TABLE I. Mean Values Obtained with Chicks of Various Ages.

Age, days	No. of chicks	Body wt, g	Plasma vol, % B. wt	Blood vol, % B. wt	Thiocyanate space, % B. wt	Hematocrit, %
13	7	121	6.7	9.9	40.7	32.2
20	6	190	6.3	9.1	39.9	31
27	7	279	7.2	10.4	43.8	30.6
35	12	411	6.4	9	46.2	30.4
Mean ± Stand. error of mean			6.56 ± .15	9.55 ± .21	43.28 ± .78	30.47 ± .20

the subsequent 10 minutes.

Results. The mean values obtained from 32 chicks which were examined in groups of different ages are shown in Table I. The values are essentially constant when expressed as percentage of body weight regardless of the age or weight of the chick. In earlier studies with rats(2), most of the fluid volumes did not remain constant at different ages when expressed as percent of body weight. However, only a relatively small range of age has been studied with chicks and these values probably will not hold for much older birds. If the present values are compared to those obtained with young rats(2), the blood volume is approximately the same but the plasma volume is larger because of the smaller red cell volume. The thiocyanate space is much larger than was found in rats and is similar to that obtained by Fellers *et al.*(3) for very small babies. This might be expected since the smallest rats studied, 60-to-70 grams, are much older actually and relatively than the chicks used in this work. Data on suckling rats which might be comparable have not been reported.

Summary. The blood and plasma volumes and thiocyanate space in normal chicks from 13 to 35 days of age have been determined.

2. Wang, C. F., and Hegsted, D. M., *Am. J. Physiol.*, 1949, v156, 218.

3. Fellers, F. X., Barnett, H. L., McNamara, H., and Hare, K., *Federation Proc.*, 1948, v7, 32.

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Demonstration that Para-aminohippuric Acid "Enters" the Human Erythrocyte.* (18993)

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Para-aminohippuric acid (PAH) has been found to penetrate the canine erythrocyte(1), but its entrance into the human erythrocyte has been denied(2-4). This report demonstrates that PAH does "enter" the erythrocyte in man.

Recent studies in this laboratory of renal blood flow (RBF) in man included a com-

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1. Phillips, R. A., Dole, V. P., Hamilton, P. B., Emerson, K., Jr., Archibald, R. M., and Van Slyke, D. D., *Am. J. Physiol.*, 1946, v145, 314.

2. Smith, H. W., Finkelstein, N., Aliminosa, L., Crawford, B., and Graber, M., *J. Clin. Invest.*, 1945, v24, 388.

3. Bradley, S. E., and Halperin, M. H., *J. Clin. Invest.*, 1948, v27, 635.

4. Barker, H. G., Clark, J. K., Crosley, A. P., Jr., and Cummins, A. J., PROC. SOC. EXP. BIOL. AND MED., 1949, v72, 616.

parison of the RBF determined by renal extraction (E) of PAH from whole blood with the RBF determined by E from plasma and the hematocrit. The latter calculation was noted to be consistently higher than the former in experiments in which the renal venous plasma was not separated from the cells immediately. In consequence the ratio of PAH in the whole blood cell water to that in the whole blood plasma water was determined. The experiments cited below show conclusively that PAH is present in the cellular fraction of whole blood. No evidence is presented for its actual location but its presence in the erythrocyte is assumed for simplicity of discussion. The practical point is the observation that PAH enters the cellular fraction, not the specific type of cell or cells involved. Since PAH is almost completely extracted from the plasma passing through normal human kidneys(5), extraction will be falsely low and renal plasma flows (RPF) will be falsely high if cell-plasma separation is delayed sufficiently to allow PAH to diffuse from the cells into the plasma. The unlikelihood of PAH extraction from the erythrocyte during the passage of blood through the kidney has been discussed previously(1).

Methods. (1) *Technics.* The analytical technics employed were the same as those previously described(1) except that in the present studies a Beckman DU Spectrophotometer was used. Arterial samples were drawn from an indwelling needle(6) and simultaneous renal venous samples were obtained from an indwelling catheter(5). After the priming infusion of PAH, a sustaining infusion was maintained for at least one hour before collection of the first blood and urine samples. This was done in order to assure a steady state. Observations for several hours at arterial plasma levels of PAH *circa* 2 mg % have been analyzed (Table I). In other experiments (Table II) arterial plasma concentration was elevated to 80-100 mg % for an hour or more before the first blood and urine samples were obtained. After these were taken the sustaining infusion was changed to one containing no PAH, and blood and urine samples were collected over the subsequent 2-3 hours. (2) *Calculations.* Hematocrits were determined as described previously(1) after centrifugation for one hour in Wintrobe tubes. The correction factor of Chapin and Ross(7), 8.5%, was applied to the observed cell column to obtain true cell volume (V_c). The true plasma volume (V_p) per volume of whole blood $= 100-V_c$. From the plasma and whole blood specific gravities(8), values for the hemoglobin concentration in whole blood (Hb_b) and the protein concentration in the plasma ($Prot_p$) were obtained. The hemoglobin concentration in the cells (Hb_c) was obtained from the formula $Hb_c = \frac{Hb_b}{V_c} \times 100$.

The apparent specific volume of protein, both plasma protein and cellular hemoglobin, was assumed to be 0.74(9). Values for cell water (C_{H_2O}) and plasma water (P_{H_2O}) are not included in the tables but were calculated as $100-(Hb_c \times 0.74)$ and $100-(Prot_p \times 0.74)$, respectively. The PAH content of the plasma fraction of 1 liter of whole blood was obtained by multiplying the plasma concentration by $\frac{V_p}{100}$. This value subtracted from PAH concentration in whole blood was recorded as the PAH content in the cell fraction of the whole blood. The cell fraction value divided by $\frac{V_c}{100}$ was taken as the cell concentration in mg/l. The PAH concentration in cells $\times \frac{100}{C_{H_2O}}$ was taken as the PAH concentration in cell water ($PAH_{c_{H_2O}}$). $PAH_{p_{H_2O}}$ was obtained by substituting p values for c

5. Warren, J. V., Brannon, E. S., and Merrill, A. J., *Science,* 1944, v100, 108.

6. Wood, E. H., Montgomery, G. E., Jr., and Geraci, J. E., *Fed. Proc.,* 1948, v7, 137.

7. Chapin, M. A., and Ross, J. F., *Am. J. Physiol.,* 1942, v137, 447.

8. Phillips, R. A., Van Slyke, D. D., Hamilton, P. B., Dole, V. P., Emerson, K., Jr., and Archibald, R. M., *J. Biol. Chem.,* 1950, v183, 305.

9. Adair, G. S., and Adair, M. E., *Proc. Roy. Soc. London, Series B.,* 1936, v120, 422.

TABLE I. Data Demonstrating That PAH "Enters" the Human Erythrocyte at Low Plasma Arterial PAH Concentrations.

Sample	Time, min	V_c cc/100 cc	V_p cc/100 cc	Hb_c g/100 cc	$Prot_p$ g/100 cc	PAH concentrations in mg/l: Whole blood	Plasma	Cells	Plasma fraction of whole blood	Cell fraction of whole blood	Plasma water	Cell water	$\frac{PAH_c}{PAH_p}\frac{H_2O}{H_2O}$
A2	130	33.8	66.2	32.9	7.1	17.15	25.21	1.33	16.70	.45	26.62	1.76	.066
3	142	33.3	66.7	32.8	7.2	17.26	25.74	.27	17.17	.09	27.18	.36	.013
	149-159		1 liter of dextran infused										
4	170	28.2	71.8	33.2	7.05	14.55	19.86	1.06	14.25	.30	20.93	1.41	.067
5	180	28.4	71.6	33.6	7.05	16.12	22.09	1.09	15.81	.31	23.29	1.45	.062
6	283	32.4	67.6	34.6	6.6	20.27	29.29	1.45	19.80	.47	30.80	1.95	.063
7	297	32.4	67.6	34.6	6.6	20.42	29.49	1.51	19.93	.49	31.00	2.03	.065
RV2						1.38	1.54	1.06	1.02	.36	1.63	1.40	.859
3						1.38	1.54	1.05	1.03	.35	1.63	1.38	.847
4						.98	1.28	.21	.92	.06	1.35	.28	.207
5						.98	1.28	.21	.92	.06	1.35	.28	.207
6						1.68	1.87	1.26	1.27	.41	1.97	1.69	.858
7						1.80	1.87	1.64	1.27	.53	1.97	2.20	1.116

TABLE II. PAH Concentration in Human Erythrocyte at High Plasma Arterial PAH Concentrations; Demonstration of the Slow *In Vivo* Diffusion of PAH from Cells to Plasma.

Sample	Time, min	V_c cc/100 cc	V_p cc/100 cc	Hb_c g/100 cc	$Prot_p$ g/100 cc	PAH concentrations in mg/l: Whole blood	Plasma	Cells	Plasma fraction of whole blood	Cell fraction of whole blood	Plasma water	Cell water	$\frac{PAH_c}{PAH_p}\frac{H_2O}{H_2O}$
A 2	183	38.2	61.8	35.9	7.33	588.5	874	127	540	48.5	923	173	.188
13	326	35.7	64.3	35.2	6.37	29.4	22.2	42.45	14.25	15.15	23.3	54.4	2.46
	332-343		1 liter 6% albumin infused										
15	433	30.7	69.3	36.4	6.67	481.5	652	96	452	29.5	686	131	.191
27	566	30	70	36.7	6.48	22.35	18.5	31.35	12.95	9.4	19.45	43	2.21
RV 2						459.5	656	142	405	54.5	693	193.5	.279
13						17.95	4.35	42.45	2.8	15.15	4.57	57.4	12.55
15						368	505	60	349.5	18.5	531	82	.154
27						11.75	2.9	32.35	2.05	9.7	3.05	44.4	14.55

values in this paragraph. (3) *Other points.* Specific gravity and hematocrit determinations were done only on arterial blood and these values were assumed for the renal venous samples. No great error could arise from this practice in these experiments for urine flow was usually less than 0.3% of RBF. The highest ratio obtained when the urine flow was 9 cc per minute and RBF was over 1800 cc per minute. Validation of the accuracy of the plasma and whole blood analyses was proven by a technic to be described elsewhere(10). The other essential point was the manner of handling the renal venous samples. They were quickly drawn (15-30 seconds) into chilled, oiled syringes, transferred rapidly to lusteroid tubes containing a dry film of heparin, inverted a few times, centrifuged in a refrigerated centrifuge at 2800 rpm for 5 minutes and the plasma immediately removed from the cells.

Results and discussion. In arterial blood at low PAH concentration, *circa* 3 mg per 100 cc, the ratio $PAH_{c_{H_2O}} : PAH_{p_{H_2O}}$ averaged about 0.06 in over 20 bloods from 4 patients; the ratio in simultaneously drawn renal venous samples was over 0.8. Representative examples are given in Table I. The inherent error (up to 1%) in the analytical methods would not permit detection of lower ratios.

Proof that PAH does "enter" the human erythrocyte does not depend solely on the results obtained from calculation of the ratio $PAH_{c_{H_2O}} : PAH_{p_{H_2O}}$. In Table II it will be noted in both arterial and renal venous samples numbered 13 and 27 that the whole blood actually contained more PAH than did the plasma. In this instance the values are well beyond experimental error, and similar results have been observed in other experiments.

Once PAH has entered the erythrocyte it leaves it slowly, *cf.* arterial values in the last column of Table II. It is obvious from comparison of the final column of Tables I and II that in the experiment cited in Table II, the diffusion gradient from cells to plasma in renal venous blood when the arterial plasma PAH has reached 2 mg % has been increased 10-20 fold over that prevailing in the steady state, Table I, and that the cell concentration of PAH in renal venous blood is 200 times that which will be present when diffusion equilibrium is attained.

An example of the magnitude of error that will occur in plasma extraction values if renal venous plasma is not separated immediately is taken from the experimental data in Table II. If renal venous samples 13 and 27 were allowed to stand until diffusion of PAH from cells to plasma has proceeded to the point where the ratio in the simultaneously drawn arterial sample prevailed, then the observed PAH plasma extractions would be 23 and 24% respectively, or 28.5% of the observed values in this individual with mild renal damage. Likewise calculation of RPF would be nearly 4 times the true value.

Conclusions. (1) Contrary to previous reports in the literature para-aminohippuric acid (PAH) has been found to "enter" the human erythrocyte at low and high plasma PAH concentrations. (2) It is pointed out that PAH plasma extraction will be falsely low and renal plasma flow estimation from the extraction values will be falsely high unless renal venous plasma is separated *as soon as is possible* after the blood leaves the kidneys. An alternate procedure is to determine the whole blood extraction and estimate the plasma extraction with the aid of the hematocrit.

10. Phillips, R. A., in press.

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