addition of sodium cyanide reduced $^{33}P_i$ distribution ratios after 40 minutes of incubation to 1 (mean ± standard deviation: 1.1 ± 0.6 for controls and 0.9 ± 0.2 for FHR patients), results indicating that P_i uptake was energy-dependent as well as concentrative in both groups. Since the ratio of $^{33}P_i$ to total ^{33}P after 40 minutes of incubation was the same in control tissue (0.8 ± 0.04) as in that from patients with FHR (0.9 ± 0.1), the reduced P_i uptake observed in FHR is not explained by a more rapid rate of conversion of P_i to organic phosphate derivatives in tissue from affected patients.

When the uptake of P_i was examined in mucosa from controls over a 1500-fold range of substrate concentration (0.002 to 3.0 m*M*), saturation kinetics were observed (Fig. 3A). The sharp break in the double reciprocal plot suggests the presence of two transport systems for P_i: a high-affinity system (I) identified at substrate concentrations below 0.03 m*M*, and a low-affinity system (II) observed at substrate concentrations greater than 0.03 m*M*. These systems have apparent Michaelis constants that differ by a factor of 100.

Kinetic analyses were also attempted in the patients with FHR (Fig. 3B). Insufficient tissue was obtained for conclusive results, but the data suggest that the high-affinity system has been lost in the affected male in pedigree C and impaired in the two heterozygous females in this kindred. In pedigree T, kinetic analyses failed to show any significant deviation from normal.

These data provide the first in vitro demonstration of a transport defect for P_i in patients with FHR. The observed defect, although incomplete in all affected patients, was of considerably greater magnitude in the hemizygous male in pedigree C than in his heterozygous female relatives. Such results are consistent with the X-linked dominant pattern of inheritance in FHR and with previous data on P_i reabsorption in the renal tubule (*9*). Mucosal P_i uptake was much less in the affected male in pedigree C than in the two males in pedigree T. This difference, which could not be attributed to previous treatment, may well be a manifestation of genetic heterogeneity so commonly observed in other inherited metabolic diseases. These observations, in conjunction with those reported by Arnaud *et al.* (*5*) and Glorieux and Scriver (*9*), lend strong support to the hypothesis that a primary defect in both renal and intestinal transport of P_i underlies FHR. The molecular nature of this long-elusive defect should now be open to direct examination.

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Cholesterol: Vitamin C Controls Its Transformation to Bile Acids

Abstract. *Cholesterol accumulates in the blood serum and in the liver of guinea pigs with chronic latent vitamin C deficiency. The reason for this is the decreased rate of transformation of cholesterol to bile acids in the liver of animals deficient in vitamin C. A significant direct correlation exists between the vitamin C concentration in the liver and the rate of cholesterol transformation to bile acids.*

There is disagreement about the effects of vitamin C on cholesterol metabolism. Some investigators have described hypocholesterolemic and atheroma-reducing effects of vitamin C in rabbits and rats (*1*), but others deny such an effect (*2*). Rabbits and rats synthesize ascorbic acid (vitamin C), and their metabolic response to massive doses of exogenous vitamin C differs from that of the organisms, including man, which are not able to synthesize ascorbic acid (*3*). Other investigators have described disturbances of cholesterol metabolism, in guinea pigs and monkeys, caused by acute scurvy; for example, there were altered cholesterol concentrations in blood and tissues (*4*), increased cholesterol biosynthesis (*5*), decreased cholesterol catabolism (*6*), and atheromatous changes in their blood vessels (*7*). Many other researchers did not find similar changes [see (*8*)]. The contradictions in results may be explained by the fact that acute scurvy is, metabolically, a very complicated state, primarily evoked by vitamin C deficiency, but secondarily by other factors, such as a refusal of food, decrease in body weight, negative nitrogen balance, hemorrhaging, and so forth. Moreover, the model of acute scurvy does not reflect the nutrition situation in developed countries, where

acute scurvy occurs rarely as compared to widespread latent vitamin C deficiency.

We have elaborated a new model of vitamin C deficiency, the model of chronic latent hypovitaminosis C (*8*), which is better defined metabolically than is acute scurvy. Increased amounts of cholesterol accumulate in the tissues of guinea pigs with chronic latent hypovitaminosis C (*8*). Studies with cholesterol labeled in the nucleus ([4-^{14}C]cholesterol) or on the side chain ([26-^{14}C]cholesterol) showed that this was due to slower conversion of cholesterol to bile acids (*9*). My results presented here show the rate of cholesterol turnover to bile acids in guinea pigs with latent vitamin C deficiency, and its correlation with the concentration of vitamin C in the liver.

Fifty-two male guinea pigs (initial weight, 330 g), with free access to a diet causing scurvy (*10*), were divided into a control group and a group deficient in vitamin C. The control animals each received 10 mg of ascorbic acid by mouth every 24 hours. To induce vitamin C deficiency, the animals were fed the diet for 14 days, and were then given, orally, a maintaining dose of 0.5 mg of ascorbic acid every 24 hours (*8*). After 3 months, the animals were all given an intraperitoneal injection of [26-^{14}C]-cholesterol (Radiochemical Centre, Amersham; specific activity, 55 mc/mmole), emulsified with Tween 20 in saline, in a dose of 1.2 μc per 100 g of body weight. Immediately afterward, three of the animals in each group were placed in ventilated cages and their 24-hour output of [^{14}C]CO_2 was measured (*11*). The guinea pigs were then decapitated. The vitamin C concentration in their liver and spleen was determined (*12*), the total cholesterol concentration in their serum and liver was determined with the Liebermann-Burchard reaction, and ^{14}C activity was determined in a chloroform-methanol extract of liver with a scintillation spectrometer (Mark I, Nuclear Chicago). As the ^{14}C activity of liver extract was identical with the activity of [^{14}C]digitonides isolated from this extract, the former amount was used as an estimate of the specific activity of cholesterol in liver. The same determinations were made from successive groups of three to four control animals, and three to four guinea pigs deficient in vitamin C killed at intervals of 1, 3, 5, 7, 9, and 11 weeks after receiving labeled cholesterol. The rate of transformation of cholesterol to bile acids was determined as the ratio of disintegrations in the [^{14}C]CO_2 expired in 24 hours to the specific activity of the cholesterol in the liver (the organ in which cholesterol is converted to bile acids). A similar method of determining the rate of cholesterol transformation to bile acids was also used successfully in man (*13*).

Table 1. Effect of latent vitamin C deficiency on the concentration of vitamin C and cholesterol, and on the rate of cholesterol transformation to bile acids. Results are expressed as means ± standard error of the mean. The number of animals observed for each determination was 26, unless indicated otherwise in parentheses. Statistical significance of results between control and vitamin C–deficient animals was $P < .001$, unless otherwise indicated.

Animal	Vitamin C (mg/100 g) in		Cholesterol in		Cholesterol → bile acids (mg/24 hours per 500 g of body weight)
	Liver	Spleen	Serum (mg/100 ml)	Liver (mg/100 g)	
Control	8.2 ± 0.4	21.6 ± 0.8	126 ± 9	359 ± 15*	11.8 ± 0.6 (23)
Deficient in vitamin C	1.6 ± 0.1	4.7 ± 0.2	218 ± 17	443 ± 19*	8.3 ± 0.4 (21)

* $P < .002$.

Latent hypovitaminosis C did not significantly influence body growth. These animals deficient in vitamin C consumed food normally and had a normal outward appearance. However, the vitamin C concentration in their liver and spleen was significantly lower than that in the control group (Table 1). The total cholesterol concentration in the serum and the liver of guinea pigs deficient in vitamin C rose significantly (Table 1), in agreement with our earlier findings (*8*). There was a decrease in [^{14}C]CO_2 output, and a corresponding decrease in specific activity of cholesterol in the liver from the first week after injection of labeled cholesterol, so that the ratio of these two values, expressing the rate of cholesterol transformation to bile acids, was constant in the two groups from the first to the last week of the experiment. Latent hypovitaminosis C significantly reduced the rate of cholesterol transformation to bile acids (Table 1).

A significant negative correlation was found between the concentration of vitamin C in the liver, and the concentration of cholesterol in the serum and liver ($P < .001$), that is, the higher the vitamin C concentration, the lower the cholesterol concentration in the serum and liver. The cholesterol concentration in the serum and liver of guinea pigs is probably controlled, therefore, by the rate of cholesterol conversion to bile acids, as there is a significant negative correlation between these two parameters ($P < .001$), that is, the greater the rate of the conversion process, the lower the cholesterol concentration in the serum and liver. Cholesterol is converted to bile acids in the liver, and the rate of this process seems to depend on the vitamin C concentration in the liver cells of guinea pigs, as there is a significant linear correlation between the rate of transformation of cholesterol to bile acids and the concentration of vitamin C in the liver ($P < .001$).

It should be emphasized that these results were obtained in guinea pigs with latent vitamin C deficiency, and not in animals with acute scurvy, which refuse food, lose weight, develop hemorrhage, and so forth. In the model of latent hypovitaminosis C, the only variable is a significant drop in the concentration of vitamin C in tissues. The decrease in the concentration of vitamin C in the liver causes a decrease in the rate of the conversion of cholesterol to its main catabolic product, the bile acids, with the result that cholesterol eventually accumulates in the serum and liver of the animals deficient in vitamin C. Conversely, restoration of vitamin C concentration in deficient guinea pigs stimulates the oxidation of [26-^{14}C]cholesterol to [^{14}C]CO_2 (*14*). The conversion of cholesterol to bile acids involves several hydroxylation reactions on the cholesterol nucleus and on the side chain. Because of the known function of ascorbic acid in hydroxylation reactions, we hypothesized that ascorbic acid is essential for the hydroxylation of cholesterol (*9*). The results of experiments (*15*) indicate that the action of ascorbic acid on the catabolism of cholesterol may be mediated by its action on the concentration of cytochrome P-450 in the liver microsomes. Another possibility is that ascorbic acid stimu-

lates the sulfation of cholesterol (*16*).

The relationship of the concentration of cholesterol in serum and vitamin C intake has been demonstrated in humans (*17*). If our conclusions are applicable to the human organism, we shall probably find that latent hypovitaminosis C can cause hypercholesterolemia, and that it may also play some role in the pathogenesis of atherosclerosis.

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Development of Grooming in Mice with Amputated Forelimbs

Abstract. *Face grooming sequences that involve coordination of the shoulders, tongue, and eyes develop remarkably normally in inbred mice with one or both forelimbs amputated from birth. This indicates endogenous control with a strong genetic component. Evidence for the maturational expression of "sensory expectations" was also obtained.*

The extent to which the development and integration of species-characteristic movements depend on endogenous versus exogenous programming has received much attention in recent years (*1*). Experiments on a variety of invertebrate and vertebrate species suggest that central motor programs which are under strong genetic control may be more important than previously supposed (*2*). However, other data indicate that peripheral and exogenous feedback are often critical for the development, if not the maintenance, of integrated movement (*3*), and generalizations must be of a limited nature at this time. Most strikingly, there are few data on the emergence and functional coupling of complex yet relatively stereotyped species-characteristic movement patterns in mammals deprived of normal feedback experience from infancy.

Previous studies have suggested that face grooming in rodents might be usefully studied in this respect. Face grooming is a common species-characteristic (and strain-characteristic) movement pattern with readily definable components (*4*). Systematic maturational stages can be plotted from the abbreviated and rudimentary single face swipes of neonatal and fetal animals (*4, 5*), and in adults the basic patterning demonstrates considerable, although not exclusive, central control (*4*). Since mature face grooming involves a complex sequential pattern of contacts of the forepaws with the face and tongue it might reasonably be anticipated that experience resulting from these contacts is critical for the development, if not the maintenance, of the adult behavior. Therefore, I performed a series of experiments in which one or both forelimbs in inbred mice were painlessly amputated at birth, and observed the developmental patterning of grooming activities. The data indicate a high degree of endogenous control.

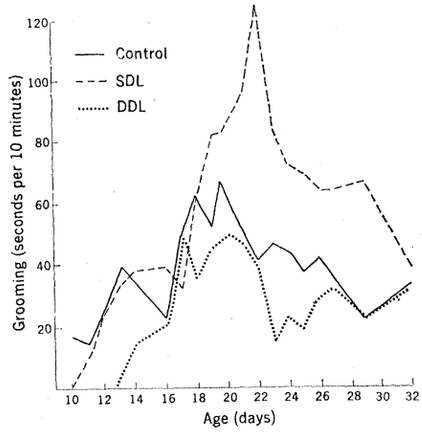

Fig. 1. Development of grooming in an observation cage by a litter of DBA/2J mice; *SDL*, single forelimb removed ($N = 2$); *DDL*, both forelimbs removed ($N = 1$); control ($N = 3$).

Three litters of DBA/2J and three litters of C57/6J mice were studied (seven to nine mice per litter). In each litter one to three infants had one forelimb amputated on postnatal day 1 [single delimb (SDL)], one to three infants had both forelimbs amputated [double delimb (DDL)], and one to three infants served as controls. Surgery was performed by anesthetizing the mice with ice and a small dose of Metofane (Pitman-Moore), sectioning one or both forelimbs between the elbow and the shoulder with a scalpel, and treating the limbs with ferric subsulfate to block bleeding. I removed an entire litter from the mother at one time and except for the actual surgery treated them identically, including the application of ferric subsulfate. They were returned to the mother simultaneously. In this way none of the infants was rejected, and only two of the operated animals died before the termination of the experiment.

Each mouse was observed at regular intervals (1 to 5 days) until it was 30 days old. Periodic observations were then continued over a period of 3 to 5 months. Sample films made at 32 feet/sec (1 foot = 0.3 m) permitted detailed single frame analysis of grooming and related movement patterns. Grooming movements could be separated without ambiguity from other behaviors by a combination of the animal's characteristic sitting posture, tongue movements, and pattern of shoulder and upper arm movements (*4*). Each mouse observed displayed obvious grooming behavior; its basic postnatal development is thus not dependent on normal contacts between the paws and face.

The broad profile of grooming probability during postnatal development was also similar for control and operated animals. For example, normal