in this paper. In view of the high early mortality observed in swine the prophylactic use of vitamin B_{12} in these conditions should be investigated.

Summary. 1. Acute uremia of the newborn rat observed on rations in which a commercial soybean protein and DL-methionine furnish the only source of amino acids can be prevented by subcutaneous administration of 0.05 μg of vitamin B_{12} shortly after birth.

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The Volume Distribution and Equilibrium Time of Para-Aminohippurate.* (17517)

HAROLD G. BARKER,† JOHN K. CLARK,‡ ARCHER P. CROSLEY, JR.,§ AND ALVIN J. CUMMINS (Introduced by I. S. Ravdin)

From the University of Pennsylvania School of Medicine and Hospital, Philadelphia.

Sodium para-aminohippurate (PAH) is widely used for measurement of effective renal plasma flow and maximal tubular excretory capacity (Tm). Its volume distribution is of importance in studies dealing with rate of change in PAH plasma levels and excretion following single injections,(1-3) or with constant infusions before equilibrium has been established.(4,5) Information concerning volume distribution is also useful in calculating optimum priming doses for rapid achievement of the desired plasma level. The literature provides very few data on the PAH space. It has been stated to be 28% of body weight in the dog(6) and estimates of 4-5 liters(4) and 20 liters(5) have been made for man. We are therefore reporting its determination in man.

Methods. All patients were free of renal, cardiac, or other diseases which might be expected to alter fluid compartments. An intravenous priming dose of PAH was given over a 7-9 minute period and this was followed by a constant intravenous infusion for 1½-3 hours. In three patients the priming dose was omitted. Infusions (both priming and sustaining) were given at an extremely constant and accurately known rate by means of a motor driven worm screw pushing on the barrel of a 50 cc syringe. Urine was collected by catheter with bladder washing. The anticoagulant was heparin powder. Total PAH was determined according to Newman.(4) This method in our hands gives complete hydrolysis of acetylated PAH. Its use, in the cases reported resulted in no destruction of free PAH, as indicated by identical values in infusion solutions analyzed as free and total. The total amount of PAH excreted was subtracted from the total amount injected and this difference divided by the plasma level to arrive at the apparent volume distribution.

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† Harrison Fellow in Surgical Research.

‡ National Institute of Health Post-Doctorate Fellow.

§ Woodward Fellow.

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TABLE I.
Degrees of Equilibrium Attained with Various Durations of PAH Infusions without Priming Doses.

Patient PAH clearance PAH space	Time, min.	Plasma conc., mg %	Out/in ratio		
			Determined	Theoretical	Deter./theoret.
K.W.	83.2		.85	.86	.99
885 cc/min.	114	1.40	.90	.93	.97
37.1 L	143	1.45	.92	.97	.95
	175	1.47	.98	.98	1.00
J.D.	48.4		.82	.78	1.05
885 cc/min.	88.7	1.36	.87	.94	.93
28.0 L	112	1.44	.92	.97	.95
	141	1.46	.96	.99	.97
J.B.	37.1		.59	.63	.94
489 cc/min.	82.8	2.38	.89	.89	1.00
18.2 L	99.3	2.43	.88	.93	.95
	121	2.51	.95	.96	.99
	144	2.59	.95	.98	.97

As a criterion of equilibrium we have divided the rate of excretion in mg/min during a 15-20 minute period by the rate of injection in mg/min thus obtaining an "out/in ratio". As equilibrium is approached, this value approaches unity.

Results. Sixteen tests were done on 14 patients (all but 1 males, ages 11 to 50) and the volume distribution was calculated at 2 points in each test. The elapsed time at the first calculation point was 83 (68-121) minutes and at the second 130 (89-192) minutes. Most of the patients were excreting PAH in the urine at the same rate it was being injected (mean out/in ratio .96, S.D. .069, S.E. .017) by the end of the second period although not necessarily at the end of the first (mean .89, S.D. .094, S.E. .024) The mean apparent volume distribution at the end of the second period was 35.9% of body weight with a standard deviation of 7.3, a standard error of the mean of 1.8 and a range of 22.8 to 47.8. In terms of liters per square meter of body surface the mean was 13.4 with a standard deviation of 2.6, a standard error of the mean of 0.6, and a range of 9.1 to 17.7. All patients had priming doses except 3. Table I shows the time required in approaching equilibrium, in patients who received no primary dose. The percentage approach toward equilibrium is indicated by the out/in ratio (% of eventual plasma level is of course an identical concept). In Table I the figures for time intervals and plasma levels correspond to the mid point of the 15 to 30 minute urine collection period used in calculating the observed out/in ratios, whereas in space calculations figures corresponding to the end of the urine collection periods were used.

Discussion. The figure 36% of body weight lies between those reported for extra cellular fluid—15%(7) to 25%(8) and total body water—53%(7) to 70%,(9) indicating that PAH probably enters cells to some degree. It does not enter human red blood cells as several workers(10,11) have shown and we have confirmed. A recent statement(12) that human whole blood and plasma PAH concentrations are equal, was probably not intended and may have been an accident of wording. An alternative explanation to cell penetration might be that PAH reaches higher concentra-

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tions in some part of its volume distribution than in plasma. We have not considered in the present communication the question of expanding and shrinking apparent volume distributions. The mean space at the first calculation point was 34% and this rose to 36% by the time of the second calculation point (some 47 minutes later). This difference is statistically significant and could represent an expanding space. We are more inclined however to think that the finding arises from the fact that our priming dose in most cases was less than optimal and plasma equilibrium had not yet been achieved at the first calculation point. It is to be expected, of course, that any space calculation made before equilibrium has been achieved will be falsely low if the plasma level is rising and high if plasma level is falling. The magnitude of the volume distribution error is not known but it is probably small when the change in plasma level is occurring at a slow rate.

In practice of course, it is impossible to know what the eventual equilibrium plasma level will be and consequently one cannot detect the lack of equilibrium by comparing a specific plasma concentration with the eventual plasma level. It is known however what the eventual rate of excretion will be (*i.e.* equal to the rate of injection) and therefore examination of the out/in ratios is a far more useful index of equilibrium than judgments reached from comparison of several plasma determinations because, during a slow rise, the difference between successive samples may be no greater than the error of the method. For technical reasons equilibrium is desirable although not essential in all clearance determinations. If however, the rate of infusion is to be substituted for the rate of excretion,(5) then equilibrium is an absolute pre-requisite. Newman has suggested recently(4) that if the PAH prime be omitted entirely, equilibrium will be reached in 20-30 minutes. On the basis of out/in ratios however, our subjects did not seem to reach equilibrium under 2 hours even with a priming dose. It can be shown by substitution of our determined PAH space and reasonable clearance values for the average person in Newman's formula‖ that, when the priming dose is omitted, it should require 77 minutes to reach 90% of the eventual plasma level, 100 minutes to reach 95% and 153 minutes for 99%. Table I shows experimental values in close agreement with these calculations. Newman's estimate of 20-30 minutes equilibrium time was based on an erroneously low value of 4-5 liters for volume distribution.

We have not attempted to determine experimentally the exact size of the optimal priming dose for rapid achievement of equilibrium. It would appear from comparison of our data with and without priming doses that use of doses of 0.5 to 0.7 g injected over a period of 7-9 minutes does not appreciably hasten equilibrium over complete omission of a priming dose. It may be that use of a considerably larger dose would be of value. Berger, *et al.*(5) seem to have had some success with priming doses of 0.8 g, frequently reaching satisfactory equilibrium in 60-90 minutes.

Summary and conclusions. 1. The apparent volume distribution of PAH is 35.9 (S.D. ± 7.3) % of body weight.

2. If the priming dose is omitted and the plasma level allowed to rise slowly during an intravenous infusion of PAH, the plasma concentration appears to reach about 90% of its eventual level in 75 minutes, 95% in 100 minutes, and 99% in 150 minutes in the average patient, so long as the plasma concentration remains below that of beginning saturation of the tubular excretory mechanism.

‖ $P = \frac{IV}{C}(1 - e^{Ct/Vc})$ where P is the plasma concentration, IV is the infusion rate, C is the clearance, t is time and Vc is the apparent volume distribution.

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