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### Research Article

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# Effect of Neonatal Modulation of Cholesterol Homeostasis on Subsequent Response to Cholesterol Challenge in Adult Guinea Pig

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**ABSTRACT** Experiments were designed to study whether or not the mechanism of handling dietary cholesterol in adulthood can be modulated by the manipulation of cholesterol homeostasis during neonatal period. The effects of enhancing cholesterol degradation (cholestyramine feeding), high dietary cholesterol intake, and early weaning during neonatal period of guinea pigs on their subsequent plasma cholesterol levels and the response to dietary cholesterol challenged in adulthood were investigated. Pretreatment of neonatal guinea pigs with cholestyramine resulted in (a) a lower plasma cholesterol level, (b) an increased excretion rate of fecal bile acids and total steroids, (c) an expanded bile acid pool, (d) an increased activity of cholesterol 7 $\alpha$ -hydroxylase, and (e) no change in the hepatic 3-hydroxy-3-methylglutaryl coenzyme A (CoA) reductase activity when challenged with cholesterol in adulthood. Cholesterol pretreatment during neonatal period resulted in (a) no alteration in the plasma cholesterol level, (b) no alteration in the fecal excretion of steroids, or (c) no alteration in the cholesterol 7 $\alpha$ -hydroxylase activity when they were challenged with a high cholesterol diet. Early weaning did not influence the fecal excretion of steroids or cholesterol 7 $\alpha$ -hydroxylase activity but resulted in a slight decrease in the hepatic 3-hydroxy-3-methylglutaryl-CoA reductase activity when they were challenged with a high cholesterol diet. These results suggest that stimulation of cholesterol catabolism rather than cholesterol feeding or early weaning during neonatal period can influence the response to dietary cholesterol challenge in adulthood.

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## INTRODUCTION

The serum cholesterol concentration of children increases from an average of 75 mg/dl at birth to 120 mg/dl by 4–5 d of life (2). A small increase in serum cholesterol concentration occurs between ages 1 and 6 yr (3, 4). Thereafter, the plasma cholesterol concentration rises gradually (4, 5). Since atherosclerosis begins in childhood and progresses in the second and third decades of life and because hypercholesterolemia is regarded as a risk factor with respect to atherosclerosis (6), it is important to determine which factors control cholesterol metabolism in infancy and childhood. Previous investigators proposed that the metabolism of cholesterol in adult life can be modulated by perturbation of cholesterol synthesis and degradation during the neonatal period. Reiser and Sidelman (7) found that rats suckled by mothers whose milk contained high levels of cholesterol were able to withstand a cholesterol challenge in adult life without developing a significant increase in their serum cholesterol levels. They suggested that the intake of rat's milk may have induced an enzyme for cholesterol degradation in rats. Hahn and Kirby (8) indicated that preweaned rats had higher plasma cholesterol levels when compared with normal weaned rats. They further demonstrated that neonatal manipulation can have long-lasting effects that appear later in life (8).

Recent studies of hepatic 3-hydroxy-3-methylglutaryl (HMG)<sup>1</sup> coenzyme A (CoA) reductase have provided evidence that perturbation of the enzyme activity early in life may be persistent in the adult rat (9). However,

<sup>1</sup> Abbreviation used in this paper: HMG-CoA, 3-hydroxy-3-methylglutaryl-coenzyme A.

these findings in rats have not been replicated in studies of normal and hypercholesterolemic infants and children (3–5, 10). In fact, Hodgson et al. (10) demonstrated that children who consumed a low cholesterol diet during the neonatal period had a lower plasma cholesterol level at 7–12 yr of age (10). These human studies suggested that low cholesterol intake in early infancy has no undesirable effect on the long-term ability to regulate plasma cholesterol concentration (10–12). Up to the present time, neonatal manipulation in animal studies was only carried out by exposing young animals to a large quantity of dietary cholesterol to establish the serum cholesterol homeostasis. The promotion of bile acid removal (stimulation of cholesterol catabolism) in early life may also have long-lasting effects on subsequent cholesterol metabolism in adult life. However, no studies have been carried out in which cholesterol catabolism has been stimulated in neonatal animals and the subsequent response to dietary cholesterol evaluated in adult life.

The 7 $\alpha$ -hydroxylation of cholesterol by the cholesterol 7 $\alpha$ -hydroxylase is the rate-limiting step in bile acid synthesis from cholesterol (13, 14). Cholesterol 7 $\alpha$ -hydroxylase is a microsomal mixed function oxidase and the reaction involves the participation of NADPH, molecular oxygen, NADPH-cytochrome P-450 reductase, and cytochrome P-450 (15, 16). The enzyme activity is modulated by biliary diversion, bile acid sequestrant feeding, and bile acid feeding in rats (17). Previously, the hydroxylation of testosterone has been shown to be a cytochrome P-450 mediated reaction (18) and the neonatal development of hepatic cytochrome P-450 has been shown to be regulated by testicular androgens (19). Therefore, it is possible that the cytochrome P-450 involved in the cholesterol 7 $\alpha$ -hydroxylation could be under the influence of "neonatal imprinting" by its metabolites and thus the control mechanism be similar to that of the androgenic metabolic enzymes.

Cholestyramine, a bile acid sequestrant that binds bile acids in the intestine, increases bile acid excretion, enhances cholesterol catabolism, stimulates the cholesterol 7 $\alpha$ -hydroxylase, and subsequently reduces plasma cholesterol levels (7, 20). The current investigation was designed to determine whether or not the mechanisms responsible for the metabolism of dietary cholesterol in adult animals could be influenced by manipulation of cholesterol homeostasis during the neonatal period. The manipulations used were: (a) enhancing cholesterol degradation by cholestyramine feeding; (b) high dietary cholesterol intake; and (c) early weaning of newborn male guinea pigs during the neonatal period with the determination of their subsequent response to a dietary cholesterol challenge and of their plasma cholesterol levels in later life.

In the studies of cholesterol homeostasis, the selec-

tion of the animal model is critical. For example, studies of the effect of a high cholesterol intake on neonatal rats is difficult to extrapolate to humans because rats are known to enhance their bile acid formation when fed a high-cholesterol diet (21). Although guinea pigs have some distinctive features of cholesterol metabolism compared with that of humans, such as low plasma cholesterol level and low cholesterol synthetic activity in the liver, the guinea pig was chosen for the current studies because of the developed state of neonates in this species and the physiological response to extrauterine environment was similar in principle to that occurring in human infants (22–24). Previous studies from this laboratory have demonstrated the details of the change in plasma cholesterol and bile acid pool size in the neonatal guinea pig (25). The activity of cholesterol 7 $\alpha$ -hydroxylase in the newborn was about one-third of that in adult guinea pigs. This finding suggested that the system for bile acid synthesis in the neonatal guinea pig is underdeveloped (26).

## METHODS

Age-matched English short-hair female guinea pigs with a body weight of 600–800 g were used. Guinea pigs were bred in our animal facility and the data of copulation was recorded after examination of the vaginal opening. Pregnancy was confirmed by examination of vaginal membrane at the end of the estrus cycle. Before the experimental period, both pregnant and nonpregnant guinea pigs were housed individually in slat-bottomed metabolic cages for 4 d before study to adapt them to this environment. Guinea pigs were fed Purina guinea pig chow (Ralston Purina Co., St. Louis, Mo.) ad lib. and had free access to drinking water. They were kept under controlled lighting with equal light and dark periods (darkness from 5:00 p.m. to 5:00 a.m.). The guinea pigs were allowed to complete their gestation (68 d). The newborn guinea pigs were nursed by their respective dams until 1 wk of age (27). The male neonatal guinea pigs were grouped for experiments. The experimental group was fed: (a) a cholestyramine-containing diet (1.1%) for 6 wk, or (b) a cholesterol-containing diet (0.25%) for 12 wk. The control group of guinea pigs were fed a Purina guinea pig chow diet. All the animals were then fed a standard diet for 6 wk. For the studies of the effect of cholestyramine pretreatment on the newborn guinea pigs, the animals were challenged with a 0.25% cholesterol diet and a 0.5% cholesterol diet for 4 wk each. This was followed in each case by a period of 4 wk on a standard diet, then they were challenged again with 0.25% cholesterol diet for 6 wk followed by 4 additional wk on a standard diet. To study the effect of cholesterol pretreatment on the newborn guinea pigs, the animals were challenged with a 0.25% cholesterol diet for 7 wk followed by a period of 8 wk on a standard diet after which they were rechallenged with a 0.25% cholesterol diet. For the studies of the effect of age of weaning on the guinea pig, one group of neonatal guinea pigs was kept with their dams until 24 d of age, the other group was weaned at 1 wk of age. Both groups of guinea pigs were fed guinea pig chow until 7 wk of age, then challenged with 0.25% cholesterol diet for 12 wk. For the studies of the effect of cholestyramine pretreatment on the adult guinea pig, one group of adult animals was fed a cholestyramine containing (1.1%) diet for 6 wk. The control group

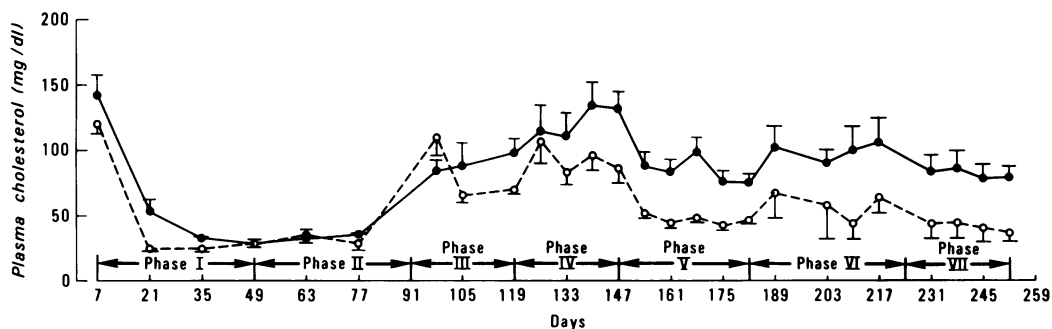


FIGURE 1 Plasma cholesterol levels in the guinea pigs pretreated with cholestyramine during neonatal period (○) and control guinea pigs (●). Phase I, guinea pigs on 1.1% cholestyramine or guinea pig chow diet. Phase II, both groups of guinea pigs on guinea pig chow diet. Phase III, both groups of guinea pigs on 0.25% cholesterol diet. Phase IV, both groups of guinea pigs on 0.5% cholesterol diet. Phase V, both groups of guinea pigs on guinea pig chow diet. Phase VI, both groups of guinea pigs on 0.25% cholesterol diet. Phase VII, both groups of guinea pigs on guinea pig chow diet.

was fed a standard chow diet. Thereafter, both groups of guinea pigs were fed a standard chow diet for 6 additional wk.

Blood samples of guinea pigs (heparinized) were taken from the femoral vein under ether anesthesia. Plasma cholesterol levels were determined by a method described by Ellefson (28).

The hematocrits in guinea pigs were determined by the method of Mitrunka and Rawsley (29). A capillary hematocrit tube was used and filled by capillary action. The sealed capillary tubes were then placed in a high-speed centrifuge (IEC Corp., Austin, Tex., model MB). After centrifugation, the volume of packed cells was read from a scale held against the capillary tube.

For acidic and neutral sterol excretion studies, guinea pigs were housed in individual cages. The total intake of food was measured and fecal output during the 4-d period was collected in preweighed containers. The fecal samples were pooled and homogenized in a known amount of water. Aliquots of the fecal homogenates were taken for the analysis of bile acids and neutral sterols.  $\beta$ -sitosterol, which is present in the Purina guinea pig chow, was used as a nonadsorbable marker to correct for variation in fecal flow and losses from incomplete stool collection. Its concentration in the diet was measured by gas-liquid chromatography before the study (30).

The fecal bile acids were analyzed by a procedure described previously (29, 31). The bile acids were extracted, methylated, and quantitated with hyocholic acid as an internal standard. Gas-liquid chromatography of their methyl esters of trifluoroacetates was done with a F & M model 402 gas chromatograph and a 3% QF-1 column (Packard Instrument Co., Inc., Downers Grove, Ill.). Column conditions were as follows: column, 220°C; flash heater, 240°C; detector, 250°C; and carrier gas, helium, 50 ml/min.

The fecal neutral sterols were analyzed by a procedure described previously from this laboratory (27, 31). After extraction, the lipid extracts were applied to a silica gel G thin-layer chromatographic plate (500  $\mu$ m, thickness) with ethyl ether:heptane (55:45 vol/vol) as solvent. The bands corresponding to cholesterol, coprostanol (5 $\beta$ -cholestan-3 $\beta$ -ol), and coprostanone (5 $\beta$ -cholestan-3-one) were eluted and quantitated as trimethylsilyl ether derivatives by gas-liquid chromatography using 5 $\alpha$ -cholestane as an internal standard. Gas chromatography was done using a Packard 409 model gas chromatograph and 3.8% w-98 column. Column conditions were as follows: column, 230°C; flash heater, 250°C; detector, 270°C; and carrier gas, helium, 50 ml/min. Losses of neutral sterols during the experimental procedure were corrected on the basis of recovery of [4-<sup>14</sup>C]cholesterol.

**Determination of biliary cholesterol and bile acids and bile acid pool size in guinea pig.** The gallbladders from the guinea pigs were dissected and the contents collected. After extraction, measurement of cholesterol and bile acid content was done by the aforementioned method for fecal cholesterol and bile acids. For determination of the bile acid pool size, intestinal content was removed and bile acid contents were extracted, saponified, and quantitated by gas-liquid chromatography as reported previously from this laboratory (24, 28, 30).

**Assay of HMG-CoA reductase (EC 1.1.1.34).** Subcellular fractionation of hepatic microsomes was performed according to the method of Shefer et al. (30) with minor modification (30, 32). The assay of HMG-CoA reductase was done according to the method of Goldfarb et al. (33) after establishment of optimum condition for the guinea pig system (26). The assay medium of 1 ml contained potassium phosphate buffer, 0.1 mmol, pH 7.4; NADP, 3.0  $\mu$ mol; glucose-6-phosphate, 10

TABLE I  
Effect of Cholestyramine Pretreatment during Neonatal Period on Bile Acid and Neutral Sterol Excretion in Guinea Pigs\*

Group	Diet	Bile acids	Neutral sterols	Total sterols
Control (n = 3)	0.25% Cholesterol	14.5 $\pm$ 3.9	20.7 $\pm$ 3.6	35.1 $\pm$ 0.2
Cholestyramine pretreated (n = 5)	0.25% Cholesterol	29.9 $\pm$ 4.2†	14.0 $\pm$ 2.0	43.9 $\pm$ 4.8

\* Data shown as mean $\pm$ SEM, milligrams per kilogram per day.

† 0.025 < P < 0.05.

**TABLE II**  
*Effect of Cholestyramine or Cholesterol Pretreatment during Neonatal Period on Hepatic Cholesterol 7 $\alpha$ -Hydroxylase Activity and HMG-CoA Reductase in the Adult Guinea Pig\**

Group	Diet	Cholesterol 7 $\alpha$ -hydroxylase	
		HMG-CoA reductase	
<i>pmol/min/mg</i>			
Control (n = 3)	0.25% Cholesterol	6.4 $\pm$ 1.0	2.2 $\pm$ 0.3
Cholestyramine pretreated (n = 3)	0.25% Cholesterol	20.9 $\pm$ 7.9 $\ddagger$	2.5 $\pm$ 0.9 $\S$
Control (n = 3)	Guinea pig chow	5.3 $\pm$ 0.3	5.8 $\pm$ 1.2
Cholestyramine pretreated (n = 5)	Guinea pig chow	9.4 $\pm$ 1.2 $\ddagger$	7.8 $\pm$ 2.0 $\S$
Control (n = 4)	0.25% Cholesterol	4.0 $\pm$ 1.3	2.3 $\pm$ 0.6
Cholesterol pretreated	0.25% Cholesterol	3.5 $\pm$ 0.6 $\S$	4.0 $\pm$ 0.1 $\ddagger$

\* Data shown as mean $\pm$ SEM.

$\ddagger$  0.025 < P < 0.05.

$\S$  P > 0.05, NS.

$\mu$ mol; glucose-6-phosphate dehydrogenase (EC 1.1.1.49) 2.5 IU; and glutathione, 50  $\mu$ mol. The assay was initiated by the addition of 0.16  $\mu$ mol of D,L-[3- $^{14}$ C]HMG-CoA (sp act, 0.53 Ci/mol) for 30 min at 37°C. The assay was stopped by the addition of 1 N H<sub>2</sub>SO<sub>4</sub> and mevalono-lactone with D,L-[5- $^3$ H]-mevalonic acid to the reaction medium. The mevalonolactone was extracted, separated by thin-layer chromatography (TLC) and subjected to liquid scintillation counting. The activity is expressed as picomoles of mevalonolactone formed per minute per milligram of microsomal protein. All enzyme determinations were carried out in triplicate.

*Assay of cholesterol 7 $\alpha$ -hydroxylase (EC 1.14.13.17).* The hepatic cholesterol 7 $\alpha$ -hydroxylase assay was based on the method of Shefer et al. (30) with slight modification (30, 32) after establishing the optimum condition for guinea pig system (26). The assay medium contained potassium buffer, 0.167 mmol, pH 7.4; MgCl<sub>2</sub>, 11  $\mu$ mol; NADP, 3.0  $\mu$ mol; glucose-6-phosphate, 6.0  $\mu$ mol; and glucose-6-phosphate dehydrogenase, 5 IU. Total substrate of [4- $^{14}$ C]cholesterol and unlabeled cholesterol was 1 mM (0.2 Ci/mol) and solubilized in 0.75 mg of cutscum (isooctylphenoxypolyoxyethylene ethanol, Fisher Scientific Co., Pittsburgh, Pa.). The assay was initiated by the addition of microsomal fraction for 20 min at 37°C in a shaker water bath. The enzymatic reaction was stopped by the addition of methylene chloride:ethyl alcohol (5:1, vol/vol). The 7 $\alpha$ -hydroxycholesterol was extracted, pooled,

and separated by TLC using the solvent system ethyl acetate: benzene (7:3, vol/vol). The activity was expressed as picomoles of 7 $\alpha$ -hydroxycholesterol formed per minute per milligram of microsomal protein. All enzyme determinations were carried out in triplicate. Protein concentrations of the microsomal fraction were determined by Lowry's method.

*Cholesterol absorption.* Cholesterol absorption was determined by a modification of the method of Sodhi (34, 35). Guinea pigs were intubated with a mixture of 5  $\mu$ Ci [1,2- $^3$ H]-cholesterol and 1  $\mu$ Ci [4- $^{14}$ C] $\beta$ -sitosterol and 500 mg of carmine red as a marker. Five daily stool samples were collected after the first appearance of the carmine marker. The  $^3$ H/ $^{14}$ C ratios in fecal neutral sterols were determined in each of these stool samples. Data from the stool samples containing the lowest  $^3$ H/ $^{14}$ C ratio were used for subsequent calculations. The fraction of dietary cholesterol absorbed was calculated from the equation  $(x + y)/x \cdot 100$ , where x and y are the ratios of  $^3$ H/ $^{14}$ C in the administered dose and the fecal samples, respectively.

## RESULTS

*Cholestyramine pretreatment during neonatal period.* Body weight gain and total food intake were similar between the cholestyramine-treated and the control

**TABLE III**  
*Effect of Cholestyramine Pretreatment during Neonatal Period on Bile Acid Pool Size in Guinea Pig\**

Group	Bile acids		
	Bile	Intestinal contents	Total
<i>mg</i>			
Control (n = 5)	1.545 $\pm$ 1.008	19.842 $\pm$ 2.496	21.385 $\pm$ 2.441
Cholestyramine pretreated (n = 3)	2.397 $\pm$ 0.740	28.575 $\pm$ 2.201	30.972 $\pm$ 2.241 $\ddagger$

\* Data shown as mean $\pm$ SEM.

$\ddagger$  0.025 < P < 0.05.

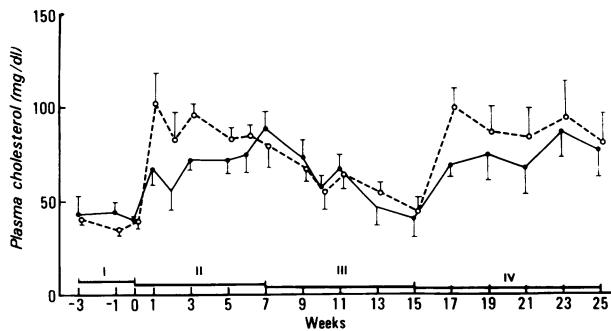


FIGURE 2 Plasma cholesterol levels in the guinea pigs pretreated with cholesterol (week 1–13) during neonatal period (●) and control guinea pigs (○). Phase I, both groups of guinea pigs (week 16) on guinea pig chow diet. Phase II, both groups of guinea pigs (week 19) on 0.25% cholesterol diet. Phase III, both groups of guinea pigs (week 25) on guinea pig chow diet. Phase IV, both groups of guinea pigs (week 33) on 0.25% cholesterol diet.

neonatal guinea pigs. Plasma cholesterol levels during the experimental period are shown in Fig. 1. In phase I, the cholestyramine-treated guinea pigs had significant lower plasma cholesterol levels when compared with the controls. Plasma cholesterol levels of the cholestyramine-pretreated group returned to a level similar to that of control guinea pigs when they were fed a standard guinea pig chow diet. In phases III and IV plasma cholesterol levels of the cholestyramine-pretreated group were lower than controls when they were challenged with a high dietary cholesterol intake (0.5 or 0.25%, respectively). In phase V the difference of cholesterol levels between cholestyramine-pretreated guinea pigs and the normally weaned controls persisted when guinea pigs were fed a standard guinea pig chow diet. In phases VI and VII the difference of cholesterol levels between cholestyramine-pretreated group and the controls remained when they were again challenged with 0.25% cholesterol and subsequently returned to a regular guinea pig chow diet.

Table I shows the fecal bile acid and neutral sterol excretion rates in the neonatal guinea pig. The daily

fecal bile acid excretion rates were significantly higher in the guinea pigs pretreated with cholestyramine than in the controls when both groups were challenged with 0.25% cholesterol-containing chow diet.

There is no difference in the cholesterol absorption between cholestyramine-pretreated guinea pigs and the controls. When challenged with a high cholesterol diet (0.25%), the cholestyramine-pretreated group had a higher hematocrit than that of the control group.

Cholestyramine pretreatment during the neonatal period led to a threefold increase of cholesterol 7 $\alpha$ -hydroxylase activity and no change in the hepatic HMG-CoA reductase activity in the adult guinea pig when fed a 0.25% cholesterol diet. When guinea pigs were fed a regular guinea pig chow diet, the cholestyramine-pretreated group also had a significantly higher cholesterol 7 $\alpha$ -hydroxylase activity (Table II).

Experiments were carried out to examine the bile acid pool sizes in these guinea pigs. The bile acid pool size in the cholestyramine-pretreated guinea pigs was found to be greater than that in the control group (Table III).

**Cholesterol pretreatment.** The body-weight gain and total food intake were similar between the cholesterol-treated and the control neonatal guinea pigs. Fig. 2 shows the plasma cholesterol level in these guinea pigs during the experimental periods. In Phase I (standard guinea pig chow diet, week 13–19) the cholesterol pretreated group had plasma cholesterol levels similar to the control group. In phase II (0.25% cholesterol diet, week 19–25), except for the initial stage of dietary alteration, the cholesterol-pretreated group stayed at a similar plasma cholesterol level to that of the control group. In phase III (wk, 25–33) the plasma cholesterol level of the cholesterol-pretreated guinea pigs returned to a level similar to that of the control group when they were fed standard guinea pig chow. In phase IV (wk, 33–43), when they were rechallenged with a 0.25% cholesterol diet, the plasma cholesterol level of the cholesterol-pretreated group still remained at a level similar to that of the control group.

TABLE IV  
Effect of Cholesterol Pretreatment during Neonatal Period on Bile Acid and Neutral Sterol Excretion in Guinea Pigs\*

Group	Diet	Bile acids	Neutral sterols	Total sterols
Control (n = 4) Cholesterol	Guinea pig chow	10.3±1.5†	3.1±0.4‡	13.3±1.1‡
pretreated (n = 4)	Guinea pig chow	10.8±0.3	3.2±0.3	13.9±0.4
Control (n = 4) Cholesterol	0.25% Cholesterol	10.2±1.5†	20.5±4.2‡	30.8±2.8‡
pretreated (n = 4)	0.25% Cholesterol	16.9±3.8	12.9±2.2	29.8±5.7

\* Data shown as mean±SEM, milligrams per kilogram per day.

†  $P > 0.05$ .

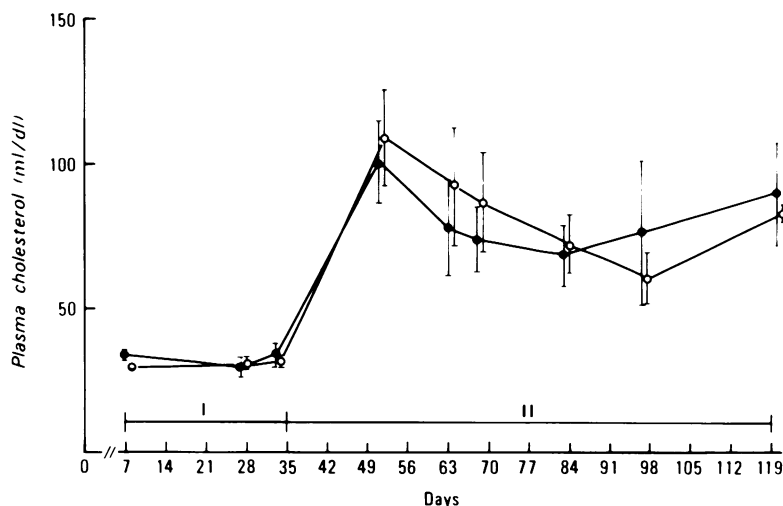


FIGURE 3 Plasma cholesterol levels in the early weaned guinea pigs (●) and normally weaned guinea pigs (○). Phase I, both groups of guinea pigs on guinea pig chow diet. Phase II, both groups of guinea pigs on 0.25% cholesterol diet.

Table IV shows the fecal bile acid and neutral sterol excretion rates in the cholesterol-pretreated neonatal guinea pigs. The fecal bile acid and neutral sterol excretion rates were studied in two periods: (a) standard guinea pig chow diet and, (b) 0.25% cholesterol-containing diet. In both periods, fecal excretion rates of bile acids, neutral sterols, and total steroids were similar in the cholesterol-pretreated guinea pigs and the control group.

When guinea pigs were fed either 0.25% cholesterol diet or a standard guinea pig chow diet, there was no difference in the cholesterol absorption or hematocrit between the cholesterol-pretreated group and the control group.

Cholesterol pretreatment during the neonatal period resulted in no alteration of cholesterol 7 $\alpha$ -hydroxylase activity and a slight increase in hepatic HMG-CoA reductase activity in the adult guinea pig (Table II).

*Age of weaning.* Fig. 3 shows the plasma cholesterol levels of these guinea pigs during the experimental periods. Early weaning had no effect on plasma cho-

lesterol levels either in those guinea pigs fed the regular chow diet or the 0.25% cholesterol diet.

Table V shows the fecal bile acid and neutral sterol excretion rates in early weaned guinea pigs. The fecal excretion rates of neutral sterols were significantly higher in the early weaned than in the normally weaned guinea pigs. However, no change in the excretion rates for bile acids and total sterols occurred when they were fed a standard guinea pig chow diet. When they were fed a 0.25% cholesterol diet, there was no difference in the fecal excretion rates of bile acids, neutral sterols, and total steroids between the early weaned group and the normally weaned group.

Early weaning during the neonatal period resulted in no alteration of cholesterol 7 $\alpha$ -hydroxylase activity and a slight increase in hepatic HMG-CoA reductase activity in the adult guinea pig (Table VI).

*Cholestyramine pretreatment during adulthood.* Table VII shows the fecal bile acid and neutral sterol excretion rates in the adult guinea pig 6 wk after administration of cholestyramine. The daily fecal bile acids

TABLE V  
Effect of Early Weaning on Bile Acid and Neutral Sterol Excretion in Guinea Pigs\*

Group	Diet	Bile acids	Neutral sterols	Total sterols
Control (n = 4)	Guinea pig chow	10.9 $\pm$ 1.3	2.7 $\pm$ 0.2	13.6 $\pm$ 1.4
Early weaned (n = 4)	Guinea pig chow	12.6 $\pm$ 1.7 $\ddagger$	3.5 $\pm$ 0.2 $\S$	15.8 $\pm$ 2.2 $\ddagger$
Control (n = 4)	0.25% Cholesterol	18.4 $\pm$ 1.7	11.6 $\pm$ 1.5	30.0 $\pm$ 2.5
Early weaned (n = 3)	0.25% Cholesterol	23.2 $\pm$ 0.3 $\ddagger$	13.5 $\pm$ 3.6 $\ddagger$	32.8 $\pm$ 1.1 $\ddagger$

\* Data shown as mean $\pm$ SEM, milligrams per kilogram per day.

$\ddagger$   $P > 0.05$ , NS.

$\S$   $0.02 < P < 0.025$ .

TABLE VI  
Hepatic HMG-CoA Reductase Activity and Cholesterol 7 $\alpha$ -Hydroxylase Activity in Adult Guinea Pigs\* Weaned on Day 7 or 24

Group	Diet	Cholesterol 7 $\alpha$ -hydroxylase	HMG-CoA reductase
<i>pmol/min/mg</i>			
Early weaned	0.25% Cholesterol	10.6 $\pm$ 1.6 $\ddagger$	5.6 $\pm$ 0.6 $\S$
Normally weaned	0.25% Cholesterol	9.0 $\pm$ 2.2	7.0 $\pm$ 0.2

\* Data shown as mean $\pm$ SEM.

$\ddagger$   $P > 0.05$ , NS.

$\S$   $0.025 < P < 0.05$ .

and total steroids were significantly lower in the pre-treated group than in the control group. Administration of cholestyramine during the adulthood leads to a reduction of cholesterol 7 $\alpha$ -hydroxylase activity 6 wk after the prior treatment (Table VIII).

## DISCUSSION

The results of our experiments show that neonatal manipulation influences cholesterol metabolism in adult guinea pigs. Pretreatment of neonatal guinea pigs with cholestyramine resulted in: (a) a lower plasma cholesterol level; (b) an increased excretion rate of fecal bile acids and total steroids; (c) an expanded bile acid pool; (d) an increased activity of cholesterol 7 $\alpha$ -hydroxylase; and (e) no change in the hepatic HMG-CoA reductase activity or in cholesterol absorption in the test group as compared with the control group when both groups were challenged with a high cholesterol diet (Fig. 1, Tables II and III). Cholesterol pretreatment during the neonatal period resulted in no change in the plasma cholesterol level except for the initial stage of the high dietary cholesterol challenge. Cholesterol pretreatment produced no alteration in the fecal excretion of steroids or in cholesterol 7 $\alpha$ -hydroxylase activity and no change in cholesterol absorption but a slight increase in the activity of HMG-CoA reductase (Fig. 2, Table II). Early weaning resulted in no alteration in the

plasma cholesterol level when challenged with a high cholesterol diet. Early weaning did not influence the fecal excretion of sterols or cholesterol 7 $\alpha$ -hydroxylase activity, but resulted in a slight decrease in hepatic HMG-CoA reductase activity in the early weaned guinea pigs (Fig. 3, Tables V and VI).

Treatment of guinea pigs with cholestyramine during adulthood resulted in a reduction of daily fecal excretion of bile acids, total steroids, and cholesterol 7 $\alpha$ -hydroxylase activity after 6 wk for both groups of guinea pigs on the standard diet (Tables VII and VIII). This change was small and in an opposite direction to our finding on cholestyramine-pretreated neonatal guinea pigs that resulted in higher bile acid excretion and cholesterol 7 $\alpha$ -hydroxylase activity even after a long period for these guinea pigs on standard diets (Tables I and II). However, the current finding will lead to the possibility that only perturbation in early life may produce "permanent" effects in the development of metabolic systems.

Previous investigators studied rats to establish whether or not high cholesterol intakes in neonatal rats would serve to maintain cholesterol anabolic and catabolic processes so that in adulthood serum cholesterol homeostasis could be maintained at low levels during a cholesterol challenge (7, 8). The adult male rats when fed cholesterol as neonates, had lower serum

TABLE VII  
Effect of Cholestyramine Pretreatment during Adulthood on Bile Acid and Neutral Sterol Excretion in Guinea Pigs\*, $\ddagger$

Group	Diet	Bile acids	Neutral sterols	Total sterols
Control (n = 3)	Guinea pig chow	12.695 $\pm$ 0.754	2.044 $\pm$ 0.246	14.738 $\pm$ 0.851
Cholestyramine pretreated (n = 4)	Guinea pig chow	8.374 $\pm$ 1.086 $\S$	1.884 $\pm$ 0.117 $\parallel$	10.257 $\pm$ 1.023 $\nparallel$

\* Data shown as mean $\pm$ SEM, milligrams per kilogram per day.

$\ddagger$  Experiment was carried out 6 wk after cholestyramine (1.1%) treatment.

$\S$   $0.025 < P < 0.05$ .

$\parallel$   $P > 0.05$ , NS.

$\nparallel$   $0.02 < P < 0.025$ .



TABLE VIII  
Effect of Cholestyramine Pretreatment during Adulthood on Hepatic HMG-CoA Reductase and Cholesterol 7 $\alpha$ -Hydroxylase Activity in Guinea Pigs\*

Group	Diet	Cholesterol 7 $\alpha$ -hydroxylase	HMG-CoA reductase
<i>pmol/min/mg</i>			
Control (n = 3)	Guinea pig chow	13.037 $\pm$ 1.795	5.078 $\pm$ 1.840
Cholestyramine pretreated (n = 4)	Guinea pig chow	7.811 $\pm$ 0.401 $\ddagger$	2.453 $\pm$ 0.336 $\S$

\* Data shown as mean $\pm$ SEM.

$\ddagger$  0.025 < P < 0.05.

$\S$  P > 0.05, NS.

cholesterol levels than rats who consumed little or no cholesterol during infancy. Glueck et al. (11) investigated variations in the intake of dietary cholesterol in infants during the 1st yr of life to establish whether or not the intake of dietary cholesterol in early infancy could determine the subsequent response to a dietary cholesterol challenge in later infancy. The plasma cholesterol concentrations in the infants of 1 yr of age did not appear to be affected by an antecedent low or moderate cholesterol intake. Furthermore, Hodgson et al. (10) demonstrated that children who received a low cholesterol diet in early infancy had a lower plasma cholesterol level at 7–12 yr of age. Thus, the human studies did not support the findings in rats. This disparity in the results between human and animal data may perhaps be explained by differences in experimental design such as timing and duration of suckling period and the life span. However, the interesting evidence in rats is not sustained by the current studies in the guinea pig. Hence, the present findings could provide some explanation for the results seen in human studies.

The current study suggests that enhancing cholesterol catabolism by cholestyramine in early infancy can improve the ability to handle a cholesterol challenge in adulthood. This is in agreement with the finding by Reiser et al. (9) that the activity of HMG-CoA reductase was permanently altered by cholesterol-free semipurified diets fed during early infancy in rats.

Our results provide evidence that possibly permanent alteration of cholesterol 7 $\alpha$ -hydroxylase activity occurred in the cholestyramine-pretreated guinea pigs. It is likely that the specific cytochrome P-450 involved in the cholesterol 7 $\alpha$ -hydroxylation is under the influence of bile acids or their metabolites and that the control mechanisms may be similar to that of androgenic metabolic enzymes (19).

Early manipulation by alteration of dietary cholesterol or by bile acid sequestration resulted in no deleterious effects on the growth or other physiological parameters of the guinea pig. These studies provide

a useful model for the study of the perturbation of cholesterol homeostasis in the neonate. Further investigation, such as studies of the role of endocrine factors in the development of the cholesterol homeostatic mechanism, is required.

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