not seen on the pre-test day in the straight channel, was indicative of a diminished ability to change a previously effective response pattern. That is, after learning to make turns through the maze, the P-group may not have been able to adapt so well as the controls to the post-test straight channel condition where swimming straight ahead was effective. Experiments designed to test the reversal learning ability of phenylketonuric rats are in progress.

Although behavioral differences have been reported by others(4,6,7,8,9), questions regarding the adequacy of the phenylalanine concentration, dietary control, or behavioral testing procedure employed remain unanswered in many of these researches. To answer these questions pair-fed experiments designed to clarify the effects of dose, duration on the diet, and weight loss *per se* are in progress using the behavioral testing method as described, except that on the 3 maze days, only 5 trials per day are given. The effects of nonspecific toxic agents, D-, DL-, and L-phenylalanine and the possibility of a "critical period" of susceptibility to excess phenylalanine will also be studied.

The present experiments also bear on the question of when the dietary regimen should begin in order to produce behavioral deficits in the rat. It is now clear that it is not necessary to feed the excess phenylalanine diet to the mother in order to provide it to the fetus *in utero,* and to the pup during the suckling stage, as was previously suggested(10). It may now be concluded that the weanling rat is a satisfactory experimental animal for use in the study of behavioral, as well as biochemical, parameters of phenylketonuria.

Summary. A behavioral testing procedure utilizing a swimming water maze has been reliable in rapidly detecting and quantifying a performance decrement in rats made phenylketonuric by feeding excessive quantities of L-phenylalanine from weaning.

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Evidence for Genetic Factors in the Resistance of the Rat to Dietary Cirrhosis.* (28501)

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When experimental animals are fed diets deficient in one or more nutrients, often only a limited number of animals develop signs of specific malnutrition. In the case of rats fed a cirrhosis-producing diet (deficient in protein and choline) the same basic diet produced a fairly predictable response: 70 to 80% of male, Sprague-Dawley rats developed

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the disease within 6 months.† The lesions were characterized by fatty infiltration, inflammatory cellular reaction, and diffuse overgrowth of connective tissue, resulting in distortion of hepatic structure. Those rats failing to develop cirrhosis became of interest. Was their resistance to the disease related to chance phenomena, to environmental factors, or to genetic determinants? The present study was designed to test the last possibility.

We have not encountered other studies concerned directly with this problem. Earlier reports, however, by Engel(1) and Copeland(2) indicate that there are strain differences in the minimal requirement for choline necessary to prevent renal damage in rats. These differences can be maintained or increased by inbreeding.

Methods. Weanling Sprague-Dawley rats were housed in individual, suspended cages with wire meshed floors. They were kept in an air-conditioned room with a mean temperature of 70°F. A nutritious chow diet (Purina Laboratory Chow-Ralston) was fed all animals until they were about 5 weeks old and weighed about 130 g. They were then placed on a cirrhosis-producing diet patterned after that devised by Daft, Sebrell and Lillie (3) and consisted of the following: casein 4.0; L-cystine 0.5; cottonseed oil 5.0; cornstarch 86.5; salt mix 3.0; vitamin mix 1.0. Details are published elsewhere(4). All rats were fed *ad libitum*. The feeding cups, with narrow necks, allowed minimal spillage. Food intake was measured twice weekly and body weights were recorded twice monthly. After 6 months the animals were subjected to laparotomy under ether anesthesia, and a wedge-shaped piece of liver measuring 0.5 cm at the base was removed. Biopsies were examined by 2 observers‡, and the sections were graded with respect to fatty change, necrosis, inflammatory cellular reaction, and overgrowth of connective tissue. Biopsies of this size were found to be representative of the liver as a whole. Changes of cirrhosis were readily recognized. Livers of resistant animals showed none of these changes or, at most, a moderate degree of fatty infiltration.

It has previously been observed that the male rat is more susceptible to certain hepatotoxins(5,6) and to nutritional cirrhosis(7, 8) than the female, and develops the disease with more regularity. On this account only male rats were tested for resistance to the disease in the present study. The experiment was carried out in 2 consecutive stages:— The first stage involved breeding resistant males and *random* females. In the second stage resistant males were crossed with *daughters* or *sisters*.§ Fig. 1 illustrates the changes in resistance to cirrhosis in one family, as these took place during the 2 consecutive stages of the study.

Stage 1. A resistant male rat (No. 83)‖ was mated with 5 random females of the same Sprague-Dawley strain (Table I). The females were about 10 weeks old and weighed 150 to 180 g. Male offspring from this mating were fed the standard, cirrhosis-producing diet for 6 months, when their livers were biopsied and examined histologically.

Nine resistant male offspring were mated with random females. However, only 5 of 9 males were fertile. The male offspring of these matings were tested for resistance to cirrhosis in the manner described above.

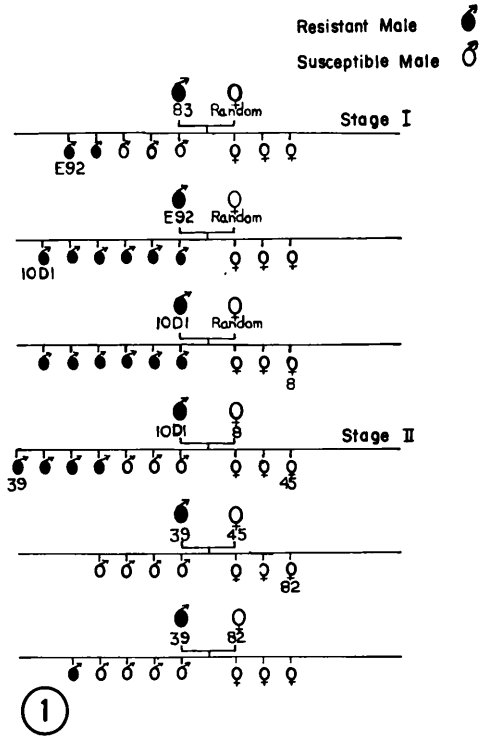
Because of an apparent increase in resistance to cirrhosis observed in this second generation, it was decided to try selective inbreeding. For this purpose a resistant male was selected from each of 3 litters which had shown a high degree of resistance. These males were bred to random females. Their offspring comprise the third generation of Stage 1.

† In previous experiments involving 20, 25, and 123 male rats of the same strain, resistance to cirrhosis occurred in 5, 20, and 24%, respectively, with a mean incidence of 22% in 168 animals.

‡ The authors wish to express their thanks to Drs. Margaret Bevans and Robert Hirsch, Dept. of Pathology, Columbia Univ. Coll. of Physicians and Surgeons, for interpreting the microscopic sections.

§ Studies on offspring from random males bred with random females were not carried out concurrently.

‖ This animal, being fed a cirrhosis-producing diet, showed no signs of cirrhosis in biopsies taken at 6 and 12 months.



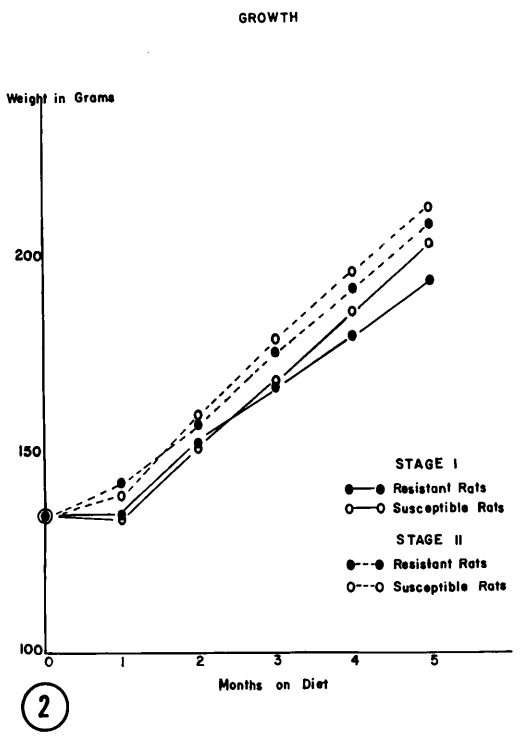


FIG. 1. Resistance to Dietary Cirrhosis of the Liver in a Single Family.
FIG. 2. Growth curves.

Stage 2. In this stage resistant males were bred to *related* females, as shown in Table II and in Fig. 1. In each instance 3 females were mated with one male. In the first generation 5 of 9 females bred to their fathers had litters suitable for testing. Sons of this mating were placed on the cirrhosis-producing diet for 6 months, when their livers were biopsied and examined histologically.

In the second generation a resistant male from each of 5 litters was mated with 3 sisters. Only 4 of 15 matings produced litters suitable for testing. The male offspring were tested for resistance to cirrhosis in the manner described. Female offspring were bred back to their fathers.

In the third generation there were 4 successful litters from 8 matings between daughters and fathers. The male offspring were tested for resistance in the usual fashion.

Results and discussion. In the first stage of the experiment the proportion of resistant offspring increased progressively when resistant males, their resistant sons and grandsons were bred to random females. (Table I, Fig. 1.) The Table shows the number of males tested in each litter, the number found to be resistant or susceptible to cirrhosis, and the total of each generation. Although individual litters were small they showed similar trends with few exceptions. In the first generation 43% were resistant; in the second, 72%; and in the third, 75%.

These findings are in sharp contrast to those in Stage 2, when female sibs and daughters were selectively bred to their resistant brothers and fathers (Table II). Here resistance to cirrhosis was found in 50% of the first generation, and in roughly 25% of the second and third generations. The incidence of resistance appeared to revert towards the usual frequency of 22% found in random rat populations†.

The high proportion of resistant offspring observed in Stage 1, when resistant males were crossed with unrelated females, suggests that genetic factors were involved. The data do not indicate whether a single gene

TABLE I. Resistance to Dietary Cirrhosis in Rats Bred from Resistant Males and Random Females. (Stage 1)

Parents			Male offspring		
Resistant ♂	Random ♀		No. tested	No. resistant	No. susceptible
83	F 9		5	2	3
83	F 6		3	2	1
83	F12		6	3	3
83	F 1		3	1	2
83	F 4		3	1	2
			20	9 (43%)	11 (57%)
Resistant ♂	Random ♀				
*E 92	10		6	6	0
*E 92	2		4	4	0
E 92	1		6	0	6
*E 63	8		6	5	1
E 63	9		5	3	2
E 62	7		7	4	3
E 62	11		4	3	1
E 62	6		2	2	0
E125	4		3	3	0
E125	5		6	4	2
E 94	3		4	4	0
			53	38 (72%)	15 (28%)
Resistant ♂	Random ♀	Male* lineage			
10D1	F10D1	E92	6	6	0
2D3	F 2D3	E92	7	5	2
8D3	F 8D3	E63	3	1	2
			16	12 (75%)	4 (25%)
		Totals	89	59 (66%)	30 (34%)

TABLE II. Resistance to Dietary Cirrhosis in Rats Bred from Resistant Males and Related Females. (Stage 2)

Parents			Male offspring		
Father	Daughter		No. tested	No. resistant	No. susceptible
10D1	7		6	3	3
10D1	8		7	4	3
2D3	3		8	3	5
8D3	4		3	3	0
8D3	5		6	3	3
			30	16	14
Brother	Sister				
53	54		1	–	1
39	45		4	1	3
33	34		5	0	5
57	62		3	2	1
			13	3	10
Father	Daughter				
53	87		4	2	2
39	82		5	1	4
33	94		3	0	3
57	98		4	1	3
			16	4	12
		Totals	59	23 (38%)	36 (62%)

was responsible or whether polygenic factors were at play.

In Stage 2, the trend toward resistance was not maintained, and it appears that other factors, either environmental or genetic in nature, had a modifying effect. Although environmental conditions may have been responsible for the change in Stage 2 these were not evident. No relation could be established with season, housing, diet, intercurrent disease, or personnel. Only 7 animals died of intercurrent disease during the experimental period which lasted 4 years.

Food intake and growth rates (Fig. 2) were similar for resistant and susceptible animals in Stages 1 and 2. Resistance in these animals appeared to be unrelated to rate of growth.

It is possible that as a result of inbreeding in Stage 2, modifying genes became homozygous and thus led to an increase in susceptibility. It also is possible that the decline in resistance was due in part to what is termed "inbreeding depression," although the nature of the latter is not clear.

Summary and conclusions. Male rats of the Sprague-Dawley strain, which were found to be resistant to the development of dietary cirrhosis, were bred with random females of the same strain. In 3 generations of rats so bred there appeared to be a sharp increase in the proportion of resistant males. However, when resistant males derived from resistant fathers and grandfathers were crossed with their daughters or sisters there was a sharp decline in the proportion of resistant male offspring. It is suggested that resistance to dietary cirrhosis in the rat may be attributable to an inherited trait or traits, and that other factors, either environmental or genetic, may exert a modifying influence.

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Carbon Dioxide in Prevention of the Deleterious Dilution Effect on Metabolism and Motility of Mammalian Spermatozoa. (28502)

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Mammalian spermatozoa, as well as spermatozoa from lower animals, have been shown to exhibit a dilution effect. Gray(7) reported that mild dilution of sea-urchin sperm stimulated motility and O_2 uptake, but was detrimental to livability. Salisbury *et al* (13) found that mild dilution of bull spermatozoa depressed livability, but did not adversely affect fertility. Later it was shown that mild dilution stimulated O_2 uptake of bull sperm depending upon the ions present in the diluting medium(1). The dilution effect and the proposed theories as to its cause have been reviewed(10,14). It is generally agreed that mild dilution stimulates motility and metabolism, but further dilution beyond an optimum sperm cell concentration depresses them both(2,3,4,11,17,18).

The depressive effect is believed to be caused by a) a loss of vital material from