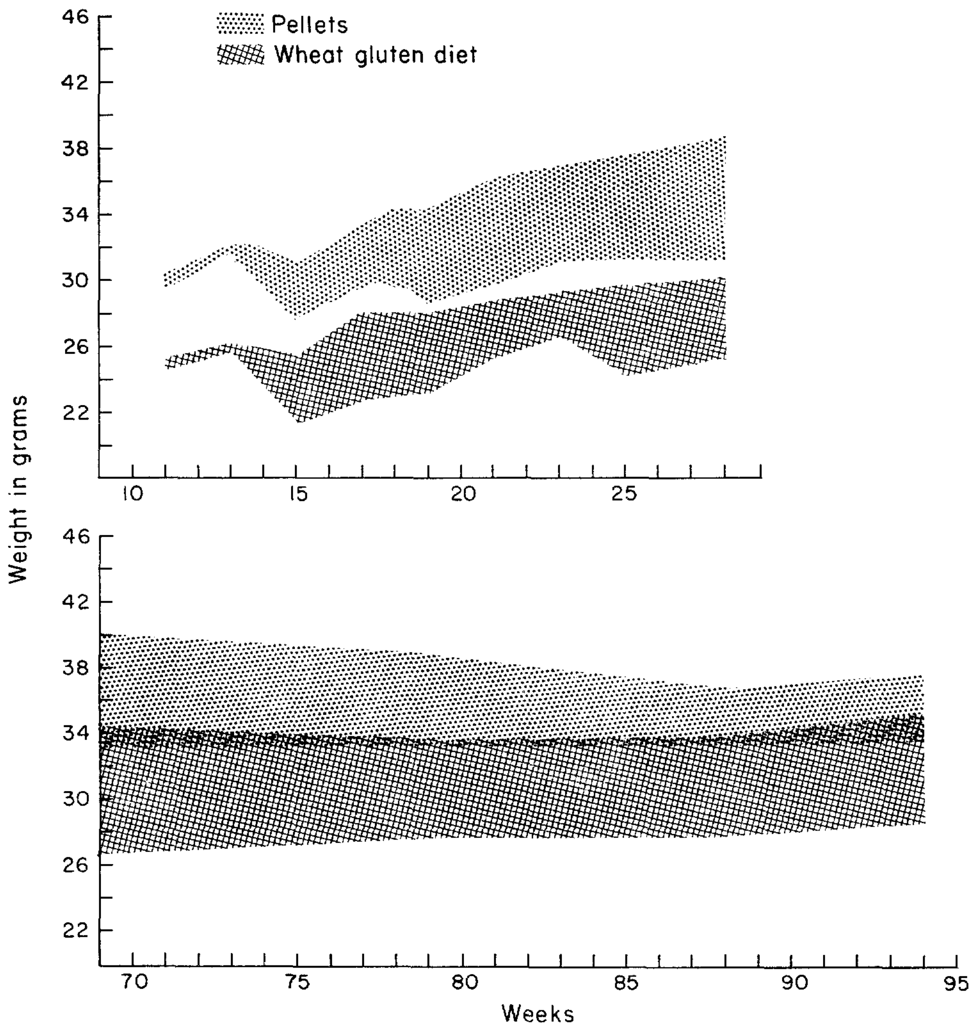
TEXT-FIG. 7. NCS mice were fed D&G pellets and mated. Approximately 1 wk before delivery of the young, half of the pregnant animals were changed to wheat gluten diet and maintained on this diet until their young were weaned at 3 wk of age. All the animals were then fed D&G pellets, for the rest of their lives.

The two graphs in this figure include males and females of the two progenies (two cages of 16 animals each per group). Males and females were separated after the 5th wk of life, but the weights of all the animals from each cage were pooled and averaged until the 14th wk of life.

ever their cause—were rapidly reversible. This is in sharp contrast to the weight effects due to early nutritional influences which appeared irreversible.

Although the animals nursed by dams fed the wheat gluten diet during gestation and lactation were much smaller than the animals of the pellet group, their life span was not shortened by this early nutritional deprivation. In fact, the first animals to die in the experiment illustrated in Text-fig. 8 were those of the pellet group and, among those, the heaviest died first.

Although the numbers of animals involved were small (16 in each group), these findings were sufficiently suggestive to warrant the initiation of another experiment designed to test the effect of early nutritional influences on longevity.



TEXT-FIG. 8. This experiment is the same as that illustrated in Text-fig. 7; from the 14th wk of life, the males were transferred to individual cages; the females were discarded.

The loss of weight on the 15th wk was caused by the fact that the animals had been transferred to individual cages on wire grids. The fluctuations of the two curves thereafter express the effects of unidentified environmental factors.

The graphs indicate the range of weight variation for each of the two diet groups.

Deaths began to occur in the D&G groups after 2 yr, the heaviest animals dying first. None of the animals survived beyond 2 yr and 7 months.

This experiment was similar in general design to the preceding ones, with the following differences: (*a*) three diets were used during the gestation and lactation periods (D&G pellets, wheat-gluten diet; casein-starch diet supplemented with corn steep liquor); (*b*) the young females were discarded immediately after weaning; the males were maintained in the original litter groups (from two to six out of the eight young per litter that had been kept) instead of being placed in individual cages. The total number of surviving males, and their average weights, at different periods, are given in Table II.

As in earlier experiments the animals nursed by mothers fed the wheat gluten diet were very much smaller than those nursed by mothers fed either pellets or the casein-starch–corn steep liquor diets. Throughout their life span, the animals of the wheat gluten group remained unusually small, even though they were fed pellets after weaning. As in the preceding experiment, however, the small animals appeared to survive somewhat longer than the others.[3]

TABLE II

Maternal Diet Effect on Weight and Life Span of Progeny

Age of progeny	Diet fed the mother* (gestation and lactation)					
	Pellets		Casein-supplemented		Wheat Gluten	
	Effect of maternal diet on progeny					
	Weight	Survivors	Weight	Survivors	Weight	Survivors
wk	*g*		*g*		*g*	
3	10.5‡	40‡	10.5‡	40‡	5.5‡	40‡
15	33.2	23	33.7	20	28.2	22
35	41.1	23	41.2	20	31.1	22
51	41.5	23	42.4	19	35.6	20
83	39.8	19	39.2	13	34.0	17
101	41.6	9	39.0	8	33.8	11
118	42.3	6	35.8	3	31.5	11

* The experimental diets were administered only during the period of gestation and lactation; after weaning all animals of progenies were fed D & G pellets.

‡ The 3-wk figures refer to males and females; from 15 wk on the weights and numbers of survivors refer only to the males.

At any given period the weights represent averages for the total number of survivors.

[3] The number of animals that were followed for their whole life span in the experiments corresponding to Fig. 8 and Table II are not large enough to warrant conclusions regarding the effect of the maternal diet on longevity of the progeny. It has seemed justified to report the suggestive but inconclusive results of these experiments, however, because more than 2 yr will necessarily elapse before the new longevity experiments now in progress have yielded data extensive enough for statistical analysis.

Experiments on the metabolic aspects of the weight depression caused by maternal diet are now being conducted in our laboratory by Dr. Chi-Jen Lee.

SUMMARY

Newborn specific-pathogen-free mice (SPF) were separated from their mothers shortly after birth and immediately reallocated at random to foster mothers, each of which received eight young. Under these conditions, the growth rate and adult size of the young were profoundly and lastingly conditioned by some unidentified influence exerted by the foster mother.

In SPF mice nursed by their own mothers, the diet of the latter during gestation and lactation, or during lactation alone, conditioned the weight of the young at weaning time, and throughout their whole life span.

Lasting depression of growth has been achieved by minor alterations of the dam's diet, for example by lowering its content in magnesium, or in lysine and threonine. The growth-depressing effect so achieved persisted throughout the whole life-span of the young, even though they were given at weaning time and constantly thereafter unlimited amounts of an optimum diet.

In contrast, the weight-depressing effect of a diet deficient in lysine and threonine administered to adult animals was completely and rapidly reversible when a complete diet was later substituted for the deficient one.

Depression of growth resulting from nutritional experiences during gestation or lactation did not seem to affect adversely the health of the young, or to decrease their longevity. In fact, the results of two experiments in which the animals nursed by mothers on different diets, were kept undisturbed and on optimum diets throughout their whole life span, suggest that the smaller animals had a greater average life expectancy than the larger ones.

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