The statement that the "pattern of urinary metabolites indicated a persistent defect in *p*-hydroxyphenylpyruvic acid oxidase" is simplistic. It certainly appears that both Faull's patients handled the [²H]tyrosine load very differently but to use this data to postulate substrate inhibition of *p*-hydroxyphenylpyruvate hydroxylase *in vivo* is fallacious. The so-called substrate inhibition is a contrivance of the enzyme assay in which oxygen inactivation of the enzyme occurs in the presence of the ketoacid. At physiologic pO₂ no inhibition is observed (5). The urinary metabolite patterns and the different handling of a tyrosine load in the two patients could just as well be explained by a difference in the amount of residual cytosol tyrosine aminotransferase activity. In this condition, the ketoacid accumulates as a result of mitochondrial transamination of tyrosine in tissues which lack the hydroxylase (4, 9).

In summary, it is regrettable that a decisive investigation of the enzyme defects was not achieved. It is clear that these patients did not have tyrosinemia type 1 but it remains uncertain as to whether they both had tyrosinemia type 2 or whether one had a primary defect in *p*-hydroxyphenylpyruvate hydroxylase.

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Letter to the Editor: Reply to Dr. Buist

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Thank you for the opportunity of replying to the criticisms of Dr. Buist and colleagues. The terminology of tyrosinemia is indeed confusing. Perhaps we should have used the term "tyrosinemia without liver damage."

We recognize deficiencies in the enzyme assays and therefore laid rather little weight upon these results in our argument; however, there were clear differences between the two patients. We can firmly dismiss the suggestion that deuterium atoms have been lost from the phenolic ring by chemical exchange during storage because such a process would alter the ratio of the D₂ to the D₁ peaks on mass spectrometry, producing an increase in the D₁ peak and a decrease in the D₂ peak. The ratio of these peaks was monitored in all samples and did not change significantly.

Absorption of the tyrosine loads used was clearly slow and probably incomplete. Parenteral administration of a smaller load would undoubtedly be preferable. Nonetheless, as our critics themselves point out, the studies of the control subject give us some idea of the amount of phenolic acids contributed

by absorption of the products of bacterial metabolism and this is clearly far too small to have any influence on the overall results obtained.

We are left with the principal observation of our study basically unaltered by the criticisms raised. One patient excreted approximately the same very large proportion of the administered label in the urine whether he was tested with high serum tyrosine or with serum tyrosine reduced by dietary treatment. In the other patient the dietary control resulted in a 100-fold reduction of the proportion of the label excreted in the urine as tyrosine and tyrosyl derivatives.

In considering these results it is important to note that the serum tyrosine level was still 2-3 times the upper limit of normal in both patients while on dietary treatment and so the rate-limiting step of tyrosine catabolism was already overloaded before the labeled tyrosine load was administered. Consequently, one should expect the handling of the load to be comparable in the two patients under these circumstances if indeed the two patients had defects of the same enzyme as

proposed by our critics. The dramatic difference observed strongly suggests different defects in the two patients and we would still maintain that secondary inhibition of *p*-hydroxyphenylpyruvic acid hydroxylase is by far the most plausible explanation in this patient and indeed in the patient with deficient soluble aminotransferase originally described by our critics.

In summary, we acknowledge the existence of certain weak-

nesses in our report and indeed these were already acknowledged in the report itself. However, none of the criticisms raised seem to seriously affect the main observation of striking heterogeneity within patients with tyrosinemia of a type which does not cause liver damage (tyrosinemia type II, in the terminology of Dr. Buist and colleagues).

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Letter to the Editor: Importance of Technique in the Induction of Glucuronyl Transferase in Gunn Rats

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We read with interest an article by Van Houwelingen and Arias entitled, "Attempts to Induce Hepatic Uridine Diphosphate-Glucuronyl Transferase in Genetically Deficient Gunn Rats by Normal Liver Tissue," which appeared in *Pediatric Research* (8). These authors apparently failed to reproduce the results of our investigation (4). It is not surprising that the authors did not reproduce the results of our experiments as there seem to be some vital discrepancies between the methodologies of the two investigations as the following paragraphs will elucidate.

One of us (J.K.) supplied six Gunn rats to the above investigators (not eight as erroneously reported). Out of those six, two rats (a mating pair) were heterozygous. Van Houwelingen and Arias (see Table 1, Reference 8) were unable to detect any enzyme activity in any of the rats provided, although they should have found activity in at least two out of the six rats. One of us (J.K.) also had an opportunity to witness some of the grafting procedures of the above investigators and felt that their animals were kept too long under ether anesthesia. This is relevant, as we found (5) that best results were obtained when the entire procedure was completed within a short time (10 min) and with minimal anesthesia.

Since our initial report in *Science* (4), we have completed some additional investigations with the Gunn rats (5), basically to answer two questions: 1) how important was the time factor and 2) what proportion, if any, of the grafted cells survived in the recipient Gunn rat liver. The results of these studies indicated that 0.9% of the prelabeled grafted cells survived at least 12 weeks after transplantation, if the surgical procedure was limited to 10 min. However, procedures requiring more than 10 min (15-30 min) had 0.02 and 0.01% cells, respectively, surviving at 12 weeks.

We proposed previously that one of the mechanisms of enzyme induction in the post-transplant Gunn rats could be due to a small number of grafted cells surviving in the recipient. These cells, in turn, produced enzyme with 100% efficiency (4). We do not have any direct evidence at this time. However, the finding that 0.9% cells survive in the recipient liver strongly supports our hypothesis. A long transplantation procedure affects the survival of the donor cells in the recipient liver. This is one of the possibilities accounting for the inability of the above authors to detect any enzyme activity.

Recently, we have been investigating the effects of the inhibitors of protein synthesis in the transplanted Gunn rats (unpublished data) using the same experimental design as reported previously (4). The grafted animals without antimetabolite treatment had enzyme activity (range 12-75 μ mol bilirubin glucuronide/min/ml homogenate) as reported earlier (4). These assays were done by different workers than the ones who did the original experiments and as previously, they had no prior knowledge of the origin of the liver tissues before the assays were performed.

Additionally, the fact that no enzyme activity was detected by the above authors (8) with either glucuronidation and/or glucosidation should have aroused suspicion as to the viability of the microsomal preparations since both of these enzymes are microsomal in origin. It has been reported for the rat that the glucosyl transferase for bilirubin is a different and a separate enzyme from glucuronyl transferase (2, 7). The Gunn rat has been shown to have both glucosyl transferase as well as glucuronyl transferase enzyme for *o*-aminophenol acceptor (6) (although bilirubin glucuronyl transferase is absent). This is particularly demonstrable when microsomes are activated by digitonin treatment (1). It would have been of much value if the liver