Effect of Binding to Hemoglobin and Albumin on Pyridoxal Transport and Metabolism*

(Received for publication, December 9, 1983)

Steven L. Ink† and LaVell M. Henderson
From the Department of Biochemistry, University of Minnesota, St. Paul, Minnesota 55108

Scatchard plot analysis indicated that pyridoxal binds to hemoglobin more than twice as tightly as it does to serum albumin. Comparison of the concentration constants for hemoglobin and albumin, using standard competitive binding equations, indicated that the distribution ratio for pyridoxal between erythrocytes and plasma should be 6.5:1. This distribution was approximately the same as that observed when pyridoxal was incubated with whole human blood, suggesting that these two proteins are the primary determinants of the pyridoxal distribution in whole blood.

With in situ perfused rat liver the uptake of [3H]pyridoxal from the perfusate was reduced by the inclusion of erythrocytes in the perfusate. This was reflected in the decreased production of 4-pyridoxic acid by the perfused liver from 3.8% to 1.2% of the dose by the addition of erythrocytes to the perfusate. The major labeled metabolites found in the liver were pyridoxal phosphate, pyridoxamine phosphate, and 4-pyridoxic acid for both types of perfusion.

In intact animals, reduction of the erythrocyte concentration to hematocrits of 30-40% increased the recovery in the urine of [H]from administered [3H]pyridoxal from control values of 27-35% to 40-50% of the dose within 48 h. Half of the label in urinary metabolites was in 4-pyridoxic acid.

Concentrative uptake of pyridoxal by erythrocytes has been reported (1, 2). This process was found to occur as a result of Schiff base formation between pyridoxal and the amino group of the NH2-terminal valine of the a-chain of hemoglobin (2). The binding of PL1 to hemoglobin may have significance in several areas of vitamin B6 transport and metabolism. Anderson et al. (3) have suggested that the distribution of PL between red cells and plasma in whole blood may be controlled by competing binders in these compartments. The binding of PL to hemoglobin may also influence metabolism of this and other B6 vitamers which are converted to PL (4). Stanulovic (5) suggested that a small increase in the PL pool might result in an increase in its metabolism to pyridoxic acid. Pyridoxal binding to hemoglobin could affect cofactor concentrations for pyridoxal phosphate-requiring enzymes in the red cell (6).

Here we report an investigation of the strength and specificity of binding of PL to hemoglobin and to various plasma protein fractions in an attempt to determine factors which are responsible for the distribution of PL between red cells and plasma in whole blood. We also report the effect of PL binding to hemoglobin on its uptake by the liver and the effect of anemia on the oxidation of PL to 4-PA.

EXPERIMENTAL PROCEDURES

Materials—Male Sprague-Dawley rats were obtained from Bio-Lab Corp., White Bear Lake, MN and Holtzman Co., Madison, WI. Pyridoxal, pyridoxamine, pyridine, 4-pyridoxic acid, pyridoxal phosphate, pyridoxamine phosphate, human and bovine serum albumin, human α, β, and γ-globulins, glyoxylic acid, and alkaline phosphatase were purchased from Sigma. Pyridoxine phosphate was obtained from ICN Nutritional Biochemicals. [3H]Pyridoxamine was prepared by the Amersham Corp. using an exchange procedure on pyridoxamine (free base). The synthesis and purification of [3H]PL starting with [3H]pyridoxamine were accomplished as previously described (7). The [3H]PL was more than 90% pure as judged by high voltage paper electrophoresis. Calbiochem-Behring was the source of polystyrene glycol. Spectrophotodiodes membrane tubing was purchased from Fisher Scientific Co. For high performance liquid chromatography, the reverse phase columns used were Rainin Instruments Microsorb C18 and Altex Ultrasphere ODS column. Guard column packing material, Pellicular C18, was obtained from Alltech Associates, Deerfield, IL.

Methods—Fresh human whole blood, collected over heparin, was centrifuged and the plasma anduffy layer were removed. The red cells were then mixed with isotonic saline and washed by centrifugation several times. The erythrocytes were lysed with an equal volume of distilled water and were kept cold for 30 min. The red cell membrane was removed by centrifugation at 15,000 × g for 25 min. The resulting supernatant hemoglobin solution was then concentrated by using an Amicon filtration cell with a PM 10 filter until the desired concentration of hemoglobin was attained. The concentration of hemoglobin was determined using molar extinction coefficients for both hemoglobin and methemoglobin at two different wavelengths (8). The methemoglobin content of the freshly prepared oxyhemoglobin was always less than 2%. The dialysis experiments were done using 6-mm spectroradiolysis tubing (M, cutoff = 14,000) and culture tubes (13 × 100 mm) with screw caps. One-mi solutions of hemoglobin were placed in the dialysis bag and dialyzed against 4 ml of isotonic saline containing various concentrations of pyridoxal and sufficient polystyrene glycol to osmotically balance the hemoglobin. The experiments were done at 4°C in the dark, allowing 20 h for the system to reach equilibrium. The tubes were rocked throughout the dialysis. The experiments were done initially by adding the pyridoxal to both the inside and outside compartments of the dialysis bag to determine if the equilibration period was sufficient and to provide evidence that reversible binding of pyridoxal to protein was occurring. Following the incubation period, the dialysis bag was removed from the culture tube and the hemoglobin concentration again determined. The distribution of [3H]pyridoxal was then determined by taking 20-μl aliquots of the solution both in and out of the bag. Forty μl of a 10% sodium tungstic acid solution, 40 μl of 0.667 N sulfonic acid solution, and 100 μl of water were added to each 20-μl aliquot. After thorough mixing and standing for 10 min, the sample was centrifuged to remove the protein and a 100-μl aliquot of the supernatant was counted.

The methodology of Hems et al. (9) was utilized for in situ liver
Pyridoxal Binding to Hemoglobin and Albumin

Results

Binding of Pyridoxal to Hemoglobin—Hemoglobin solutions of various concentrations were placed inside a dialysis bag for dialysis against a saline medium containing appropriate concentrations of PL and polyethylene glycol to provide the same osmolarity as the inside contents. Fifteen hours was found to be sufficient time for the system to reach equilibrium whether the PL was originally inside or outside of the dialysis bag, so 20 h was used for all dialysis experiments.

The Scatchard plot of PL binding to hemoglobin is shown in Fig. 1, where the line was fitted by method of least squares. The slope of the line which corresponds to the formation constant (Kf) for the PL-hemoglobin complex was 730 M⁻¹. The x axis intercept indicated that there are about 2 PL binding sites/molecule of hemoglobin. This is in agreement with previous results (2) which indicated that PL binds to the NH₂-terminal valine of the a-chain of hemoglobin, thus providing 2 binding sites/tetramer of a and b subunits.

The binding of PL to hemoglobin could play a role in the transport of the vitamin from the blood to various tissues. Compounds which are found in the blood may affect the binding of PL to hemoglobin and this may influence the transport process. Table I shows the results of dialysis experiments where various compounds were placed with PL outside the dialysis bag while hemoglobin was inside. Although a more complete analysis of the effect of a given competitor on PL binding would require Scatchard plot construction, a general idea of competitor influence on PL binding can be obtained by examining the relative levels of PL accumulation in the dialysis bag. The presence of plasma or serum albumin outside the dialysis bag reduced the accumulation of [³H]PL in the bag substantially (Table I). Acetaldehyde at concentrations much higher than physiological levels decreased [³H]PL accumulation somewhat while PLP, glucose, and galactose reduced accumulation of [³H]PL only moderately. It should be pointed out that when the fraction of protein which has a ligand bound reaches low values, as when PL concentration was about 10 µM and hemoglobin concentration was greater than 1 mM, the concentration ratio of [³H]PL inside:outside the dialysis bag, when no competitor was present, was lower than would be predicted from the formation constant obtained from the Scatchard plot. This may be due to the presence of traces of impurities which compete with PL for the binding site on hemoglobin.

As a result of the effects found for serum albumin and plasma on [³H]PL accumulation in these dialysis experiments, the binding of PL to albumin was examined via the same dialysis system used for hemoglobin. A Scatchard plot of the data (Fig. 2) showed that the formation constant for PL binding to albumin was 340 M⁻¹. The x axis intercept was 2.4 indicating that there may be two major binding sites, with participation by a weaker third site at higher PL concentrations.

Bovine serum albumin forms three types of complexes with

Table I

| Additions       | Hemoglobin | Pyridoxal | Experimental ratio (in/out) of [³H] | Theoretical ratio (in/out) of [³H] (no competitor) |
|-----------------|------------|-----------|-----------------------------------|---------------------------------------------------|
| Plasma          | 1.7 µM     | 10 µM     | 1.1                               | 2.5                                               |
| (A₂₅₀nm = 0.7)  |            |           |                                    |                                                   |
| Albumin (25 mg/ml) | 2.7 µM     | 10 µM     | 3.9                               | 4.8                                               |
| Albumin (50 mg/ml) | 1.5 µM     | 10 µM     | 1.8                               | 3.2                                               |
| Acetaldehyde (10 mM) | 3.1 µM     | 10 µM     | 4.1                               | 5.5                                               |
| Acetaldehyde (50 mM) | 3.0 µM     | 10 µM     | 3.3                               | 5.3                                               |
| PLP (10 µM)     | 4.1 µM     | 10 µM     | 5.9                               | 6.9                                               |
| PLP (100 µM)    | 4.1 µM     | 10 µM     | 6.3                               | 6.9                                               |
| Glucose (10 mM) | 2.5 µM     | 10 µM     | 3.6                               | 4.6                                               |
| Glucose (50 mM) | 2.3 µM     | 10 µM     | 4.3                               | 4.3                                               |
| Galactose (10 mM) | 2.6 µM     | 10 µM     | 4.3                               | 4.7                                               |
| Galactose (50 mM) | 2.2 µM     | 10 µM     | 3.5                               | 4.2                                               |

Fig. 1. Scatchard plot for pyridoxal binding to human hemoglobin.
PL phosphate (12). Two are formed by binding at unique sites with high formation constants while the third results from additional binding sites with lower affinity and not identified. All seem to bind by reaction with the ε-amino group of lysine. PL accumulation in a dialysis bag containing albumin was examined in the presence of PLP. The accumulation of PL in the dialysis bag was reduced only when the concentration of PLP was 100-fold greater than PL indicating that these two vitamers are not competing for the same binding sites on albumin.

The relative binding affinities of PL for albumin and hemoglobin should determine the distribution of this Be vitamer in an equilibrium dialysis situation and could determine the distribution of [3H]pyridoxal in whole blood. Therefore, standard competitive binding equations (13, 14) were used to predict what the distribution of [3H]PL would be in an equilibrium dialysis system where hemoglobin is inside a dialysis bag and albumin is outside the bag. The results of calculations using these binding equations are compared with experimental results in Table II. The experimentally determined concentrations of PL inside and outside the dialysis bag agreed quite well with the values calculated from the binding equations. These equations were then used to obtain an expected distribution of PL in whole blood by substituting the appropriate physiological concentrations of hemoglobin and albumin into the equations. The predicted concentration ratio of PL inside/outside was 6.5:1. The distribution of [3H]PL found in whole blood has been experimentally determined by incubating [3H]PL with whole blood for 60 min followed by centrifugation and washing of packed red cells at 4°C. The concentration ratio of [3H]PL in the red cell relative to outside was found to be 4.5:1.

Additional experiments were done to investigate the possible influence of plasma protein fractions other than albumin on the distribution of PL in whole blood. Equilibrium dialysis was done as before using concentrations of the various globulins fractions which were about twice those of plasma. The results are presented in Table III. None of the plasma protein fractions studied bound PL as evidenced by concentration ratios of 1.0 or less.

Liver Perfusion—The binding to hemoglobin inside the erythrocyte may modify the movement of PL between the blood and an organ or tissue. If this transport is influenced by binding then subsequent metabolism may also be influenced since the PL pool is small and a small change in this pool may affect the fate of the vitamin (15). Therefore, in situ liver perfusions were done to determine the uptake and metabolism of PL by the liver. When no erythrocytes were included in the perfusate, 19.7 ± 1.1% of the total isotope (the perfusate was initially 0.2 μM [3H]pyridoxal) had accumulated in the liver in 15 min. In contrast, 12.6 ± 1.4% of the total isotope had accumulated in the liver in 15 min when erythrocytes were included in the perfusate (23% hematocrit). The distribution of [3H]metabolites in the liver and perfusate when the experiments were done with erythrocytes are shown in Table IV. The metabolites of PL were determined in the initial 15-min perfusate, in the 7-min “wash” perfusate which followed, in the final 15-min “release” perfusate, and in the liver. These data show that even in the initial 15-min perfusate metabolites of PL had been released by the liver following uptake and metabolism of PL. The “wash” perfusate contained a smaller percentage of PL and a greater percentage of the other metabolites when compared to the initial 15-min perfusate. The final 15 min of the perfusion was done with fresh perfusate to study the distribution of [3H] in compounds released by the liver following uptake of [3H]PL. As shown in Table IV, 49.9% and 14.8% of the label in this release perfusate was PL and 4-PA, respectively. The major [3H]-labeled compounds found in the liver were PL, pyridoxamine phosphate, 4-PA, pyridoxine phosphate, and PLP representing

---

**Table II**

Calculated and observed distribution of pyridoxal between the hemoglobin and serum albumin after equilibrium dialysis

| ([Hb]o) | ([Albumin]o) | [PL] initial outside | [PL] final outside | [PL] final inside |
|--------|-------------|---------------------|-------------------|-----------------|
| mM     | μM          | Experimental        | Calculated        | Calculated*     |
| 3.5    | 0.75        | 10                  | 5.1              | 4.9             | 19.7           | 19.4           |
| 3.3    | 0.37        | 10                  | 4.6              | 4.1             | 21.6           | 23.0           |
| 7.0    | 0.7         | 10                  | 2.4              | 2               | 12             | 13             |

*These calculations were done utilizing the following binding equations:

\[
N_1 \text{Hb} + PL \leftrightharpoons HbPL
\]

\[
N_1 \text{Al} + PL \leftrightharpoons A1PL
\]

Where \(N_1\) = binding sites/molecule of hemoglobin and \(N_2\) = binding sites/molecule of albumin.

\[
\text{SP}_{PL} = \frac{(N_1)[Hb]}{1 + K_{PL}(Hb)}
\]

\[
\text{SP}_{Al} = \frac{(N_2)[Al]}{1 + K_{PL}(Al)}
\]

where \(SP_{PL}\) = moles of hemoglobin-pyridoxal complex/total moles of hemoglobin and \(SP_{Al}\) = moles of albumin-pyridoxal complex/total moles of albumin. From the Scatchard plot results: \(N_1 = 2; N_2 = 2.4; K_{PL} = 730 \text{ M}^{-1}; K_{Al} = 340 \text{ M}^{-1}.

---

**Table III**

Equilibrium dialysis of PL and various proteins

| Protein                  | Concentration of [H] (inside: outside) at PL concentrations of 10 μM | 100 μM | 1 mM |
|--------------------------|-------------------------------------------------------------------|--------|------|
| Bovine γ-globulin (11 mg/ml) | 1.00                                                              | 1.04   | 0.90 |
| Human Cohn Fraction II (20 mg/ml) | 0.82                                                            |        |      |
| Human Cohn Fraction III-1 (50 mg/ml) | 0.82                                                            |        |      |
| Human Cohn Fraction IV-2 (20 mg/ml) | 0.85                                                            |        |      |
| Human hemoglobin α-chain (1 mM) (0.4 mM) | 3.1                                                           |        |      |
| Human hemoglobin β-chain (1.3 mM) (0.9 mM) | 1.6                                                           |        |      |

---
**Pyridoxal Binding to Hemoglobin and Albumin**

**TABLE IV**

Distribution of $^{3}$H in various B$_{6}$ vitamers in the perfusates and liver following perfusion with whole blood, hematocrit 23%, containing $^{3}$H/PL.

Values are mean ± S.E. for 3 experiments. Experiments were initiated by adding $^{3}$H/PL to 60 ml of perfusate. After 15 min, the perfusate was removed and the perfusion system was washed (single pass) with 70 ml of fresh erythrocyte-free perfusate. Then a 15-min perfusion was done with 60 ml of fresh perfusate, designated release perfusate. The red blood cells in the wash perfusate are from the initial erythrocyte-containing perfusate.

| Metabolite     | Initial 15-min perfusate | Wash perfusate | Isotope distribution | Red blood cells |
|----------------|--------------------------|----------------|----------------------|-----------------|
|                | %                        |                | %                    |                 |
| Pyridoxamine   | 1.9 ± 1.6                | 2.9 ± 0.9      | 1.6 ± 0.2            | 1.1 ± 0.7       |
| Pyridoxine     | 8.5 ± 1.0                | 6.1 ± 2.1      | 5.6 ± 0.6            | 2.1 ± 1.3       |
| PL             | 86.6 ± 13.1              | 51.9 ± 2.5     | 49.9 ± 5.3           | 67.2 ± 18.0     |
| Pyridoxamine-P | 6.6 ± 3.9                | 11.0 ± 2.6     | 10.1 ± 0.8           | 3.2 ± 1.9       |
| 4-Pyridoxic acid | 5.0 ± 3.7               | 7.7 ± 0.5      | 14.8 ± 6.0           | 6.2 ± 3.1       |
| Pyridoxine-P   | 6.1 ± 4.6                | 11.6 ± 0.7     | 8.3 ± 3.4            | 4.5 ± 2.3       |
| PLP            | 6.1 ± 1.6                | 6.8 ± 1.2      | 7.7 ± 0.8            | 13.1 ± 1.2      |
| Other          | 1.8 ± 0.8                | 2.0 ± 0.9      | 2.2 ± 1.8            | 2.8 ± 0.4       |

18.4%, 26.7%, 11.8%, 16.9%, and 17.2%, respectively, of the isotope found in the liver. The distributions of $^{3}$H-labeled compounds in the red cells from the initial perfusate and the wash perfusate were similar to those of the media in which they were suspended. One noteworthy exception was PLP, where the percentage of $^{3}$H recovered in the erythrocytes was approximately twice that in the corresponding suspending medium. The distribution of $^{3}$H-metabolites found in the perfusates done without erythrocytes was very similar to those in perfusates with added erythrocytes. The major $^{3}$H-compounds found in the liver were PL, pyridoxamine phosphate, 4-PA, and PLP and they accounted for 22.1%, 24.2%, 23.5%, and 15.7%, respectively, of the total isotope found in the liver after 32 min.

The percentage of the original perfused dose of PL (0.8 nmol) which was oxidized to 4-PA by the liver was calculated from the results for isotope accumulation by the liver and metabolite distribution data. Almost 4% of the total isotope perfused was converted to 4-PA by the liver when no red cells were present compared to 1.2% when the hematocrit of the perfusate was 23%.

**Urinary Excretion of PL and Its Metabolites in Anemic Rats**—The results with perfused rat livers indicated that red cells may reduce the percentage uptake of PL by the liver from the portal circulation. The consequence of this in the whole animal may be increased delivery of PL to peripheral tissues when erythrocytes accumulate this compound. Conversely, more PL may enter the liver to be metabolized to compounds such as 4-PA in those animals with compromised ability to bind PL in the blood. To check this hypothesis, rats were made anemic by bleeding.

Eight rats were divided into two groups of four each. Group I was bled initially and 2 days later both groups were injected intraperitoneally with $^{3}$H/PL. Fourteen days after Group I had been bled, Group II was bled and 2 days after that both groups were again injected with $^{3}$H/PL. The urinary excretion results for this switchover experiment are shown in Table V. Group I had hematocrits of about 33% for the first injection and excreted in the urine in 48 h approximately 48% of the $^{3}$H injected as PL (Table V). Group II had hematocrits which averaged approximately 50% for the initial injection and they excreted 27.2% of the injected dose. Group II was made anemic prior to the second injection. The hematocrits of Group I rats were now between 45 and 48% while Group II rats averaged 38.5% (Table V). The urinary excretion of $^{3}$H-compounds following the injection of PL averaged 35.6% of the injected dose of $^{3}$H in Group I while Group II excreted 46.9% of the dose in the urine.

The distribution of $^{3}$H-compounds in the urine collected in the 24 h following the second injection was determined. The results for Group I and Group II were quite similar. Pyridoxic acid accounted for about one-half of the isotope in the urine and PLP for another 20%. Plasma protein levels were determined in both groups before they were made anemic and at the time of urine collection when they were anemic. Both groups of rats regenerated plasma proteins to normal concentrations during the 5-day pre-experimental recovery period.

**DISCUSSION**

The equilibrium dialysis experiments clearly demonstrated a relationship between concentration of hemoglobin in the

---

TABLE V

Urinary excretion of $^{3}$H during the 48 h following intraperitoneal injections of $^{3}$H/PL into rats with normal and reduced hemoglobin in switchover design.

Averages were analyzed by Student's t test. **, significantly lower than * (p < 0.04) and **** (p < 0.04); ***, significantly lower than **** (p < 0.09) and * (p < 0.06).

| Intraperitoneal injection on day 3 (0.4 mg: 3.76 µCi $^{3}$H/PL) | Release from perfusate | Liver | Wash from perfusate |
|---------------------------------------------------------------|------------------------|-------|---------------------|
| Rat Weight Group I hematocrit 48-h urinary $^{3}$H excretion   | g µCi                  | g µCi |
| 1 325 31 1.99 5 328 51 1.14                                 |
| 2 235 33 1.61 6 258 53 0.41                                 |
| 3 330 34 2.13 7 303 45 1.51                                 |
| 4 301 33 1.45 8 275 50 1.03                                 |
| Average per cent of dose excreted in 48 h                     | 47.7%**                | 27.2%** |

| Intraperitoneal injection on day 14 (0.4 mg): 4.72 µCi $^{3}$H/PL | Release from perfusate | Liver | Wash from perfusate |
|---------------------------------------------------------------------|------------------------|-------|---------------------|
| Rat Weight Group I hematocrit 48-h urinary $^{3}$H excretion       | g µCi                  | g µCi |
| 1 359 46 2.00 5 369 38 2.02                                      |
| 2 306 48 1.86 6 315 39 2.06                                      |
| 3 351 46 1.47 7 323 38 2.81                                      |
| 4 363 45 1.40 8 333 39 1.96                                      |
| Average per cent of dose excreted in 48 h                         | 35.6%***               | 46.9%**** |
Pyridoxal Binding to Hemoglobin and Albumin

The observations recorded in Table V are consistent with the vitamin B₆ conserving effect of erythrocytes. More severe anemia might cause a greater elevation of urinary excretion of 4-PA, but added complications might also be expected. The size of the dose of PL injected (0.4 mg) in these studies is nearly three times the daily requirement for rats of the body weight used (19). At a dose of pyridoxine approximating the daily requirement, Contractor and Shane (20) reported that pregnant rats excreted 43% in the urine about one-half as 4-PA. At high dosage pyridoxine is excreted as pyridoxine, PL, and 4-PA, and PL as 4-PA (21) by the human. Likewise at a dose of 1.5 mg of pyridoxine 50% of intake was excreted as 4-PA (22). The 4-PA-forming enzymes aldehyde dehydrogenase and aldehyde oxidase are widely distributed in animal tissues including erythrocytes (23) so an increase in the freely diffusible pool of PL in the blood should result in increased metabolism to 4-PA. This mechanism would lead to the increased 4-PA excretion observed in those rats with reduced erythrocyte volumes.

REFERENCES

1. Mehansho, H., and Henderson, L. M. (1980) J. Biol. Chem. 255, 11901–11907
2. Ink, S. L., Mehansho, H., and Henderson, L. M. (1982) J. Biol. Chem. 257, 4753–4757
3. Anderson, B. B., Newmark, P. A., and Rawlins, M. (1974) Nature (Lond.) 250, 502–504
4. Fonda, M. L., and Harker, C. W. (1982) Am. J. Clin. Nutr. 35, 1391–1399
5. Stanulovic, M. (1980) in Vitamins, Metabolism and Role in Growth (Tryfiates, G. P., ed) pp. 115–136, Food and Nutrition Press, Westport, CT
6. Solomon, L. R. (1982) Enzyme (Basel) 28, 242–250
7. Mehansho, H., Hamm, M. W., and Henderson, L. M. (1979) J. Nutr. 109, 1542–1551
8. Antonini, E., and Brunori, M. (1971) Hemoglobin and Myoglobin in Their Reactions with Ligands, pp. 19 and 46, North-Holland Publ. Co., Amsterdam
9. Hems, R., Ross, B. D., Berry, M. N., and Krebs, H. A. (1966) Biochem. J. 101, 284–292
10. Mehansho, H., Buss, D. D., Hamm, M. W., and Henderson, L. M. (1980) Biochim. Biophys. Acts 631, 112–123
11. Cooper, J. G. (1977) The Tools of Biochemistry, p. 54, John Wiley and Sons, New York
12. Dempsey, W. B., and Chrisiensen, H. N. (1962) J. Biol. Chem. 237, 1113–1120
13. Steinhardt, J., and Reynolds, J. A. (1969) Multiple Equilibria in Proteins, Academic Press, New York
14. Hedlund, B., Danielson, C., and Lovrien, R. (1972) Biochemistry 11, 4660–4668
15. Colombini, C. E., and McCoy, E. E. (1970) Biochemistry 9, 533–538
16. Benesch, R. E., Benesch, R., Renthai, R. D., and Maeda, N. (1972) Biochemistry 11, 3576–3582
17. Bunn, H. R., and Higgins, P. J. (1981) Science (Wash. D.C.) 213, 222–224
18. Savitz, D., Sidell, V. W., and Solomon, A. K. (1964) J. Gen. Physiol. 48, 79–94
19. Nutrient Requirements of Laboratory Animals (1978) No. 10, National Academy of Sciences, Washington, D.C.
20. Contractor, S. P., and Shane, B. (1971) Biochim. Biophys. Acta 230, 127–136
21. Rabinowitz, J., and Stell, E. E. (1949) Proc. Soc. Exp. Biol. Med. 70, 235–240
22. Kelsay, J., Baysal, A., and Linkswiler, H. (1968) J. Nutr. 94, 490–494
23. Stanulovic, M., Jeremic, V., Leskovac, V., and Chaykin, S. (1976) Enzyme (Basel) 21, 357–369

dialysis bag and the distribution of PL in the dialysant and dialysate. The formation constant found in these dialysis studies (730 m⁻¹) is lower than that reported for the binding of PLP to oxyhemoglobin (16) which was 2.2 × 10⁵ m⁻¹. However, the competition of 2,3-diphosphoglycerate and other compounds for the β-chain site where PLP binds, reduces the concentration of PLP in the erythrocyte below that predicted from the formation constant. Competition with PL for the α-chain NH₂ terminus was investigated using compounds known or suspected to bind to this subunit (17). Glucose and galactose had little or no effect on the distribution of PL in the dialysis system. PLP or acetaldehyde caused a slightly lower in-out concentration ratio for PL than was predicted from the models, but the values were not lower than controls with the same low concentrations of hemoglobin. The results with glucose and PLP are in agreement with previous conclusions (2) based upon experiments with intact erythrocytes.

Plasma proteins may bind PL and thereby reduce the accumulation of PL in the red cells. The presence of albumin in the dialysate reduced the accumulation of PL in the hemoglobin compartment as predicted from the formation constants of each protein for PL. The calculated distribution of PL between the erythrocytes and plasma in whole blood, based upon the competitive binding equations of Steinhardt and Reynolds (13) using the experimentally determined binding constants, was 6.5:1. The ratio determined in whole human blood varied from 4 to 5 in several experiments. This deviation from the calculated value cannot be explained by the effect of other plasma proteins, since albumin alone gave evidence of PL binding in separate equilibrium dialysis experiments. Two other explanations can be offered for the reduced concentration ratio for whole blood. The washing of the pelleted red cells with isotonic saline at 4°C as was done in these studies may remove some of the intracellular PL. The temperature sensitivity (1) of the transport of PL through erythrocyte membranes would suggest that this is not a major cause for leakage. The second factor might be the water of solvation. Not all of the water space in cells is available for equilibration of solutes (18). When the concentration of PL in the erythrocyte is expressed as a function of the volume of intracellular water of solvation, the concentration ratio for whole blood would be corrected upward by a factor of 1.3. This correction would bring the ratio to a value in good agreement with the ratio generated from the binding model. Therefore, these data suggest that hemoglobin and plasma albumin are competing binders of PL and that these proteins alone determine the distribution of PL between red cells and plasma.

The liver perfusion experiments demonstrated that erythrocytes in the perfusate reduce the uptake of PL by the liver. This was presumably caused by the binding to hemoglobin inside the erythrocytes, thus effectively reducing the concentration of PL in the plasma. The major change in the pattern of labeled metabolites in the liver caused by the lack of erythrocytes was the increase in 4-PA from 1.2 to 4% of the dose. The liver has the capacity to oxidize PL to 4-PA via a nonspecific aldehyde oxidase and is the organ most responsible for this metabolic inactivation of vitamin B₆. The presence of erythrocytes in the perfusate may be reducing the PL available for metabolic inactivation in the liver.
Effect of binding to hemoglobin and albumin on pyridoxal transport and metabolism.
S L Ink and L M Henderson

J. Biol. Chem. 1984, 259:5833-5837.

Access the most updated version of this article at http://www.jbc.org/content/259/9/5833

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/259/9/5833.full.html#ref-list-1