serums (*2*) containing antibody to HSV type 2 were confusing. If correctly absorbed human cancer serums should contain a specific tumor antibody reacting only with specific herpesvirus-coded nonvirion antigens, this finding would also help to prove that the tumor cell contains herpesvirus genetic information and indicate an etiological relationship between the HSV and the tumor.

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## Familial Hypophosphatemic Rickets: Defective Transport of Inorganic Phosphate by Intestinal Mucosa

Abstract. *Uptake of inorganic phosphate is impared in intestinal mucosa from hemizygous males and heterozygous females with X-linked familial hypophosphatemic rickets. Considerable intrafamilial and interfamilial variation in uptake of inorganic phosphate is observed in affected patients. Uptake by normal mucosa is concentrative and energy-dependent, and is mediated by at least two systems with widely different affinities. These results lend direct support to the thesis that the primary metabolic disturbance in this disease results from impaired transport of inorganic phosphate in kidney and gut.*

A reduced serum concentration of inorganic phosphate ($P_i$) is the most constant biochemical hallmark of familial hypophosphatemic rickets (FHR), a human X-linked dominant trait. Affected hemizygous males exhibit hypophosphatemia and severe rickets; heterozygous females tend to have a higher serum $P_i$ concentration and less severe bone disease (*1*). For more than 30 years investigators have sought a unifying biochemical and genetic defect which would explain the findings that characterize this disorder: hypophosphatemia, hyperphosphaturia, reduced intestinal absorption of calcium and phosphorus, and rickets (*2*).

Two conflicting theories of pathogenesis have evolved. One view holds that a primary defect in endogenous conversion of vitamin D to its active metabolite is responsible for intestinal malabsorption of calcium (*3*); this in turn leads to secondary hyperparathyroidism resulting in decreased renal tubular reabsorption of $P_i$, hypophosphatemia, and rickets. Recent evidence has weakened this argument. Hadded *et al.* reported that the concentration of 25-hydroxycholecalciferol in serum of patients with FHR was normal (*4*). Furthermore, Arnaud *et al.* (*5*) and Roof *et al.* (*6*) showed that serum parathyroid hormone concentrations were normal or reduced in affected patients, not increased.

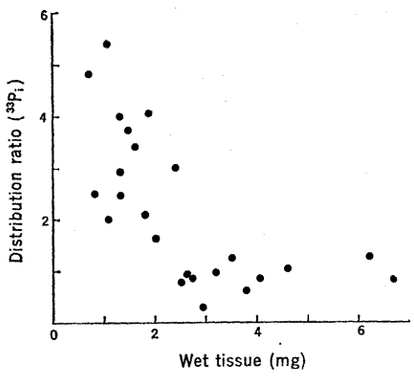



Fig. 1. Relation between weight of jejunal biopsy specimens and distribution ratio for radioactive inorganic phosphate ($^{33}P_i$). Specimens were obtained from eight controls and were incubated for 20 or 40 minutes at a $^{33}P_i$ concentration of 0.003 m*M*. Distribution ratios, corrected to 20 minutes of incubation, are expressed as counts per minute of $^{33}P_i$ per milliliter of cell water divided by counts per minute of $^{33}P_i$ per milliliter of incubation medium.

The alternate view proposes a primary membrane transport defect for $P_i$ in the proximal renal tubule (*7*). This hypothesis has been challenged because it fails to explain either the capacity of calcium infusion to reverse the hyperphosphaturia (*8*), or the intestinal malabsorption of calcium. Glorieux and Scriver (*9*) have resolved the objection raised by calcium infusion studies. Their data indicate that renal tubular reabsorption of $P_i$ is normally accomplished by two discrete mechanisms. The first is a parathyroid hormone–sensitive system responsible for reclamation of about two-thirds of filtered $P_i$; this system is defective in FHR. The second is a parathyroid hormone–resistant but calcium-responsive system that mediates reabsorption of the remaining $P_i$ and accounts for the augmented $P_i$ reabsorption observed in FHR patients during calcium infusion.

Intestinal calcium malabsorption in FHR can also be explained if one proposes that the functionally similar jejunal mucosal cell shares with the renal tubule cell the defect in phosphate transport (*10*). Such a primary disturbance in intestinal absorption of $P_i$ might lead to the formation of insoluble calcium-phosphate complexes in the intestinal lumen, and, hence, to malabsorption of calcium. In vivo evidence for impaired intestinal absorption of $P_i$ in FHR is derived from metabolic balance studies (*11*) and estimation of serum $P_i$ during an oral phosphate tolerance test (*12*). Such in vivo studies, however, cannot distinguish primary intestinal malabsorption of $P_i$ from malabsorption secondary to a defect in calcium transport. Therefore, the present in vitro studies of intestinal $P_i$ transport were undertaken to examine $P_i$ transport directly.

Inorganic phosphate flux and accumulation by rat intestine in vitro has been studied (*13*), but, to our knowledge, in vitro analysis of $P_i$ transport

by human intestine has not been reported previously. We examined $P_i$ uptake by jejunal mucosa from five adult normal volunteers and from five adult patients with FHR (two females and one male in pedigree C; two males in pedigree T). Four of these patients had not been treated with either vitamin D or phosphate supplements for several years before study; the fifth had received vitamin D until 3 weeks before biopsy.

Peroral specimens of jejunal mucosa, obtained from the region of the ligament of Treitz with a hydraulic biopsy tube (*14*), were placed in chilled, $P_i$-free saline-bicarbonate buffer (*p*H 7.4) containing 1.25 m*M* $Ca^{2+}$ for no more than 45 minutes before transport studies were initiated. Single specimens were then incubated for 10 to 40 minutes at 37°C in fresh buffer containing 0.003 m*M* $P_i$ as $[^{33}P]NaH_2PO_4$. Uptake studies were terminated by homogenizing the tissue in 8 percent trichloroacetic acid. Portions of trichloroacetic acid supernatants from incubation medium and tissue were extracted for $P_i$ by a modification of the method of Lippman and Tuttle (*15*). Distribution ratios (defined as the ratio of radioactivity per milliliter of cell water to radioactivity per milliliter of incubation medium) for total $^{33}P$ and for $^{33}P_i$ were calculated by using previously described methods (*16*).

Uptake of $P_i$ by mucosa from normals was inversely proportional to tissue weight (Fig. 1). This result, which was not noted in previous studies of amino acid or glucose uptake by jejunal mucosa (*14, 17*), suggests that the increased weight of submucosal tissue present in the larger specimens produces an artifactual lowering of the distribution ratio. Since biopsy specimens weighing more than 2 mg routinely yielded a distribution ratio of 1, only pieces weighing less than 2 mg were analyzed in the subsequent studies.

Uptake of $P_i$ by mucosa from controls increased linearly during the 40-minute interval, reaching a maximal mean distribution ratio of 7.4 (Fig. 2A). Uptake of $P_i$ by mucosa from patients with FHR was considerably less than that in controls at each of the three time points tested, these differences being statistically significant at 20 minutes ($P < .05$) and 40 minutes ($P < .005$). Although no significant difference in mean $P_i$ uptake was noted between female and male controls or patients, mucosa from the affected male in pedigree C accumulated much less $P_i$ than did tissue from his affected mother and sister (Fig. 2B), or did that from the two affected males in pedigree T whose values differed significantly from controls only at 40 minutes. In controls and patients,

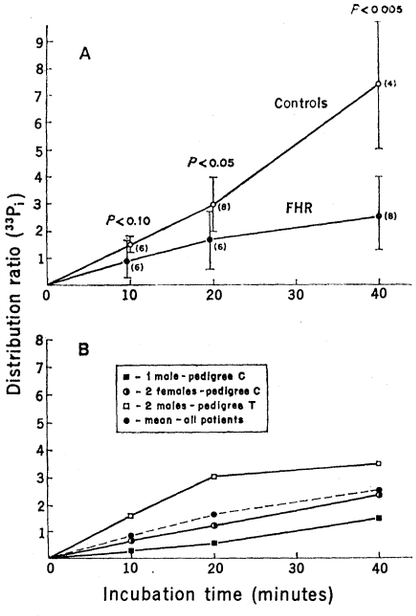
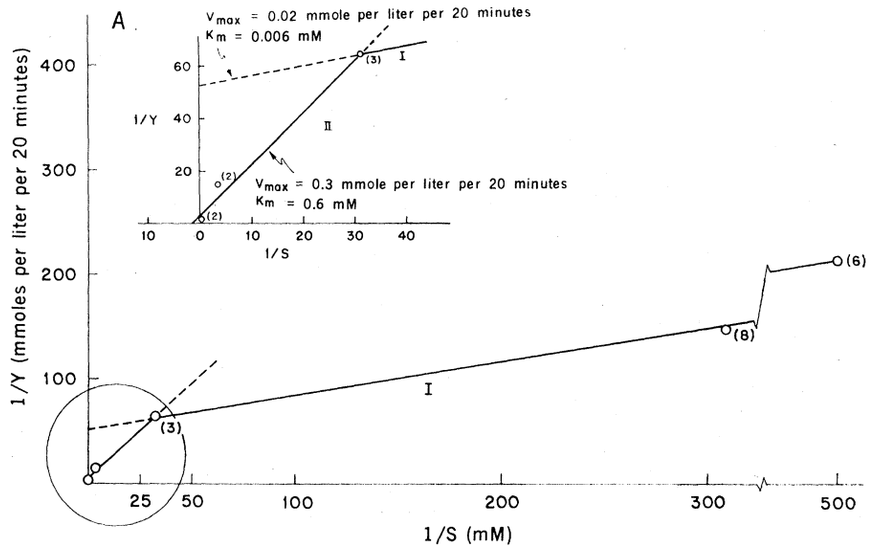



Fig. 2. Time course of uptake of radioactive inorganic phosphate ($^{33}P_i$) by human jejunal mucosa from five controls and five patients with familial hypophosphatemic rickets (FHR). Biopsies were incubated with 0.003 m*M* $^{33}P_i$ in Krebs-Ringer bicarbonate buffer (*p*H 7.4) at 37°C. (A) Means ± 1 standard deviation are shown; the number of determinations is in parentheses. Statistical significance of differences was determined by Student's *t*-test. (B) $^{33}P_i$ uptake in pedigrees C and T. Values for females in pedigree C and males in pedigree T represent the means of closely agreeing separate studies in two affected patients in each kindred.



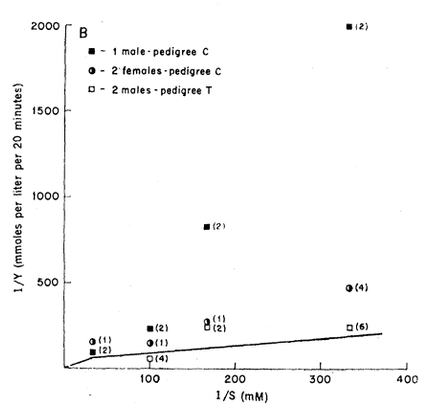




Fig. 3. (A) Double reciprocal plot of $^{33}P_i$ transport by jejunal mucosa in normal controls. Biopsy specimens were incubated for 10 to 40 minutes with substrate concentrations (*S*) from 0.002 to 3 m*M*. A diffusion constant could not be calculated by the method of Akedo and Christensen (*17*) because diffusion equilibrium had already been achieved at the shortest incubation interval used (10 minutes). Therefore, mediated velocity (*Y*) was calculated by subtracting 1 from the observed distribution ratios for $^{33}P_i$ at each substrate concentration (*18*). Values shown are the means, and the number of determinations is in parentheses. No correction was made in calculation of the Michaelis constant ($K_m$) and maximum velocity ($V_{max}$) of the low-affinity system (*II*) for the contribution of the high-affinity system (*I*), and vice versa. (B) Double reciprocal analysis in patients with FHR. The solid line depicts the two transport systems defined in normals (A). Values shown are the means, and the number of determinations is in parentheses. The values for females in pedigree C and males in pedigree T are again the means of closely agreeing studies in two affected patients.

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