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Determination of *in vivo* Distribution Volumes in Man With Non-reactive Compounds[1]

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INTRODUCTION

Recently Christensen and co-workers (1) reported on the distribution of the non-metabolizable amino acid α-amino*iso*butyric acid (AIB) in man. They described a method by which the apparent distribution volume of AIB-C^{14} was determined after its intramuscular injection. The same method was later applied by Kowalczyk (2) in the study of various endocrine disturbances, renal failure, and growth. These authors collected all urine for a standard interval of 19 hours after the intramuscular injection of the labeled AIB. At this specified interval a plasma sample is drawn, counted for radioactivity and the apparent volume of distribution is calculated, that is, the volume required to contain the AIB not yet excreted if concentration were uniformly that found in plasma. In one patient an increase of the distribution volume of AIB on the second day over that of the first day was noted. From this experiment Christensen and co-workers (1) concluded that AIB displayed a continued gradual entrance into less accessible compartments and that the increase in the distribution volume could be explained, at least in part, by the neglect of minor routes of AIB loss.

In the present investigation an intravenous route of injection was used and the apparent distribution volumes of AIB were calculated in two ways as will be discussed later (equations 1 and 2); henceforth, these spaces will simply be referred to as distribution volumes. Our original goal was to investigate the magnitude of the AIB distribution volume in patients with some forms of mental retardation, especially phenylpyruvic oligophrenia and mongolism. It became apparent, however, as the work progressed, that some conflict of data existed in the calculation of the distribution volumes. The main purpose of this paper is to report these conflicts.

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EXPERIMENTAL

All patients selected for these experiments were young adults. From 0.15 to 0.50 μmoles of AIB-C^{14} per kg. body weight, specific activity between 2.9 and 5.2 μC per μmole, were injected intravenously in the morning after a light breakfast. The radioactive AIB solution was autoclaved in ampoules at 120°C. for 20 minutes. After injection, heparinized blood samples were collected at definite intervals, that is; 5 minutes, 2 hours, 4 hours, 6 to 7 hours, 12 hours, and every 24 hours thereafter for the next 6 to 10 days. The blood samples were drawn and centrifuged at once and 0.5 ml. aliquots of the supernatant fluid were plated on stainless steel planchets. Urine specimens were collected at various intervals for the first 24 hours and thereafter at 24-hour intervals. Urine aliquots were also plated on stainless steel planchets and dried. The radioactivity of the samples was measured in a Gas Flow Counter with micromil window, corrected for self-absorption and expressed in counts per minute as specified in the figures and plotted on semilogarithmic paper. The lines were drawn by visual inspection.

The self absorption factor for radioactivity in plasma was determined individually for each patient by adding a known amount of radioactivity to the plasma obtained before the injection of AIB.

AIB-1-C^{14} was purchased from Isotopes Specialties or New England Nuclear Corporation. The manufacturers gave evidence of no detectable radio impurities in one-dimensional paper chromatograms with butanol-acetic acid-water (4:1:1). This finding was confirmed in our experiments wherein one-dimensional chromatograms were carried out with n-butanol-acetic acid water

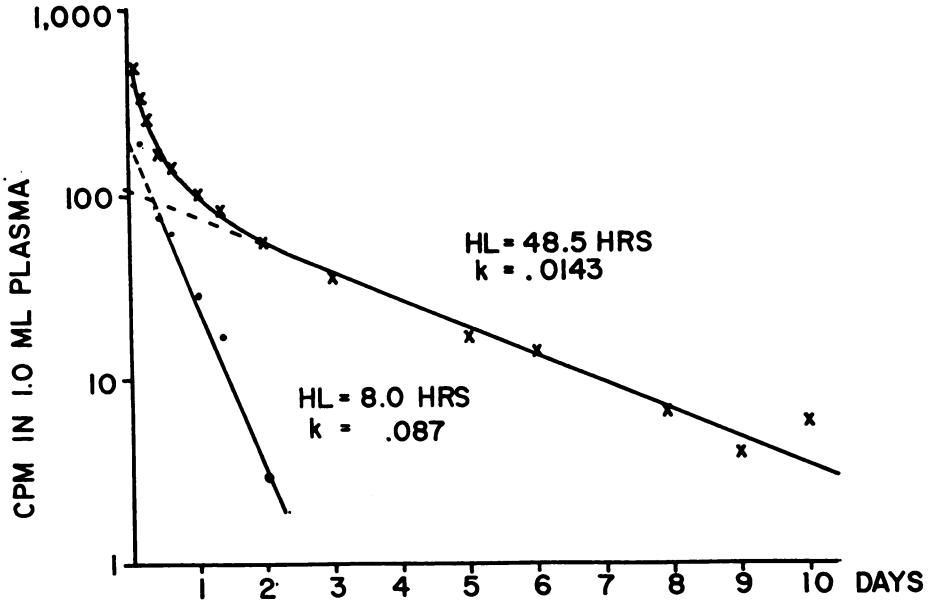

Fig. 1. Disappearance of AIB-C^{14} from plasma in the hypogonadic patient #37. X - X - X experimental data, - . - . - . values obtained by graphical analysis.

(5:1:4) and with n-propanol water (8:2). In order to exclude the presence of substantial amounts of carbonate-C^{14}, an appropriate quantity of the AIB-1-C^{14} was incubated in a closed system with a small amount of non-labeled $NaHCO_3$ as carrier. A well was suspended containing hyamine hydroxide (Packard) for the absorption of CO_2. 0.2 ml of 3 *N* sulfuric acid was injected into the main chamber through the rubber stopper. The incubation was started at once in a metabolic shaker at room temperature for 20 minutes. The content of the well was counted for radioactivity in a liquid scintillation counter.

RESULTS AND DISCUSSION

The rate of AIB disappearance from plasma is illustrated in Fig. 1. The slope of the straight line segment of the experimental curve was in agreement with a process of a first-order reaction. The time required for equilibration of AIB among various compartments was usually from 12 to 24 hours as judged from the time of appearance of the final straight line in plasma.

The spaces were calculated either by equation 1:

$$\text{distr. vol.}_1 = \frac{\text{radioactivity injected}}{\text{radioactivity in 1.0 ml. plasma extrapolated to } t_0} \quad (1)$$

or by equation 2, in the way proposed by Christensen and co-workers (1):

$$\text{distr. vol.}_2 = \frac{\text{Radioactivity injected} - \text{accumulated radioactivity excreted into urine at } t_x}{\text{radioactivity in 1.0 ml. plasma at } t_x} \quad (2)$$

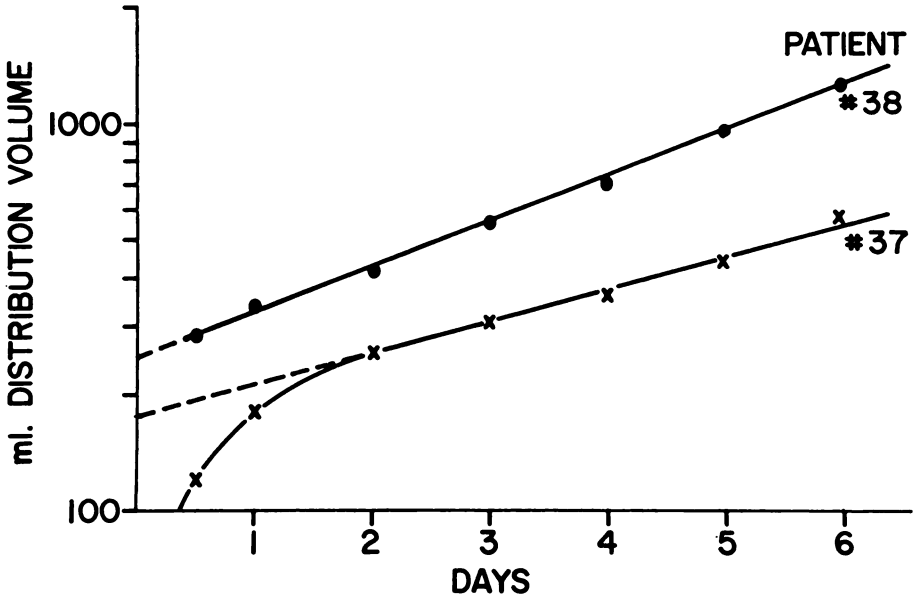

Fig. 2. shows the distribution volumes for 70 kg. body weight which were obtained in patient #37 and #38. The values were calculated by equation 2. The increase of the volumes was also found in previous observations; the magnitude of the extrapolated zero times volumes of these plots are reported in the text and in Table I.

where t_0 is zero time and t_x denotes any time after the injection of AIB. When equation 2 was used, steadily increasing distribution volumes of AIB were obtained even over a period of weeks (Fig. 2). The continued increase over such a long period of time seemed unlikely. Since calculations according to equation 2 require that the urinary removal of the injected substance be the sole source of elimination, we woundered how much of the injected AIB-C^{14} could be recovered in the urine. The hypogonadic patient #37 and the mongoloid patient #38, were subjects for these studies and the time of observation was extended to about three weeks until no significant amount of radioactivity was excreted (Fig. 3). The accumulated amount of labeled AIB found in urine was 83 and 74 per cent, respectively, of the dose injected. In further studies in which standard amounts of radioactivity was added to urine of various individuals, it was realized that our standard self-absorption curve did not correct sufficiently for the urinary excretion of radioactivity.

TABLE I

				Equation 1		*Equation 2*
No.	*Diagnosis of mental deficiency*	*Age*	*Sex*	*D.V./70 kg. AIB ml. × 10^5*	*Elimination constant of final slope $1/t_t$ (hrs)*	*D.V./70 kg. AIB ml. × 10^5 at t_o*
23	Familial Type	25	F	2.29	.0215	1.40
23A				3.08		
26	Familial Type	26	M	6.44	.0127	4.56
30	Chronic Brain Syndrome	22	M	2.62	.0230	2.00
31	Familial Type	23	F	2.30	.0134	1.97
32	Unknown Origin	33	F	2.62	.0139	1.83
33	Phenylketonuria	19	F	4.26	.0142	
34	Phenylketonuria	18	M	4.39	.0165	
35	Mongolism	27	M	2.63	.0099	2.28
36	Mongolism	21	F	2.61	.0158	1.73
37	Hypogonadism	24	M	2.96	.0143	1.78
38	Mongolism	18	F	3.68	.0152	2.40
Average				3.32		2.22

Summary of values obtained after the i.v. administration of AIB-C^{14}. The distribution volumes (D.V.) in column 5 were obtained according to Equation 1 and in column 7 according to Equation 2. Using various times for t_x after equilibration Equation 2 results in a straight line. Extrapolation of this line to the intercept permits the calculation of the hypothetical distribution volume at t_o as shown in Fig. 2.

The AIB-C^{14} did not contain any significant radioimpurity as evidenced by paper chromatography. Neither was there any evidence of contamination with C^{14}-labeled material which could have been easily removed as respiratory CO_2, nor was there any significant radioactivity of isolated and washed plasma proteins 12 hours after the injection. The fecal excretion of AIB-C^{14} in two patients was found not to exceed 0.35 per cent of the injected dose for the first 24 hours; these values are similar to those found in rats (3). Also, very little labeling of respiratory CO_2, that is 0.002 per cent of the injected dose, was demonstrated by Christensen and co-workers (1,3) for the first six hours after the injection of AIB-C^{14}. All these experiments indicate the non-existence of significant alternate routes of AIB-elimination, other than urine. If the radioactivity not recovered in urine (Fig. 3) is subtracted from the experimental curve, a line is obtained which has within the experimental error the same rate constant as the experimental disappearance curve in plasma. An additional component which approaches insignificantly low values within the first 24 hours can be demonstrated by a second subtraction. This finding shows that the initial rate of AIB elimination from serum into urine is the result of at least two exponential functions and not of a single exponential function only, as equation 1 implies. Therefore, the distribution volumes of AIB obtained from equation 1 are somewhat

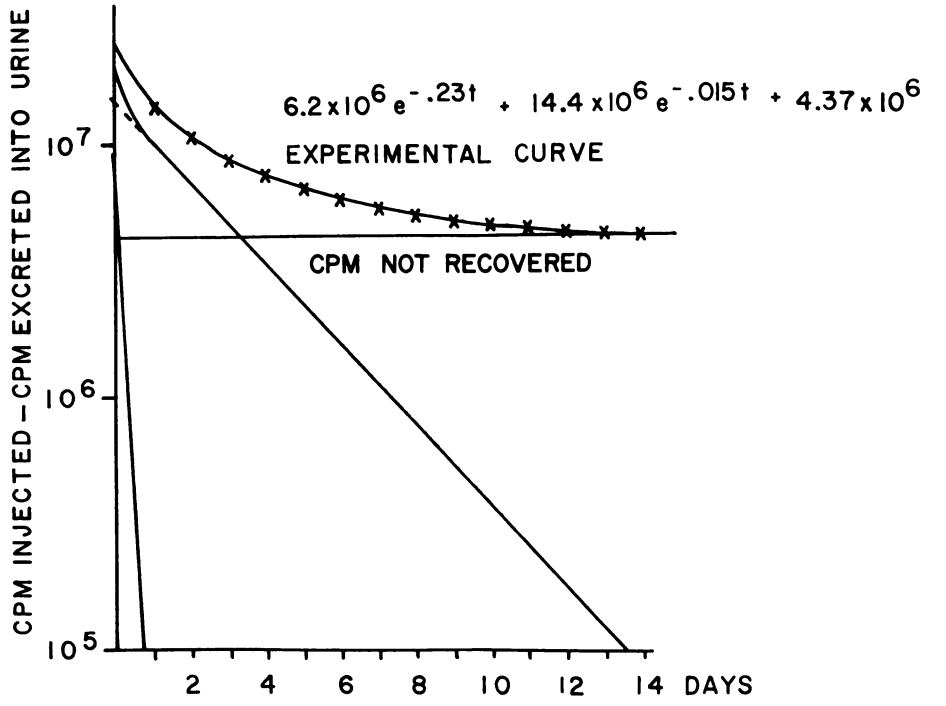

Fig. 3. Decline through urinary excretion of the amount of AIB in the body. Graphical analysis of the experimental curve (x-x-x) of the hypogonadic patient #37 reveals a curve with the value $14.4 \times 10^6 e^{-.015t}$. A second subtraction results in a component with the value $6.2 \times 10^6 e^{-.23t}$. t refers to time in hours. 25.0×10^6 counts per minute (cpm) of AIB-C^{14} were injected. For interpretation of these components see text.

larger than those obtained from extrapolation of equation 2 to zero time (Table 1).

CONCLUSION

The above experiments with a non-reactive compound are a warning not to rely solely on distribution volumes estimated at a single time interval, unless proof by other means has been obtained to do so. When using equation 2, the experiments are best performed over a sufficient period of time to permit the determination of the distribution volume by graphic extrapolation to zero time as shown in Fig. 2. In case the distribution volume according to equation 2 increases with time, the possibility of other metabolic routes or incomplete recovery of the compound in question should be suspected. If no complete urine specimens can be collected, equation 1 may be useful for comparative studies as long as they are performed under standardized conditions and no estimate of the absolute distribution volume is attempted. The spaces, according to equations 1 and 2, correlate reasonably well as shown in Table 1, justifying the use of equation 1 for comparing distribution volumes of a nonreactive compound in various clinical disorders. Since it may be difficult to collect complete urine specimens, as for instance, from mentally retarded patients, the use of equation 1 is then the only suitable approach to this particular problem.

SUMMARY

The apparent distribution volume of α-amino*iso*butyric acid-1-C^{14} in patients with various forms of mental retardation is reported. The elimination of radioactivity of intravenously injected AIB-C^{14} from plasma and into urine was followed up to 10 days and, in the case of two patients, until no significant radioactivity could be demonstrated in urine. The size of the apparent distribution volume was dependent upon which of two methods of calculation were used. Some procedures are discussed for overcoming the difficulties in determining the distribution volume of AIB or other non-metabolizable compounds *in vivo*.

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