than 1 PFU per ganglion). Typical ultrastructural features of the infection in neuronal somas and satellite cells from ganglia processed 4 days after infection are presented in Fig. 1. Morphologically complete and incomplete virions are present in both cell types. Figure 2 depicts a section from a similar ganglion stained by immunofluorescent methods with HSV-specific rabbit antibody. Viral antigen is present in several neuronal somas and satellite cells.

Animals that had recovered from paralysis for periods of 2 weeks to 3 months were killed, and the spinal ganglia associated with sciatic nerves were explanted and maintained as organ cultures on glass cover slips in 2-ounce French square bottles at 37°C, standard methods and media (*8*) being used. Supernatant fluids from the organ cultures were assayed on RK_{13} cells for infectious virus at 7 and 14 days after explantation. Similar ganglia were ground in a Ten Broeck homogenizer and directly assayed for virus. In these latter tissues, infectious virus could not be found. However, HSV was detected in supernatant fluids after ganglia had been cultured in vitro (Table 1). Thus some form of the virus was present in ganglia of essentially all mice tested at the time of explant, and it had persisted for at least 4 months after the time of initial infection (the maximum time interval that we have examined). In addition, ganglia contralateral to the inoculated foot from about one half the animals supported viral replication in vitro. Extensive morphologic study of ganglia from recovered mice with ultrastructural and immunofluorescent techniques has failed to reveal evidence that virions or virus-specified antigens persist. An electron micrograph of neuronal cytoplasm and a supporting cell in a ganglion explanted from a latently infected mouse and maintained for 10 days in vitro is presented in Fig. 3. Many morphologically complete virions are seen. Finally, it should be noted that these "recovered" mice possess significant levels of neutralizing antibody (a 1:25 dilution of serum neutralizes $\geqslant 75$ of 100 PFU of virus).

To determine whether other tissues in the nervous system also harbor latent virus, portions of left and right sciatic nerve trunks, thoracic spinal cord, and medulla oblongata from 13 mice with latent infection (six recovered for 3 months and seven for 2 weeks) were cultured as organ cultures by use of methods identical to those used for spinal ganglia. In no case were we able to recover infectious virus. In additional experiments, similar tissues from 11 mice that had been recovered for 2 weeks were minced and maintained on monolayer cultures of RK_{13} cells for 1 week. These cultures failed to reveal the presence of virus. When spinal ganglia from the same mice were cultured in this fashion, virus was replicated in ganglia and the RK_{13} cells were destroyed. From these results, we tentatively conclude that, in this system, latent infection is limited to spinal ganglia.

The immunologic relation between virus recovered from ganglia and the parental virus was compared by neutralization kinetics (*9*) by using the specific antiserum used in immunofluorescent studies. This serum possessed a neutralization constant of 74 ml/min at 37°C against the homologous virus, and the mean neutralization constant against six separate isolates from spinal ganglia was 78 ml/min (standard deviation, 12.6). Thus all virus isolates tested have similar if not identical neutralizable antigens.

Cell types involved and the physical state of virus in latently infected ganglia are unknown, and maintenance of infection by continued replication of a small amount of infectious virus is a possibility that certainly cannot be ruled out by our data. As noted above, our extensive searching of ganglia in latently infected mice by means of ultrastructural and immunofluorescent methods has given no evidence for the presence of viral-specific products in any cell. Thus, it is also possible that the viral genome is maintained in a subviral state in ganglionic neurons or supporting cells.

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Serum Parathyroid Hormone in X-Linked Hypophosphatemia

Abstract. *Serum immunoreactive parathyroid hormone (IPTH) is normal in patients with X-linked hypophosphatemic rickets who are not treated with phosphate salts. Phosphate raises IPTH in these patients. Endogenous IPTH does not influence the existing defect in tubular reabsorption of phosphate in male patients.*

X-linked hypophosphatemic rickets is a dominant disease in which a low concentration of orthophosphate in plasma is the most constant index of the phenotype (*1*). In males, the mutant allele also causes bone disease, while in carrier females it is variably expressed, some showing only hypophosphatemia, while others have bone disease as well. Impairment of renal tubular reabsorption of phosphate has long been recognized as another important phenotypic feature of this trait.

Two opposing views have evolved concerning the pathogenesis of X-linked hypophosphatemia. One opinion favors a primary disorder of intestinal calcium absorption due to impaired endogenous conversion of vitamin D (*2*) to normal biologically active polar derivatives (*3*). This would result in secondary hyperparathyroidism, renal loss of phosphate, hypophosphatemia, and bone disease as an ultimate consequence.

The well-known suppressive effect of intravenous calcium infusion upon the hyperphosphaturia (*4*) appeared to support this hypothesis. The failure of X-linked hypophosphatemia to respond

Table 1. Response of serum IPTH to change in dietary phosphate in patients with X-linked hypophosphatemic rickets and acquired secondary hyperparathyroidism.

Subject	Sex	Dietary regimen					
		Phosphate (3 g/day); vitamin D_2 (< 25,000 unit/day)			Reduced phosphate (1 g/day); vitamin D_2 (100,000 unit/day)		
		Serum values		IPTH (μl eq/ml)	Serum values		IPTH (μl eq/ml)
		P*	Ca*		P*	Ca*	
M.C.	F	5.4	8.9	290	3.9	10.4	40
L.A.	M	5.0	9.3	100	3.8	11.4	67
E.M.	M	4.4	7.4	750	3.9	10.6	43
	Mean	4.9	8.5	380	3.9	10.8	50

* Values in milligrams per 100 milliliters.

unambiguously to treatment with 25-hydroxycholecalciferol (*5*) is against defective hepatic biosynthesis of vitamin D metabolites; a failure to synthesize the derivative active in the intestine (*3*) has not yet been examined.

The other opinion favors a primary disorder of phosphate transport in the renal tubule (*6*), and perhaps also at the general cellular level (*7*). The ability to heal the bone disease more effectively with phosphate administration alone (*8*), or with phosphate and modest supplements of vitamin D (*9*), than with very large amounts of vitamin D alone in either its precursor or hydroxylated forms (*5*) emphasizes the apparent primacy of the phosphate leak in the pathogenesis of the complete phenotype. If the calcium-transport hypothesis, including its essential codicil about secondary hyperparathyroidism, could be eliminated by direct measurements of circulating parathyroid hormone, investigative efforts could be focused on the phosphate transport hypothesis. The development of a new radioimmunoassay for the measurement of human parathyroid hormone in serum (*10*) provided an opportunity to study this problem.

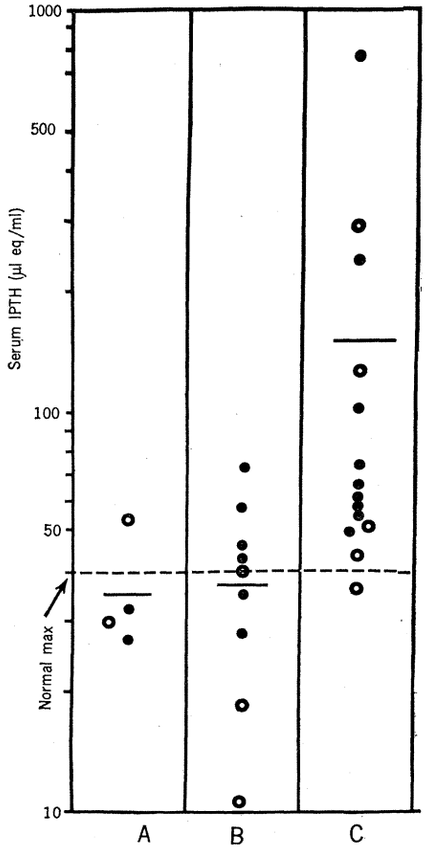

Fig. 1. Serum IPTH in patients with X-linked hypophosphatemic rickets. Column A, no treatment; column B, treatment with large doses of vitamin D_2 alone (about 100,000 unit/day); column C, treatment with a phosphate supplement by mouth (3 g/day, as orthophosphate) and vitamin D_2 (up to 100,000 unit/day). Open circles; female patients; closed circles; male patients. All patients have bone disease.

Serum was obtained from patients with familial hypophosphatemic rickets meeting the criteria for the X-linked trait (*11*). Untreated patients had serum immunoreactive parathyroid hormone concentrations (IPTH) within or close to the normal range (Fig. 1). Patients who had been receiving vitamin D but who still retained their hypophosphatemia, hyperphosphaturia, and active bone disease, also had normal serum IPTH. Only those patients receiving large quantities of therapeutic phosphate salts by mouth had increased serum IPTH. Three patients on the phosphate regimen developed roentgenographic and biochemical signs of hyperparathyroidism; reduction of the phosphate intake and supplementation of their diet with calcium, as calcium gluconate (1 to 3 g/day), and vitamin D_2 (100,000 unit/day) for at least 4 weeks suppressed serum IPTH to normal or near normal in these patients (Table 1).

Tubular reabsorption of phosphate [μmole/100 ml glomerular filtration rate (GFR)] was measured (*12*) on many occasions in four male probands. The results were plotted in relation to the serum IPTH at the same time (Fig. 2). Tubular reabsorption of phosphate was greatly impaired [mean for group = 36.8 μmole/100 ml GFR, normal, > 90 μmole/100 ml GFR (*1*)]. The serum phosphorus concentration was 3.3 ± 1.4 mg/100 ml (mean and S.D.) for the group at the time these determinations were done and when the patients were taking varying amounts of phosphate by mouth. There was no relationship between tubular reabsorption of phosphate and serum IPTH, indicating that the defect in phosphate transport in male patients is not influenced by endogenous serum IPTH.

We believe that this is the first unambiguous evidence of normal serum IPTH activity in patients with untreated X-linked hypophosphatemia. Serum IPTH is increased only when these patients are treated with phosphate supplements. The mechanism involved in producing this increase in serum IPTH is probably the negative feedback control of parathyroid hormone secretion by the concentration of calcium in serum (*13*). Serum calcium decreased in the patients whose serum phosphorus had been increased by daily oral phosphate administration (Table 1).

A phosphate transport defect is present in male patients with X-linked hypophosphatemia even when their serum IPTH is normal (Fig. 2). The insensitivity of residual phosphate transport to endogenous IPTH suggests to us that the renal tubule of patients with

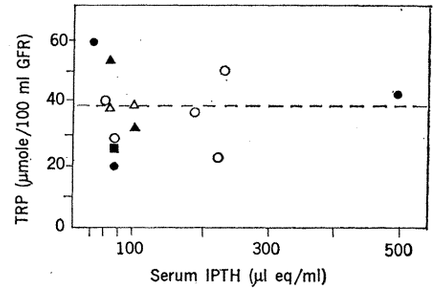

Fig. 2. Relationship of TRP [tubular reabsorption of phosphate, μmole/100 ml glomerular filtration rate (GFR)] to endogenous serum IPTH in four male probands with X-linked hypophosphatemic rickets, at serum phosphate values for group of 3.3 ± 1.4 mg/100 ml (mean ± S.D.). The dotted line indicates the regression of TRP on serum IPTH; the latter does not influence TRP in these patients. Maximum normal serum IPTH, 40 μl eq/ml.

X-linked hypophosphatemia contains a second parathyroid hormone–insensitive phosphate-transport system. We also suggest that this component of phosphate transport in kidney is responsive directly to calcium, perhaps in a manner analogous to that documented in canine kidney by Lavender and Pullman (*14*). This would account for the well-known effect of hypercalcemia on tubular reabsorption of phosphate in X-linked hypophosphatemia (*4*).

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11. The criteria for diagnosis of X-linked hypophosphatemia were: proven hypophosphatemia for age on a morning blood sample after overnight fast; absence of hypocalcemia and hyperaminoaciduria in the untreated state; no male-to-male transmission; female clinical phenotype not more severe than male in same pedigree.
12. Tubular reabsorption of phosphate (TRP) was measured after an overnight fast. A timed urine collection lasting about 180 minutes was obtained, and venous blood was obtained at the end of the period. Phosphorus and creatinine determinations were made and the TRP value was calculated. TRP was also determined with inulin clearance rates to monitor GFR on several occasions. Inulin and creatinine clearances were comparable in the same subject.
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Effects of Long-Term Reserpine Treatment on Brain Tyrosine Hydroxylase and Behavioral Activity

Abstract. *Treatment of rats with reserpine (for 8 or 9 days) produced a temporally related increase in behavioral activity and in tyrosine hydroxylase activity in the midbrain. Weight loss resulting from such treatment was not sufficient, by itself, to account for either the behavioral or enzymatic changes. The results support the role of catecholamines in behavioral arousal.*

The depletion of brain catecholamines (CA's) by reserpine is believed to play an important role in reserpine-induced sedation (*1*). However, animals treated for extended periods with appropriate doses of reserpine exhibit normal gross behavior (*2*) and, in fact, eventual hyperactivity (*3*); this delayed reversal into hyperactivity occurred when the CA concentrations in the brain were maintained substantially below normal by the long-term drug treatment. Haggendal and Lindqvist (*2*) have attempted to explain this apparent lack of correlation between the concentrations of CA in the brain and the behavior by suggesting that the CA's exist in two pools: (i) a large "storage" pool that is not directly involved in the mediation of neuronal activity and (ii) a smaller pool that more directly influences synaptic function. This hypothesis is based on their finding that after the depletion of the large "physiologically inert" CA pool, reserpine-induced fluctuation in the residual CA concentrations ("functional" pool) correspond to behavioral changes. A number of other findings are consonant with the existence of a relatively small, labile pool that mediates neuronal activity (*4*). This functional pool appears to be maintained primarily by synthesis de novo because newly synthesized CA's have been shown to be released preferentially when adrenergic neurons are activated (*5*).

Although the existence of a small functional pool may partially explain the restoration of normal behavior during long-term treatment with reserpine, it does not by itself explain the hyperactivity that appears after several days of such treatment. However, recently it has been shown that tyrosine hydroxylase, the rate-limiting enzyme in CA biosynthesis (*6*), can be induced in the adrenal medulla and sympathetic ganglia after reserpine treatment (*7*). This elevation of tyrosine hydroxylase activity appeared to be due to an increased synthesis of new enzyme, mediated by the prolonged reflexive increase in sympathetic nerve activity. In addition, in the case of the adrenal medulla, the increase in tyrosine hydroxylase activity, as measured in tissue homogenates, correlates with an increase in CA biosynthesis in the intact animal (*8*). If a similar increase in tyrosine hydroxylase activity occurs in the brain concomitant with the behavioral activation produced by long-term treatment with reserpine, an increase in brain tyrosine hydroxylase and the resulting increase in functional norepinephrine levels (provided that tissue tyrosine levels are maintained such that the enzyme is saturated) would be consonant with the alleged role of norepinephrine in behavioral arousal (*1*, *9*).

Male Sprague-Dawley rats (250 to 275 g) were injected intraperitoneally with reserpine (0.5 mg/kg) at 24-hour intervals for 9 days, or with distilled water for 1, 2, or 6 days and reserpine (0.5 mg/kg) for the remaining 8, 7, or 3 days, respectively. Control animals were injected with distilled water for 9 days. Behavioral activity of crossovers in a free-field situation (*10*) was determined during a 1-hour interval, 23 hours after each injection. In order to obtain a stable level of responding, all animals were exposed to the experimental chambers for hourly intervals during the 5 days immediately prior to the onset of drug administration (*11*).

In agreement with previous findings, long-term treatment with reserpine pro-