Pediat. Res. *2:* 329–331 (1968)

Correspondence

Effect of Free and Peptide Hydroxyproline

Sir:

After reading the article by YOUNOSZAI *et al.* (Pediatric Research *1:* 266 [1967]), I feel several discrepancies in interpretation of data might be discussed.

There are many papers as noted by the authors that correlate rate of collagen metabolism with excretion of the imino acid hydroxyproline. Most of these studies have determined total hydroxyproline in the urine rather than peptide hydroxyproline. In the subject over 6 months of age, more than 95 % of the urinary hydroxyproline is in the bound peptide form so that the terms total or peptide hydroxyproline can essentially be used interchangeably. However, in the newborn and particularly in the premature, a significant amount of the hydroxyproline is excreted as the free imino acid [1]. For example, the authors found at 9–20 days of age that infants were excreting 38.8 mg/day of total hydroxyproline. Our reported values [2] are quite similar; i.e., at 10–17 days of age, total excretion was 43.0 mg/day. Of the 43.0 mg/day, however, 15.3 mg or approximately 36 % was free hydroxyproline. In prematures 47–77 days of age, the percentage of free hydroxyproline was 47 %.

The increase in excretion of free hydroxyproline during the newborn period reflects several factors [2]. Newborns have an increased clearance of the free imino acid. During the first 6 months of life, oral tolerance tests of free hydroxyproline [2] also reveal a decreased ability to catabolize the free imino acid [2]. Peptide excretion/kg/day falls slightly during the first year of life, reflecting a decrease in the relative collagen turnover of the infant. However, during that same period there is a 60-fold decrease in free hydroxyproline excretion. These changes must be accounted for by factors [2] other than changes in collagen turnover.

Since urinary excretion of peptide hydroxyproline reflects collagen metabolism more accurately than excretion of free hydroxyproline, the latter should be discounted [3] when one talks of correlating growth and hydroxyproline excretion. This fact is especially important in the infant less than 6 months of age and particularly in the premature infant.

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Effect of pCO_2 on the Oxyhemoglobin Dissociation Curve

Sir:

I found the paper by BATTAGLIA *et al.* (Pediatric Research *2:* 193 [1968]) most interesting and its area of emphasis most appropriate. Two points have arisen upon which I should like to comment. The authors state that others have demonstrated the effect of pCO_2 independent of pH to be negligible in determining the position of the oxyhemoglobin dissociation curve. This seems to me to state the relation in a misleading fashion. The authors quote two references in support of their statement. In one, ASTRUP *et al.* [1] point out that their calculation of the Bohr effect produces results similar to those obtained by a variety of other investigations. Their point is that since their measurements were done with pCO_2 constant with added acid and base, and all other measurements were made with variable pCO_2 with constant buffer base content, there appears not to be an independent effect of CO_2 on oxygen affinity. This conclusion is compatible with those observations but by no means it is the sole explanation. An equally valid possibility is that the technique for calculating the Bohr effect is faulty in either or both cases. The observation of ASTRUP *et al.* is compatible with, but does not prove their hypothesis.

In the second reference, using dog blood, ROSSING and CAIN [2] were able to calculate a significant coefficient for a pCO_2 term in their regression equation and further demonstrated that its use improved the goodness of fit of the resulting equation to observed measurements. They opted against use of the pCO_2 term on the

basis that the added computational work was not justified in view of the small improvement in fit.

In the only work which I know bears directly on this point [3, 4], an independent effect of pCO_2 on dissociation curves in human blood is established. The effect is certainly not large in arterial blood, but may be considerable in fetal blood. Using NAERAA's nomograms, I calculate that at pO_2 approximately 32 millimeters of mercury and BE-10 mEq/l, failure to consider the pCO_2 effect results in an approximately 5 % error in pO_2 calculated from saturation. Whether this error is large or not in the sense of influencing the results in this paper, I cannot say. It does seem to me that the authors' position that the effect of pCO_2 independent of pH is negligible requires some support. One would like some assurance that pCO_2 in the gas phase during tonometry did not vary widely from one measurement to another. The text gives no sign that CO_2 tension was controlled in any way.

It appears to be the authors' position that the precision with wh ch experimental data fits the general equation for .he dissociation curve over the range of variables studied is sufficient evidence for proving a linear relation between pH and log pO_2, since this is one of the assumptions of that equation. I think there is a serious argument with this point. One may certainly produce any sort of mathematical function he wishes, based on any assumptions he cares to make, in an effort to fit data. If he satisfies himself that the function does indeed fit observed data, it is supportive, but hardly conclusive evidence for the assumptions which they have made. Important assumptions require independent laboratory justification. The nicest example which comes readily to mind is the Hill equation itself. This function is derived from physical definitions, mass action law, and the assumption of uni-molecular binding of oxygen to hemoglobin. Experimental data obtained in the range of saturation, which is the same in which Dr. BATTAGLIA works, certainly fits the Hill equation nicely, despite the fact that the assumption of uni-molecular binding is entirely invalid. Independent investigation of that assumption has demonstrated it to be false, and further investigation of the Hill function in areas of extreme values of oxygen saturation has confirmed that direct experimental refutation. Nevertheless, the function is a useful one in terms of expressing data within the appropriate range of saturation.

Dr. BATTAGLIA is correct in that his general equation is a combination of the old Hill equation and the Dill correction factor, rearranged so that coefficients are differently defined. Certainly there is no reason why it should not fit dissociation data at least equally as well as the Hill function in the range of saturation which he studies. This does not mean that the assumption of linearity between pH and log pO_2 is any more conclusively supported by this degree of fit than is the proposition of uni-molecular oxygen binding of hemoglobin. The relation between pH and pO_2 requires independent investigation to verify its validity. To my knowledge, wherever investigators have looked at this relation on the basis of laboratory data derived solely for that purpose, they have found it non-linear. I have the strong feeling that failure to grasp this point in mathematical modeling is an important one.

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References

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Sir:

Dr. KIRSCHBAUM is correct in stating that the Hill equation has no theoretical basis. To our knowledge, no one in recent times is ascribing any such basis in its use, nor have we done so in this report. The usefulness of this empiric equation is clearly brought out in the discussion in Handbook of Respiration, Vol. 1, p. 767, 1964, by F.J.W. ROUGHTON.

While the question of an effect of pCO_2 independent of pH is an interesting one, it is not crucial to this study since the pCO_2 in the tonometers for the THAM and bicarbonate bloods were within 1 or 2 mm/Hg of each other. We should like to point out that ROSSING and CAIN calculated a *statistically* significant coefficient for pCO_2. This is not the same as saying that the effect of pCO_2 was *physiologically* significant. In fact, it was their judgment and one with which we concur that the effect of pCO_2 is so small as to be of little physiologic signifi-

cance, and they did not include such a correction in their constructed nomograms, nor have others in similar nomograms. Occasionally, in the literature, there have been reports of wide variations in pO_2 at constant saturation and pH, and among the largest variations reported are the data of KIRSCHBAUM *et al.* (Amer. J. Obstet. Gynec. *96:* 741 [1966]) in which, at a constant saturation of 80 % and pH of 7.4, fetal blood pO_2 ranged from 20 to 40 mm/Hg. Variations of pO_2 of this degree at constant saturation and pH have certainly not been our experience.

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