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Research Article

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STUDIES OF I^{131}-ALBUMIN CATABOLISM AND DISTRIBUTION IN NORMAL YOUNG MALE ADULTS *

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Studies of I^{131}-albumin catabolism and distribution are appearing in rapidly increasing numbers in current literature. The data for normal human subjects reported from various laboratories differ considerably in mode of analysis and in results obtained. In normal subjects the observed "half-life" of I^{131}-albumin has ranged from below 10 to above 20 days (1–15), and the ratio of the amount of albumin contained extravascularly to that present in the plasma has been calculated as ranging from 1 to 4.5 (6, 7, 15–17). Although these wide variations are due to many factors, the major causes appear to be these: *1*) variable degrees of denaturation incurred during the preparation of the protein and its radioisotopic labeling, perhaps detectable only through biological testing; *2*) variable lengths of study periods, many being too short to allow metabolic trends to be fully realized; and *3*) differing mathematical approaches to the analysis of data, each requiring assumptions of variable validity. With the availability of improved I^{131}-albumin preparations, it can be anticipated that greater efforts will be directed toward the investigation of albumin catabolism and distribution in various disease states. In the present work, results are reported from 20 studies lasting 28 days each in 13 healthy young males, utilizing a specially prepared I^{131}-albumin. The data have been scrutinized as to compatibility with a number of differing models describing distribution and degradation in the body. The results have bearing on three important aspects of studies of this kind: *1*) the question of biological homogeneity of the labeled material; *2*) the question of the presence of irreversible trapping of iodine in the body; and *3*) the question of reproducibility and variation of the estimates in the normal male.

METHODS

Subjects

The subjects were 13 carefully selected white male students between the ages of 21 and 32. None had a history of metabolic disease, jaundice, or recent illness. They were not obese, nor did they have any significant abnormalities upon physical examination. Body weights, taken weekly, were constant during the study. Initial hematocrit (Wintrobe), urinary protein (sulfosalicylic acid), stool occult blood (guaiac), free electrophoresis of serum proteins, serum total protein (micro-Kjeldahl), serum albumin (based upon free electrophoretic analysis), sulfobromophthalein (BSP) excretion, and thymol turbidity determinations were normal for each. All but the BSP and thymol turbidity tests were repeated midway and at the end of each study, and showed no significant variation from the initial values. Each subject took orally 10 drops of a saturated solution of potassium iodide twice daily throughout the study to minimize thyroidal uptake of I^{131}.

Radioactive albumin

Albumin was obtained from the pooled plasma of three healthy young adult males. Approximately 2 years before donation for this study these donors had given transfusions with no ill effects upon the recipients. The albumin was isolated [1] by a modified Cohn fractionation

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[1] Generously prepared by Dr. John H. Hink, Jr. of Cutter Laboratories.

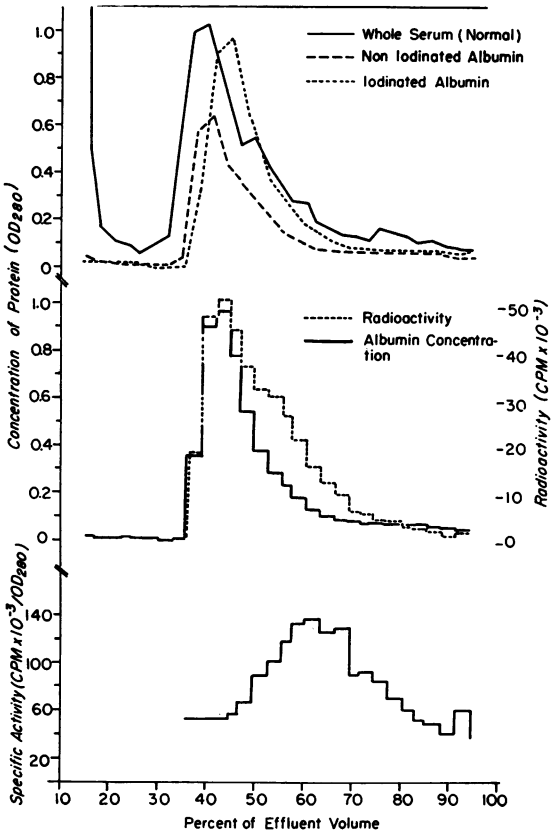

FIG. 1. DEAE CELLULOSE COLUMN CHROMATOGRAPHIC ANALYSIS (BY DR. JOHN L. FAHEY). Top: comparison of elution patterns of Cohn-fractionated albumin, before and after radioiodination, with albumin in normal whole serum. Middle: elution pattern of one representative preparation of radioiodinated albumin, showing the position of radioactivity relative to the position of protein. Bottom: elution pattern of specific activity of radioiodinated albumin, calculated from the middle figure.

method 6 (18), in which *heat treatment was specifically avoided.* By ultracentrifugation and free electrophoresis, the unlabeled albumin was found to be homogeneous. After diethylaminoethyl (DEAE) cellulose column chromatography,[2] this unlabeled protein appeared indistinguishable from albumin of normal whole serum (Figure 1). The albumin was radioiodinated[3] 5 days prior to administration by a modification of the T-4 method described by Steinfeld and associates (19). The final product contained 125 μc per ml (2.5 μc per mg), with a ratio of 1 atom of iodine to 5 molecules of albumin (molecular weight 65,000). By dialysis and chromatographic methods, more than 98 per cent of the radioactivity was shown to be bound to protein. Paper electrophoretic analysis showed the peak of radioactivity to closely approximate the position of the peak of albumin concentration, the maximum difference in migration being 6 mm after migration of 75 cm in 18 hours (Figure 2). A minute hump of unknown significance appeared on the trailing edge of the albumin peak in the free electrophoretic pattern after radioiodination (Figure 3). The peak of I^{131}-albumin concentration occurred one or two fractions later than that of the unlabeled albumin during elution from the DEAE cellulose column, indicating minimal alteration during the iodination (Figure 1, top graph), and slight nonhomogeneity of labeling.

Isotope administration, sample collection, and counting

After preparation of appropriate radioactivity standards, a weighed amount of I^{131}-albumin–saline solution, containing from 75 to 150 μc and less than 100 mg of albumin, was injected rapidly through a 25 gauge needle into an antecubital vein.

After the determination of plasma volume, described later, blood samples of 8 ml were drawn at approximately 12-hour intervals during the first 5 days, then daily until the fourth week, after which samples were drawn on alternate days for the remainder of the 28-day study period. The blood was transferred to glass tubes containing dry heparin, mixed, and centrifuged. Duplicate

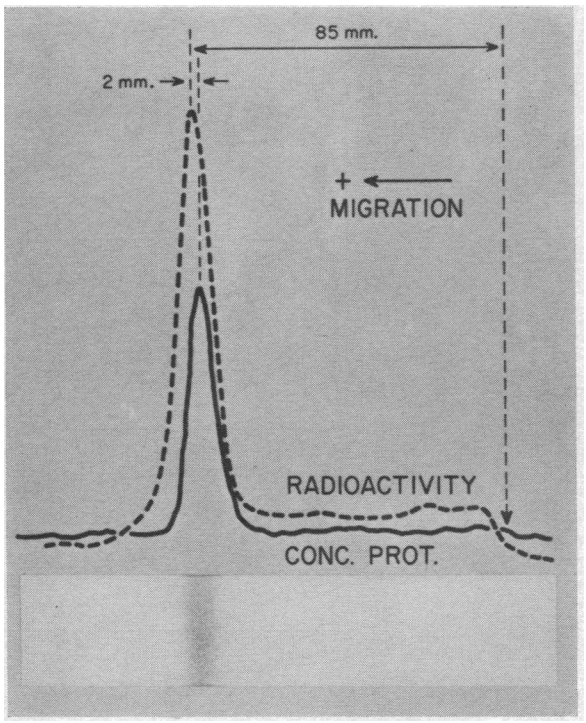

FIG. 2. ELECTROPHORETIC DISTRIBUTION OF ALBUMIN AND RADIOACTIVITY. Immediately after radioiodination, this product was diluted with a large amount of nonlabeled human albumin which was prepared from the plasma of different donors and which had been heat-treated after separation by Cohn fractionation.

[2] Kindly conducted by Dr. John L. Fahey of the National Cancer Institute.

[3] Abbott Laboratories.

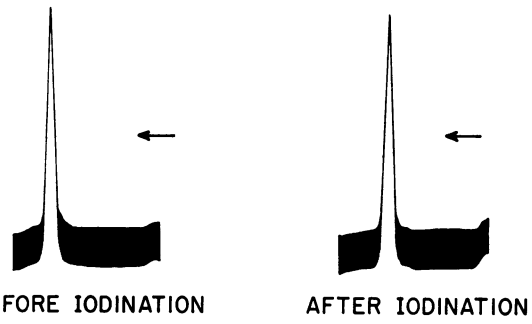

FIG. 3. FREE ELECTROPHORETIC PATTERN OF ALBUMIN SOLUTION. See legend to Figure 2 for details regarding albumin solution.

1-ml aliquots of plasma were transferred to 4-ml glass counting vials (Kimble 60910-L). The total amount of urine excreted during each interval was collected, measured, and mixed separately, and duplicate 4-ml aliquots were pipetted into the counting vials. Daily 24-hour creatinine excretion was determined as a measure of the completeness of urine collection.

All samples were counted with an error of less than 5 per cent by a Nuclear-Chicago scintillation detector (model DS-3), recording through a Tracerlab Auto-scaler (model SC 1 B). To correct for differences in counting geometry between the 1-ml plasma and 4-ml urine volumes, the urinary radioactivity was multiplied by a determined factor of 1.085.

Plasma volume measurement

The plasma volume was determined at the beginning of each study by the isotope dilution technique. After isotope administration, 12 minutes were allowed for rapid mixing, then 5 blood samples were drawn without stasis at exactly timed intervals of about 5 minutes each. The radioactivity per milliliter of plasma was plotted upon semilogarithmic graph paper as a function of time. Using the law of least squares, a straight line was fitted to these points and the extrapolated intercept value at zero time determined. The radioactivity per milliliter at zero time, thus determined, was then divided into the administered radioactivity to obtain the plasma volume.

Corrections for sampling

Deficits of radioactivity created by plasma sampling result in a spuriously rapid fall in plasma radioactivity if no correction for this artificial loss is made. Accordingly, each plasma radioactivity value was appropriately corrected subsequent to the plasma volume determination.

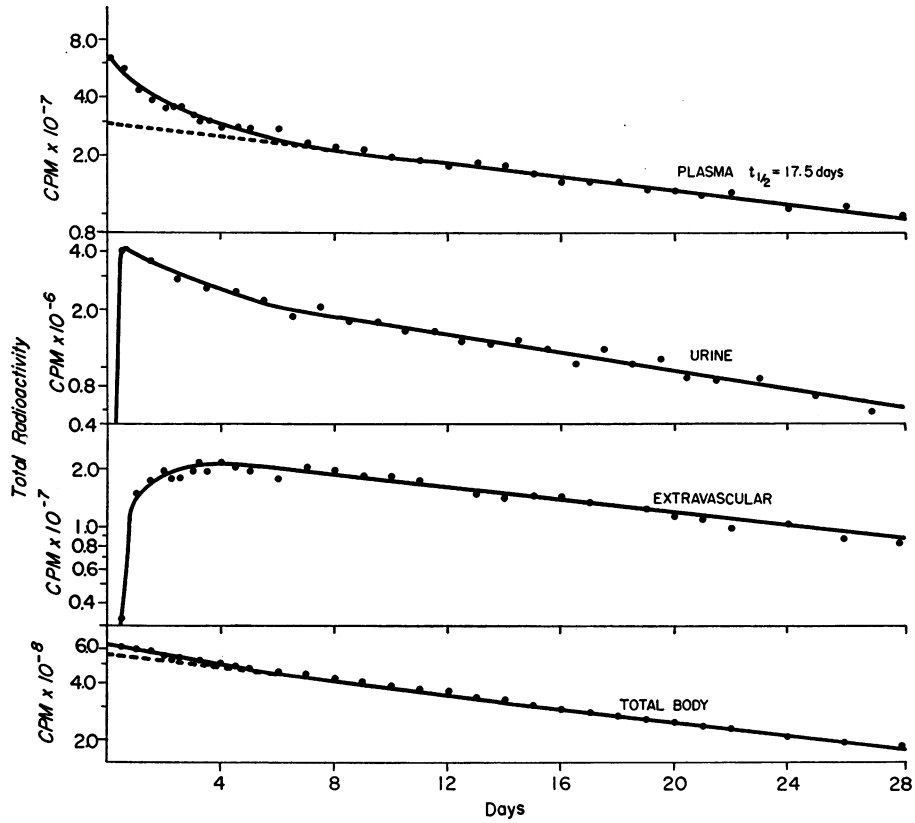

FIG. 4. RADIOACTIVITY CURVES OF I^{131}-ALBUMIN (SUBJECT 8). Plasma and urine radioactivity are observed data; extravascular and total body radioactivity are calculated.

This sampling deficit was ultimately shared by the total volume of I^{131}-albumin distribution. Therefore, the amount of radioactivity in each sample was divided by 2.5 times the plasma volume, an approximate theoretical total volume of distribution, assuming a uniform concentration of albumin extravascularly equal to that in the plasma. This gave an approximation of the radioactivity deficit per milliliter of plasma. Had the I^{131}-albumin removed in samples remained in the body, it would have been catabolized at the same rate as the I^{131}-albumin not removed. Therefore, each deficit was finally decreased exponentially at the rate observed for the first approximation of the catabolic portion of the uncorrected plasma activity curve. Samples taken for the plasma volume determination were not corrected in this manner because the time span over which these were drawn was too short for the effect of distribution and catabolism to appear. Since from 7 to 16 per cent of the plasma radioactivity was excreted daily in the urine, urinary radioactivity levels were corrected for the effect of plasma sampling by the addition of an arbitrary value of 10 per cent of the total plasma deficit to the appropriate urine radioactivity value. A mean maximum deficit of 3 per cent (range, 2 to 7 per cent) of the corrected value justified the necessity for these corrections (see Appendix).

Analysis of data

Plasma radioactivity was plotted as a function of time upon semilogarithmic graph paper. A single exponential began to emerge near the end of the first week. A straight line was statistically fitted (law of least squares) to this portion of the curve, and extrapolated to zero time (Figure 4). The plasma radioactivity values of the extrapolated line (exponential 1, Figure 5) were then subtracted graphically from the plasma radioactivity values of the initial nonlinear portion of the curve, and the differences used to construct a second curve (exponential 2). Since this derived curve was nonlinear in its initial portion, the process of extrapolation and graphical subtraction was repeated on this first derived curve to obtain a second derived curve (exponential 3), which in each instance appeared linear. In all studies, the plasma radioactivity curves could be thus reduced to a minimum of three exponential components.

Urinary radioactivity values for each of the 24- or 48-hour collection intervals were plotted in the middle of the respective interval (Figure 4).

Extravascular radioactivity was calculated for each interval from the equation: administered radioactivity − (cumulative urinary activity + total plasma activity) = extravascular radioactivity (Figure 4).

Total body radioactivity was represented as the sum of the plasma and extravascular radioactivity (Figure 4).

Urinary excretion rates were plotted upon linear graph paper, expressing daily urinary radioactivity as per cent of: *1*) total plasma activity, *2*) extravascular activity, *3*) total body activity, and *4*) total administered radioactivity (Figure 6).

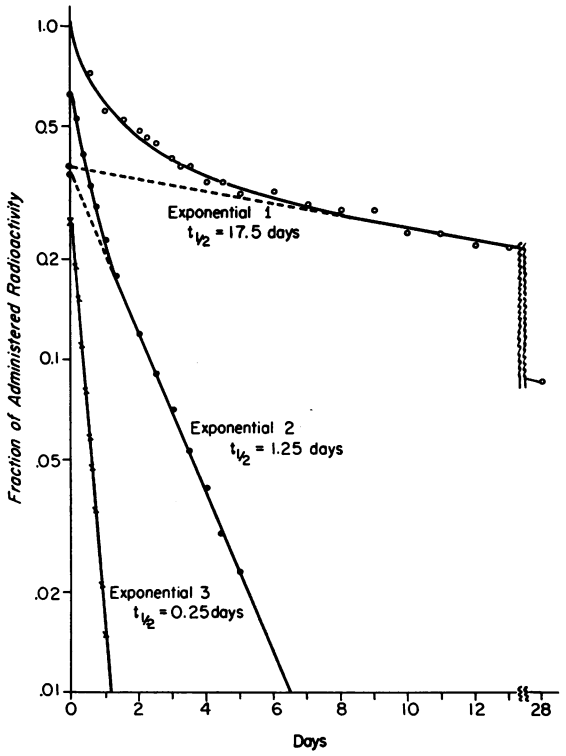

FIG. 5. GRAPHIC ANALYSIS OF PLASMA RADIOACTIVITY (SUBJECT 8). The nonlinear portion of the plasma curve (top) has been reduced to its constituent exponentials—1, 2, and 3. Open circles represent actual data points; other points were graphically derived.

Cumulative urinary excretion was examined for reflections of prolonged bodily iodide trapping by the mathematical approach of Lewallen, Berman and Rall (20). For each study, the per cent of administered dose excreted in the urine for each day was plotted semilogarithmically against time. The points of the data between days 10.5 and 27 fell upon a reasonably straight line, fitted to the points by freehand. The slope of this urinary curve was then determined, and the fraction of the administered dose accumulated in the urine at infinite time calculated. This was done by substituting into the equation of Lewallen and associates (20) the cumulative urinary excretion data for each day (Figure 12 and Table II). It was assumed that the slope of this "terminal" urinary curve was the terminal slope in each case; since observations were not continued beyond day 28, the validity of this assumption was not tested.

Albumin catabolic rates were calculated in three ways: *1*) as the product of the final slope of the plasma radioactivity curve ($0.693/t_{\frac{1}{2}}$) and total albumin mass (grams); *2*) as the product of the urinary radioactivity excretion rate on day 20 and the plasma albumin mass; *3*) as the product of the albumin mass of the assumed degradation compartment and the corresponding constant defining the rate of degradation.

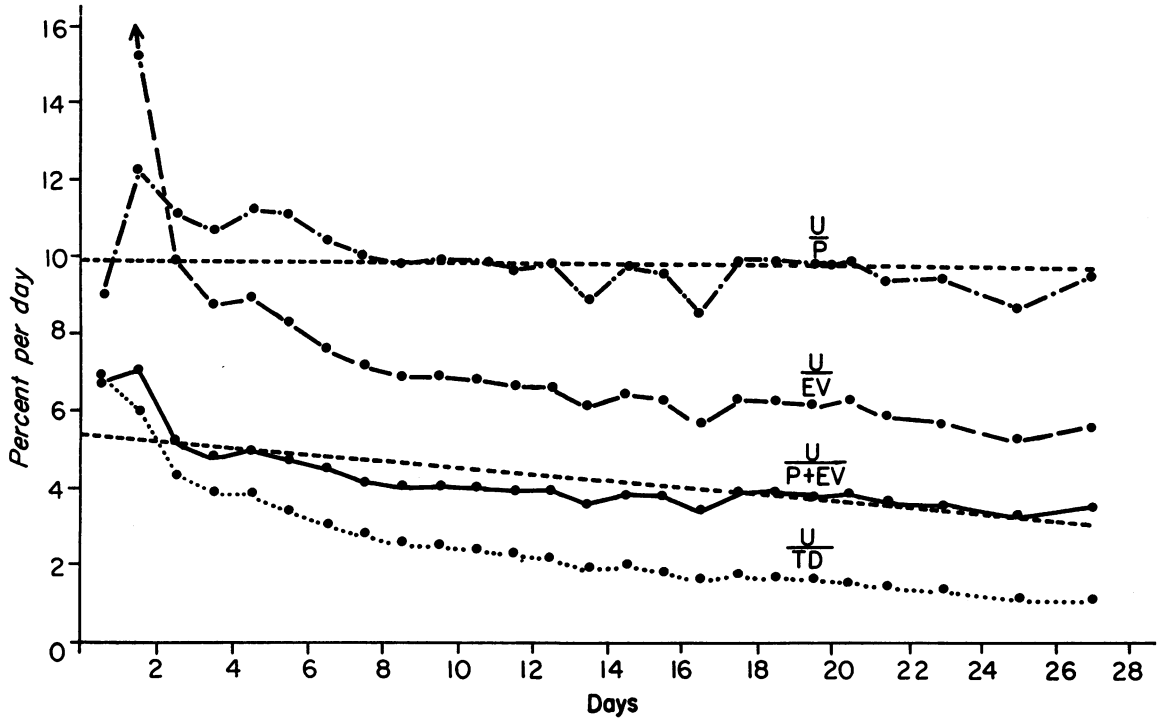

FIG. 6. URINARY EXCRETION RATES IN SUBJECT 2A. The numerators of the fractions represent observed 24- or 48-hour urinary radioactivity values. The denominators are values taken from smoothed radioactivity curves of the designated compartments. P = plasma, EV = extravascular, TD = total dose.

Distribution of albumin

Four methods were used to calculate the extravascular-intravascular (EV–IV) albumin mass distribution ratio: I) extrapolation method, II) equilibrium time method, III) activity distribution ratio method, and IV) multicompartmental analysis. The last method was additionally used to describe the configuration of the distribution system for the total bodily albumin.

I. *The extrapolation method,* as described by Sterling (1), requires extrapolation of the straight portion of the total plasma radioactivity curve to zero time to find the theoretical total plasma activity at the time of injection,

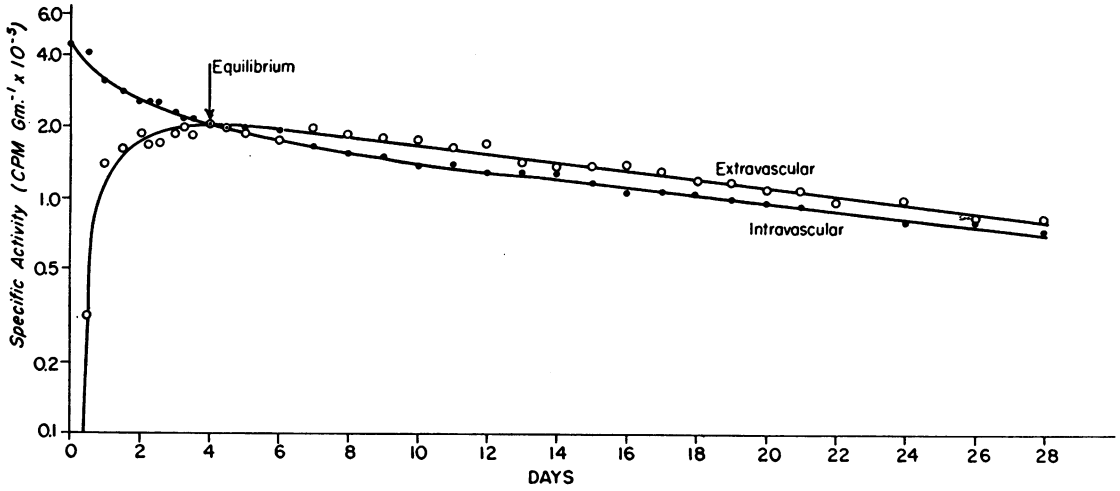

FIG. 7. EV–IV EQUILIBRIUM SYSTEM IN SUBJECT 8. Plasma specific activity was obtained from observed data. Extravascular specific activity was obtained by dividing the calculated total extravascular radioactivity by the extravascular albumin mass in grams, calculated by means of the equilibrium time method (8).

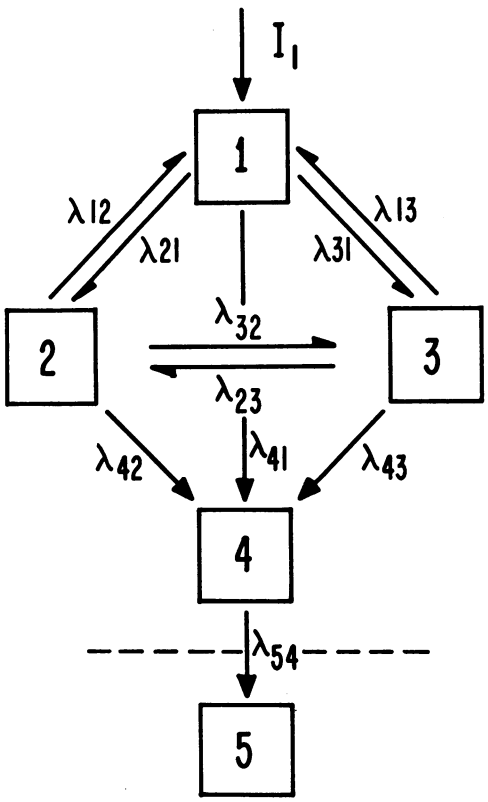

FIG. 8. THREE-COMPARTMENT REFERENCE MODEL. Pool 1 = plasma; pools 2 and 3 = extravascular compartments; pool 4 = iodide and iodinated tyrosine residues delay excretion pool; pool 5 = urine. I_1 = flow of newly synthesized albumin; λ = rate constant relating corresponding flows and pool sizes; ----demarcates limit of body pools. Specific models analyzed: model 1, $\lambda_{13} = \lambda_{31} = \lambda_{42} = \lambda_{41} = 0$; model 2, $\lambda_{13} = \lambda_{31} = \lambda_{42} = \lambda_{43} = 0$; model 3, $\lambda_{13} = \lambda_{31} = \lambda_{41} = \lambda_{43} = 0$; model 4, $\lambda_{23} = \lambda_{32} = \lambda_{42} = \lambda_{43} = 0$; model 5A, $\lambda_{23} = \lambda_{32} = \lambda_{41} = \lambda_{43} = 0$.

had there been instantaneous mixing and distribution throughout all body spaces (Figure 4). The extravascular activity at zero time is taken to be the difference between the administered activity and the total plasma activity at zero time. Provided that the distribution of radioactivity is identical to the distribution of albumin, the EV–IV radioactivity is proportional to the EV–IV albumin mass distribution ratio.

II. *The equilibrium time method,* as proposed by Campbell and associates (21) and McFarlane (8), derives the EV–IV albumin mass ratio from the corresponding radioactivity values at the time when the calculated content of radioactivity in the extravascular compartment is maximal (Figure 7). At this "equilibrium time," the specific activities of the albumin of both intra- and extravascular compartments are identical, and the rate of change of the plasma albumin specific activity is equal to the urinary excretion rate, the net transfer into and from the extravascular compartment being momentarily zero. This approach requires a simple (two-compartment) system with synthesis and catabolism proceding principally in the plasma space.

III. *The radioactivity distribution ratio method,* as described by Campbell, Cuthbertson, Matthews, and McFarlane (21), derives the EV–IV albumin mass ratio from the equation:

$$\frac{\text{urinary excretion rate} - \text{apparent replacement rate}}{\text{apparent replacement rate}} = \frac{\text{EV albumin mass}}{\text{IV albumin mass}}$$

where urinary excretion rate is taken as the total urinary radioactivity divided by the total plasma activity of the respective smoothed curves, late enough in the period of experimental observations to be free of the effects of initial distribution. In the studies herein presented, the values for day 20 in each experimental subject have therefore been arbitrarily selected for these calculations. The apparent replacement rate is $0.693/t_{\frac{1}{2}}$. This

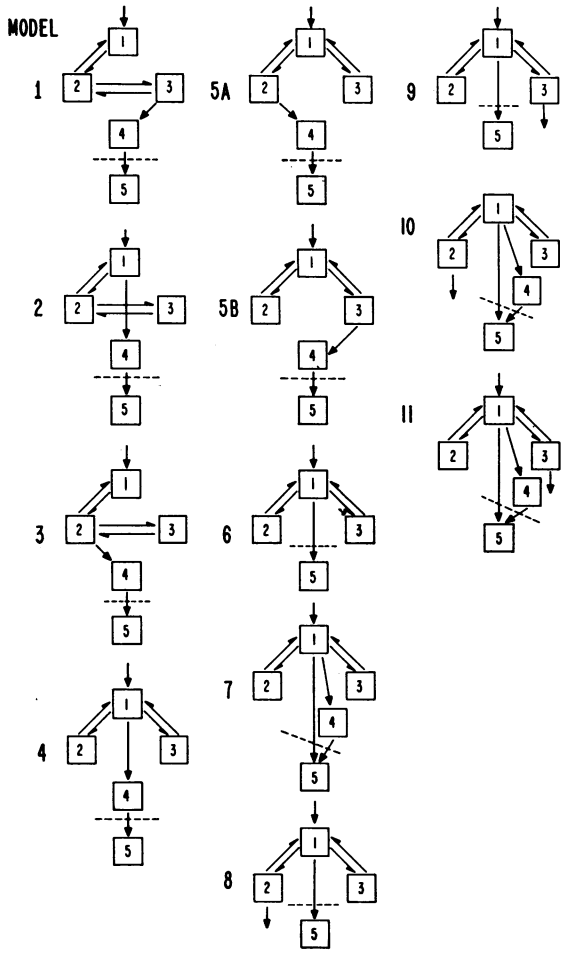

FIG. 9. SPECIFIC MODEL SYSTEMS ANALYZED.

TABLE I

Experimental data

Subject no.	Study period	Radio-albumin lot no. (Abbott)	Wt	Mean serum albumin	Plasma vol		IV albumin mass		Urinary excretion of administered radio-activity		
							Total		day 1	day 2	day 3
			kg	*g/100 ml*	*ml*	*ml/kg*	*g*	*g/kg*	%	%	%
1A	Nov.	1045-	80	4.43	3,030	37.9	134	1.68	7.66	7.22	5.07
2A	Dec. 1957	127-1	81	4.49	3,460	42.7	156	1.93	6.95	5.95	4.38
3	Jan.	1290-	71	4.61	2,880	40.6	133	1.87	5.37	5.94	4.48
4A	Feb. 1958	132-1	86	4.57	3,410	39.7	156	1.81	6.54	5.67	5.18
5	Mar.	1045-	90	4.40	3,650	40.6	161	1.79	9.04	7.47	6.68
6A	Apr. 1958	141-1	72	4.28	2,260*	31.4*	97	1.35	8.38	7.77	5.49
1B	Jul.	1045-	80	4.28	2,870	35.9	123	1.54	6.26	6.52	5.71
2B	Aug.	150	83	4.79	3,350	40.4	160	1.93	5.45	4.59	4.63
4B	1958		88	4.59	2,460*	28.0*	113	1.28	7.08	4.27	5.03
6B			72	4.37	2,840	39.4	124	1.72	6.32	6.23	5.02
7			80	4.39	2,910	36.4	127	1.59	6.13	6.45	5.77
8			67	4.38	3,220	48.1	141	2.10	6.45	5.69	4.71
9	Nov.	1045-	94	4.54	3,920	41.7	178	1.89	6.88	6.40	5.58
10A	Dec.	168-2	82	4.78	3,060	37.3	146	1.78	6.30	6.80	5.52
11A	1958		85	4.26	3,140	36.9	134	1.58	7.63	5.82	5.77
12A			72	4.84	2,570	35.7	124	1.72	7.47	6.47	5.25
10B	Feb.	1314-	84	4.78	2,930	34.9	140	1.67	9.25	8.22	5.91
11B	Mar.	035	84	4.41	2,750	32.7	121	1.44	8.69	7.68	5.49
12B	1959		72	4.60	2,320	32.2	107	1.49	8.89	7.20	5.74
13			73	4.49	2,310	31.6	104	1.42	9.28	7.37	5.47
Mean									7.30	6.49	5.34

* Coarse error suspected.

method assumes that the plasma and degrading compartments have the same specific activity. As employed by Campbell and co-workers, the method assumes the presence of a two-compartment system.

IV. *Multicompartmental analysis,* adapted to albumin studies by Matthews (7) and by Lewallen and associates (20), entails fitting the experimental data to a biologically plausible multicompartmental system of the appropriate number of spaces. The plasma radioactivity data could in each case be described as the sum of three exponential terms, as was also found in the studies of others (2, 9, 10, 22). These data are therefore compatible with an open system consisting of three compartments. For reasons to be discussed, it was assumed that entry of newly synthesized albumin occurs only into the plasma compartment. The partially restricted three-compartment system thus obtained is shown in Figure 8. In this figure additional compartments representing the bodily pool for iodide and iodinated tyrosine residues, and the urinary excretion pool have been included. This basic reference system was then modified into different, more specific models by setting the value of various of the flow rate constants equal to zero. The resulting models 1 through 5B (Figure 9) were analyzed by using the plasma radioactivity data according to the method of Skinner, Clark, Baker and Shipley (23). In this manner, values for the rate constants for each model were obtained, as well as a value for the rate of synthesis. By using the relations [4]

$$\frac{dq_5}{dt} = \lambda_{54} q_4$$

and

$$\frac{dq_4}{dt} = \lambda_{4i} q_i - \lambda_{54} q_4 \quad (i = 1,2, \text{ or } 3)$$

a cumulative urinary excretion curve was generated for each model. These calculations were performed with a digital computer (IBM model 709). These generated curves were then compared with the experimentally determined curves. The value of rate constant λ_{54} was

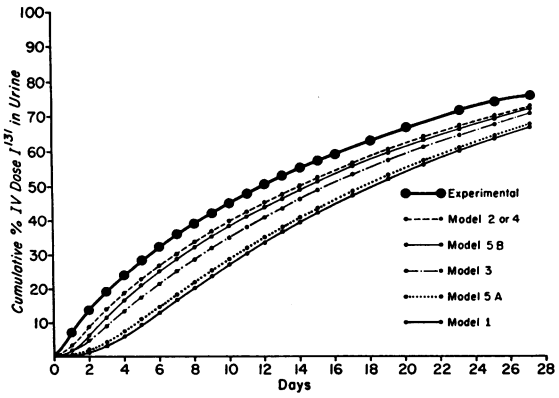

FIG. 10. EXPERIMENTAL AND GENERATED CURVES FOR CUMULATIVE URINARY EXCRETION OF I^{131}. Each point is the mean value of all 20 studies. Standard error of the mean ($\sigma/\sqrt{n}$) varies from 0.02 to 1.0 per cent of mean values.

[4] q = Amount of radioactivity present at any time in the respective pool i.

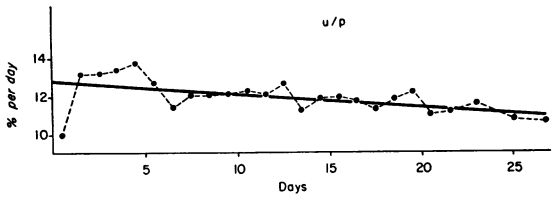

FIG. 11. VARIATION OF MEAN U/P RATIO FOR ALL SUBJECTS.

taken from the data of Lewallen, Rall and Berman (24) (1.0 per day, mean of nine experimental subjects, their Table III, page 92).[5]

An electronic analog computer (Reeves electronic analog computer C-302) was utilized to analyze the experimental data in terms of models 6 through 11 (Figure 9). Curves which were the output of the computer were compared to the experimental curves. The rate constants

[5] In that study the calculated values of λ_{54} were little different as average values between the two groups of euthyroid subjects and subjects with abnormal thyroid function. Substitution of values of 0.5 and 2.0 for λ_{54} in our data for all studies did not significantly alter the curves for models 1 and 4 in Figure 10; thus, the exact value for λ_{54} does not seem critical within this range of variation, as applied to the data of the studies herein reported.

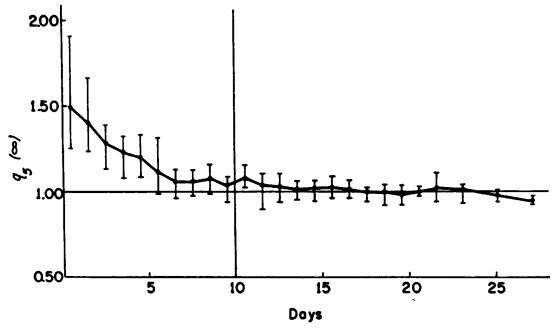

FIG. 12. FRACTION OF TOTAL ADMINISTERED DOSE OF I^{131} ACCUMULATED IN URINE AT INFINITE TIME AS CALCULATED FOR EACH EXPERIMENTAL DAY IN SIX REPRESENTATIVE STUDIES (SUBJECTS 2B, 5, 6B, 9, 10A AND 11A). Plotted points are means for these subjects. Bars indicate range of variation in these six studies. Only those fractions calculated for days > 10 were finally employed to determine the fraction of administered dose accumulated in the urine at infinite time.

TABLE II

Asymptotic values of cumulative urinary I^{131} excretion *

Study	b	$dq_5^{(27)}$	$\frac{dq_5^{(27)}}{dt}$	$q_5(\infty)$
1A	0.0588	1.10	77.6	77.6 + 18.7 = 96.3
2A	0.0473	1.05	65.2	65.2 + 22.2 = 87.4
3	0.0409	1.25	66.1	66.1 + 30.6 = 96.7
4A	0.0464	1.25	71.7	71.7 + 26.9 = 98.6
5	0.0612	1.00	78.8	78.8 + 16.3 = 95.1
6A	0.0445	1.10	75.8	75.8 + 24.7 = 100.5
1B	0.0479	1.15	77.9	77.9 + 24.0 = 101.9
2B	0.0433	1.20	67.0	67.0 + 27.7 = 94.7
4B	0.0525	1.05	72.6	72.6 + 20.0 = 92.6
6B	0.0561	1.05	75.6	75.6 + 18.7 = 94.3
7	0.0527	1.05	70.1	70.1 + 19.9 = 90.0
8	0.0588	0.95	70.8	70.8 + 16.2 = 78.0
9	0.0615	1.05	78.4	78.4 + 17.1 = 95.5
10A	0.0709	0.95	79.2	79.2 + 13.4 = 92.6
11A	0.0624	1.00	78.6	78.6 + 16.0 = 94.6
12A	0.0727	0.90	76.5	76.5 + 12.4 = 88.9
10B	0.0594	1.05	81.9	81.9 + 17.7 = 99.6
11B	0.0518	1.10	80.3	80.3 + 21.2 = 101.5
12B	0.0564	1.10	82.3	82.3 + 19.5 = 101.8
13	0.0515	1.05	79.7	79.7 + 20.4 = 100.1
				Mean value 95.7
				SEM $\left(\frac{\sigma}{\sqrt{n}}\right)$ 1.14

* $q_5(\infty) = q_5(t_1) + \left[\frac{dq_5(t_1)}{dt}\right] \cdot \frac{1}{b}$, where $q_5(\infty)$ is the value at infinite time expressed as per cent of administered dose, $q_5(t_1)$ is the cumulative amount excreted through day t_1, and b is the final slope of the urinary excretion curve. Calculations are made for $t_1 = 27$ days.

TABLE III

Calculated data

Subject no.	Graphic analysis of plasma radioactivity curve: Half-time: Exponential 1	Half-time: Exp. 2	Half-time: Exp. 3	Slope: Exp. 1	Slope: Exp. 2	Slope: Exp. 3	Ordinate intercept: Exp. 1	Ordinate intercept: Exp. 2	Ordinate intercept: Exp. 3	U/P* day 20	Albumin catabolized: a†	Albumin catabolized: b‡	Albumin catabolized: c§
	days	*days*	*days*	*days*$^{-1}$ $\times 10^2$	*days*$^{-1}$ $\times 10$	*days*$^{-1}$	$\times 10$	$\times 10$	$\times 10$	$\times 10$	*g/day*	*mg/kg/day*	*g/day*	*g/day*
1A	14.16	1.21	0.23	4.89	5.73	3.01	3.77	4.38	1.85	1.13	15.7	196	15.1	17.4
2A	17.09	1.28	0.25	4.06	5.41	2.77	3.71	3.45	2.84	0.94	15.7	194	14.7	17.1
3	18.23	1.13	0.19	3.80	6.13	3.65	3.51	3.73	2.76	0.98	13.4	189	13.0	14.4
4A	14.32	0.99	0.15	4.84	7.00	4.62	3.88	4.26	1.86	1.20	17.9	208	18.7	19.5
5‖	13.87	1.27	0.32	5.00	5.46	2.17	3.61	2.90	3.49	1.11	20.3	226	17.9	22.3
6A‖	16.52	2.70	0.32	4.19	2.57	2.17	2.51	1.62	5.87′	1.45	14.1	196	14.1	16.2
1B	13.22	1.08	0.30	5.24	6.42	2.31	3.69	4.80	1.51	1.30	15.6	195	16.0	17.5
2B	16.84	1.18	0.26	4.12	5.87	2.67	3.82	3.60	2.58	0.89	16.0	193	14.2	17.3
4B	15.16	1.03	0.13	4.57	6.73	5.33	2.63	3.36	4.01	1.51	17.9	203	17.1	19.6
6B	13.59	0.85	0.12	5.10	8.15	5.78	4.09	4.35	1.56	1.19	14.5	201	14.8	15.5
7	13.71	1.34	0.24	5.05	5.17	2.89	3.80	3.52	2.68	1.09	15.4	192	13.8	16.9
8	18.17	1.30	0.30	3.81	5.33	2.31	4.58	4.79	0.63	0.73	10.9	163	10.3	11.7
9	14.69	1.03	0.20	4.72	6.73	3.46	3.95	4.69	1.36	1.06	19.4	206	18.9	21.3
10A	12.84	0.83	0.10	5.40	8.35	6.93	3.74	4.16	2.10	1.26	15.8	193	18.4	21.1
11A‖	14.28	1.50	0.32	4.85	4.62	2.17	3.16	3.10	3.74	1.33	18.2	214	17.8	20.6
12A‖	13.15	1.20	0.22	5.27	5.78	3.15	3.49	2.94	3.57	1.22	17.1	238	15.1	18.7
10B	12.70	1.20	0.26	5.46	5.78	2.67	3.75	1.82	4.43	1.27	19.0	226	17.8	20.4
11B	14.18	1.34	0.21	4.89	5.17	3.30	3.11	3.10	3.79	1.37	17.1	204	16.6	19.0
12B	15.20	1.36	0.21	4.56	5.10	3.30	3.04	2.64	4.32	1.18	14.7	204	12.6	16.0
13	14.22	1.61	0.33	4.87	4.30	2.10	3.26	2.06	4.68	1.39	14.0	192	14.5	15.5
Mean	14.81	1.27	0.23	4.73	5.79	3.34	3.56	3.46	2.98	1.18	16.1	202	15.6	17.9

Subject no.	Equilibrium time *hrs*	EV/IV mass ratio: Extrapolation method I	EV/IV mass ratio: Equilibrium time method II	EV/IV mass ratio: Activity distribution method III	EV/IV mass ratio: Multicompartmental analysis method IV	Total body albumin in extravascular compartments: Method I	Method II	Method III	Method IV, model 6: Space 2	Space 3	Spaces 2+3
						%	%	%	%	%	%
1A	84	1.65	1.09	1.33	1.18	62.3	52.1	57.1	7.9	46.2	54.1
2A	79	1.69	1.21	1.24	1.31	62.9	54.8	55.3	12.0	44.7	56.7
3	60	1.85	1.30	1.61	1.48	64.9	56.5	61.7	11.2	48.5	59.7
4A	49	1.58	1.05	1.48	1.22	61.2	51.2	59.7	8.1	46.8	54.9
5‖	49	1.77	1.00	1.24	1.32	63.9	50.0	55.4	14.2	42.7	56.9
6A‖	37	2.98¶	1.33	2.46	2.05¶	72.3	57.1	71.1	33.8	33.4	67.2
1B	84	1.71	1.22	1.46	1.20	63.1	54.9	59.4	5.2	49.4	54.6
2B	78	1.62	1.16	1.27	1.27	61.8	53.8	55.9	10.5	45.5	56.0
4B	72	2.79	2.02¶	2.33	2.16¶	73.7	66.9	69.9	16.0	52.4	68.4
6B	84	1.45	1.16	1.33	1.15	59.1	53.7	57.1	7.0	46.5	53.5
7	72	1.63	1.20	1.16	1.18	62.0	54.5	53.6	12.3	41.9	54.2
8	96	1.18	0.78¶	0.89	0.89	54.2	43.8	47.0	2.6	44.4	47.0
9	84	1.54	1.11	1.25	1.15	60.7	52.6	55.5	5.4	48.2	53.6
10A	60	1.67	1.32	1.31	1.38	62.6	56.9	56.7	9.2	48.7	57.9
11A‖	48	2.17	1.19	1.70	1.51	68.4	54.3	63.0	15.2	44.9	60.1
12A‖	60	1.86	1.26	1.31	1.41	65.1	55.7	56.6	16.4	42.1	58.5
10B	48	1.66	1.08	1.33	1.34	62.5	51.9	57.1	23.2	34.1	57.3
11B	66	2.22	1.29	1.80	1.62	68.9	56.3	64.3	16.7	45.1	61.8
12B	60	2.29**	1.38	1.59	1.75	69.6	58.0	61.4	20.1	43.5	63.6
13	54	2.07	1.22	1.85	1.54	67.4	55.0	64.9	22.3	38.3	60.6
Mean		1.76††	1.17††	1.40††	1.33††	64.3	54.5	59.1	13.4	44.4	57.8

* $\frac{\text{Total urinary cpm/24 hrs (from drawn curve)}}{\text{plasma cpm/ml (from drawn curve)} \times \text{plasma volume.}}$

† As calculated from all three compartment models.

‡ U/P day 20 × total IV albumin mass.

§ Slope of exponential 1 × total body albumin mass ≡ slope exponential 1 × total IV albumin mass/ordinate intercept exponential 1.

‖ A fourth exponential is possible.

¶ Rejected by Dixon's ratio criterion.

** Possible experimental error.

†† Values from Subjects 4B and 6A omitted.

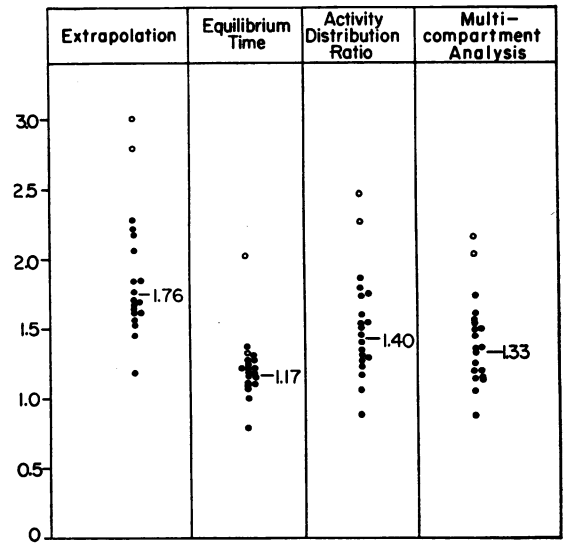

FIG. 13. SCATTER DIAGRAMS OF THE RATIOS OF THE EXTRAVASCULAR (EV) TO INTRAVASCULAR (IV) ALBUMIN MASSES FOR ALL STUDIES CALCULATED BY THE FOUR DIFFERENT METHODS, SHOWING DISTRIBUTION OF VALUES. Those designated by circles (studies 4B and 6A) were excluded in calculating the means, because of probable coarse error in determining the plasma volumes.

for a specific model were varied until the actual plasma data curve matched the curve from the computer representing the plasma compartment. Then the computer curve representing the urine compartment was compared to the actual urine data curve. An inspection of the other extravascular compartments of the specific model being tested was also accomplished by means of the computer curves for the respective compartments.

The plausible model 6 recommended by Matthews (7), because of the emphasis placed upon it and detailed work conducted with it by others (7, 9, 15), was selected for calculations not only of the size and rates of exchange of the principal albumin compartments for all subjects but also for a detailed statistical comparison of the calculated EV–IV mass ratio with such ratios obtained from methods I, II, and III. Except for omission of an iodide-delay excretion pool, this model is identical with model 4. The algebraic methods for making these detailed calculations on model 6 have been well described (7).

RESULTS

The principal experimental data for the 20 studies conducted in 13 normal subjects are summarized in Table I. The observed cumulative urinary excretion of I^{131} is shown in Figure 10.

The variation of the mean urine/plasma (U/P) ratio for all subjects with respect to time is shown in Figure 11. In the construction of this curve, all values on day 0.5 were first normalized to 10.0. The equation of regression is $Y = -0.0659\ x + 12.83$; the error of the slope is 0.020.

The urinary excretion data were examined for evidence of nonurinary excretion or bodily losses, according to the method of Lewallen and colleagues (20). Because of the slight negative slope of the plotted mean U/P values (Figure 11), an overestimate of the urinary accumulation would have resulted if the final slopes of the plasma radioactivity curve had been used; to avoid this error, values for the final slope (after day 10) of the urinary curve were substituted for each subject. The results are shown in Table II and in Figure 12. The fraction of the administered dose of I^{131} accumulated in the urine at infinite time gave values consistently near 1.0.

A comparison of the proportion of the body's albumin in intravascular and extravascular compartments (EV–IV ratio) is presented for all mathematical approaches (Table III and Figure 13). Extravascular albumin comprised from 55 to 64 per cent of the body's total albumin mass, according to mean values obtained from each of the four methods of calculation.

The relationship between the catabolic rate and body weight, as calculated from all models, is presented in Figure 14.

Finally, mean flow rates and pool sizes were calculated in terms of grams of albumin for models 1 through 5B of Figure 9, to provide representative examples of the arrangement of the body's albumin mass in several possible systems of distribution (Figure 15).

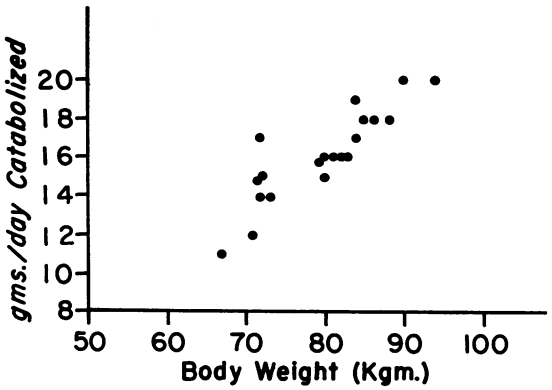

FIG. 14. RELATIONSHIP BETWEEN THE CATABOLIC RATE AND BODY WEIGHT, AS CALCULATED FROM ALL MODELS.

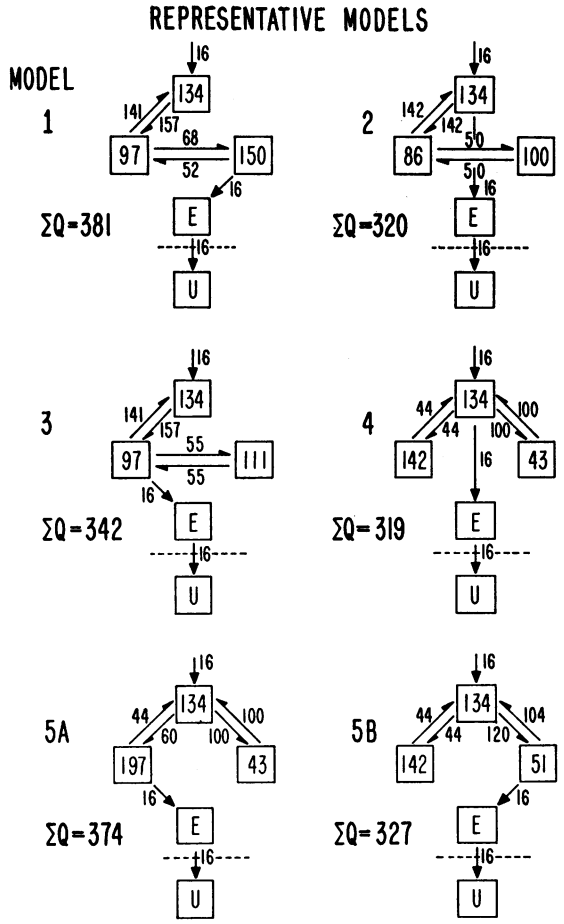

FIG. 15. MEAN VALUES FOR POOL SIZES, SYNTHESIS, CATABOLISM, AND INTERCOMPARTMENTAL FLOWS, AS CALCULATED FOR SPECIFIC THREE-COMPARTMENT MODELS FROM FIGURE 9. ΣQ = total body albumin mass.

Although the use of the actual urinary data is not required in these algebraic calculations for ascertaining the size of the principal extravascular compartmental albumin masses and the catabolic rate, the suitability of a chosen physical model must be evaluated by comparing the theoretically derived urinary excretion curve throughout the entire experimental period with the actual urinary radioactivity data. This is especially necessary since one cannot directly sample the extravascular compartments, and the plasma data alone are compatible with all models depicted in Figure 9. The generated urinary excretion curves for models 1 through 5B (Figure 9) are shown in Figure 10.

By means of the electronic analog computer, a comparison of the observed urinary data with the theoretical values derived from fitting the experimental plasma data to models 6 through 11 of Figure 9 was pursued for representative subject 8. Only very general conclusions concerning the best fit of the urinary data could be drawn. Of the six models investigated, models 6 and 9 gave the best fit of both plasma and urinary data. Model 8 seemed to give the least satisfactory fit of both curves. It was readily apparent that model 6 did not represent a unique system for the data, and that the experimental results could reasonably fit also several of the other physical models explored.

Statistical analysis of EV–IV mass ratios. In spite of the inability to select a unique multicompartmental model to fit the experimental data, methods of statistical treatment for comparing values for the EV–IV mass ratios, as obtained by the four general mathematical approaches, were explored. As representative values for method IV (the multicompartmental analysis), the results of the algebraic calculations for model 6 of Figure 9 (shown in Table III) were employed. It was desired to make some statistical statements about the relationships of the four methods with respect to average values, variability, correlations, and, if possible, reproducibility in the same subjects at different times.

The data consist of the EV–IV mass ratios computed by each of four general mathematical methods for 13 subjects. For seven of the subjects a second observation was made several months after the first. Thus, there were 20 sets of ratios, of which seven sets were "duplicates."

The first problem that required statistical treatment was the appearance of some unusual values for certain subjects (Table I). For this purpose, the measurements were divided into two series: the A series consisted of those data obtained during the first experiment upon each subject; data resulting from a second observation upon a subject comprised the B series. Six of the 13 subjects were studied only once, hence their values appear only in the A series.

In the first (A) series of measurements Subject 6A had the highest ratio by all four methods. This subject's ratios appeared unusually high for all but the equilibrium time method. A test for outlying values, using Dixon's ratio criterion r_{21} (25), rejects this subject's value at the 5 per cent

level of significance for the extrapolation method and the multicompartmental analysis method.

The value for Subject 8 in the A series is the lowest by all four methods, but is rejected by Dixon's test at the 5 per cent level only for the equilibrium time method.

In neither of these instances was there any reason to suspect a coarse error of measurement at the time of the observation. However, it is interesting to note that the plasma volume for Subject 8 appears unusually high for his height and weight.

In the second (B) series of measurements on seven of the subjects, the value for Subject 4B is the highest by all four methods, and appears unusually high in all but the extrapolation method. If the B series is combined with the single values of the remaining six subjects (Subjects 3, 5, 7, 8, 9, and 13), then Dixon's test rejects the value of Subject 4B at the 5 per cent level for the equilibrium time method and for the multicompartmental analysis method.

As in the case of Subject 6A, there was no reason at the time of the measurements to suspect a coarse error in Subject 4B's values. However, in both instances the discrepancy between the corresponding values in the A and B series supports the conclusion from Dixon's test that coarse errors had occurred.

The "duplicate" values must be interpreted cautiously because the two series of observations were made at different times. Therefore, the discrepancies may reflect sources of variation other than error of measurement. With this in mind, it was noted that the values of Subjects 4 and 6 presented unusual discrepancies between the duplicates—for Subject 4 by all four methods, and for Subject 6 by all but the equilibrium time method. A rejection criterion based upon the range (26) was used to test these discrepancies. The discrepancy of Subject 4 in the equilibrium time method is rejected at the 5 per cent level of significance. If Subject 4's values are then removed from the set of seven duplicates, the discrepancies for Subject 6 become significant for the extrapolation and distribution ratio methods.

If the values for both Subjects 4 and 6 are removed from the duplicate series, then the range criterion also rejects Subject 12's values (which show the next largest discrepancy) for the extrapolation method. The value of 2.29 for Subject 12B is known to have been exposed to possible additional error of measurement due to difficulty encountered in the administration of the labeled albumin.[6]

A retrospective search for possible causes of coarse errors in the suspected measurements of Subjects 4B and 6A suggests that the determinations of plasma volume were in error. Determinations of plasma volume made for a third time agreed closely with the higher value previously obtained for each subject. However, these discrepancies were not statistically significant when tested by the rejection criteria used for the EV–IV mass ratios.

For purposes of describing the relationships among the four methods, the means, variances, and covariances were computed for single values of the 13 subjects. The suspected values of Subjects 4B, 6A, and 12B were excluded from this analysis. One of the duplicate values was selected at random for the remaining four subjects who had two sets of measurements; these data were from studies 1A, 2B, 10A, and 11B. A statistical analysis was undertaken to answer the following questions based upon single values for the 13 normal subjects.

1. Are the extrapolation, equilibrium time, and distribution ratio methods "parallel," and are they symmetrically related to the multicompartmental analysis method in the sense that: *a*) they exhibit the same variability from subject to subject; *b*) the correlation between any two of the first three methods is the same, and *c*) the correlations between the multicompartmental analysis method and each of the other three methods are equal; *d*) the three first methods yield equal mean values?

2. If the answer to question 1 is "no," then are the first three methods symmetrically related to the multicompartmental analysis method in the sense that: *a*) they exhibit the same variability from subject to subject, *b*) the correlation between any two of the first three methods is the same, and *c*) the correlations between the multicompartmental analysis method and each of the other three methods are equal?

3. Irrespective of the mean value for the multicompartmental analysis method, do the other three methods have equal mean values?

[6] It was necessary to calculate the administered radioactivity indirectly from the determined plasma volume.

TABLE IV

Statistical evaluation of methodology *

	Multicompartment Analysis	Extrapolation	Equilibrium Time	Distribution Ratio
Multi–compartment Analysis (Mean=1.29)	s^2=.0383 s =.196	Cov =.0500	Cov =.0230	Cov =.0439
Extrapolation (Mean=1.70)	r = .96	s^2=.0707 s =.266	Cov =.0275	Cov =.0592
Equilibrium Time (Mean = 1.15)	r = .79	r =.70	s^2=.0221 s =.149	Cov =.0245
Distribution Ratio (Mean = 1.37)	r =.86	r =.86	r =.63	s^2=.0676 s =.260

* In the diagonal boxes the upper figure (s^2) is the estimated variance, and the lower figure (s) is the estimated standard deviation for the method corresponding to the row and column headings. Each entry above the diagonal is an estimated covariance (Cov) for the two methods indicated by the row and column headings. The corresponding entries below the diagonal are estimated correlation coefficients (r).

The logical hierarchy of these three questions implies that, if the answer to question 1 is "yes," then the answers to questions 2 and 3 must also be "yes." If the answer to question 1 is "no," the asymmetry may be due to differences in variability and correlations, or due to different mean values, or both. If the asymmetry is due only to differences in variability and correlations, the answer to question 2 is "no." If, however, the answer to question 2 is "yes," then the asymmetry is interpreted as due only to differences in mean values, and the answer to question 3 should be "no."

The tests of statistical hypotheses which were used to answer these questions are likelihood-ratio tests developed by Votaw and his associates, Kimball and Rafferty (*27, 28*), in which the variability of the EV–IV mass ratios obtained by a given method is measured as the variance, and the correlations are measured as covariances. The estimated means, variances, and covariances for the four methods are given in Table IV.

The pattern of symmetry to be tested is indicated by the double lines which set off the rows and columns of Table IV into quadrants. The variance, 0.0383, for the multicompartmental analysis method, is in the upper left quadrant. The covariances between the multicompartmental analysis method and each of the other three methods appear in the upper right quadrant. The variances of the latter three methods and the covariances among them appear as diagonal and off-diagonal entries, respectively, in the lower right quadrant. If question 1 is answered "yes," the following hypotheses are accepted apart from sampling variation: *a*) the three variances in the lower right quadrant are equal; *b*) the three covariances in the lower right quadrant are equal; *c*) the three covariances in the upper right quadrant are equal; *d*) the three mean values adjacent to the lower left quadrant are equal. Corresponding statements hold for positive answers to questions 2 and 3.

Applying Votaw's test criteria at the 5 per cent level of significance, the answers to questions 1 and 2 are both "no." Inspection of the table suggests that this statistically significant asymmetry

is due largely to the relatively small variances and covariances associated with the equilibrium time method. This finding is not necessarily a desirable feature of the equilibrium time method, since it may result from the relative crudeness of the method and simply reflect a lack of sensitivity to physiological differences among the subjects.

If one prefers to apply the significance tests at the 1 per cent level, then question 1 is still answered "no," but question 2 is answered "yes." In this case, question 3 can be answered "no," attributing the asymmetry to differences among the mean values for the three methods.

In any event, the data for the 13 subjects of this study present evidence of differences in the performance of the four methods for determining the EV–IV mass ratios. The practical importance of these differences and the implications for further study depend, of course, on the use to which the methods may be put. For example, if it were desired to calibrate one of the simpler methods for use in place of the multicompartmental method, something should be learned about the functional relationships among the apparently different phenomena which the various methods are measuring. The scatter diagrams (Figure 16), relating the ratios determined by the multicompartmental method to each of the other methods, suggest that simple linear models would serve well for this purpose. However, the presumed linear relationships are obscured by fluctuations due to error of measurement in both variables. An additional difficulty arises from correlation among these fluctuations, which one would expect to find, because some of the same basic observations, such as plasma volumes, are used in all four methods. Madansky (29) and Acton (30) have recently reviewed these statistical problems and discussed methods available for attacking them.

The simple scatter diagrams presented in Figure 13 provided a brief appraisal of the relation of the means of the EV–IV mass ratios from the four mathematical methods, and also indicate the relative range of individual values within each of the four methods. The relationships indicated must be considered as holding only for I^{131}-albumin preparations of the type employed in these studies, and for normal male adult subjects only; the relationships would be especially likely to be quite different in abnormal pathological states exhibiting abnormal metabolism and distribution of albumin.

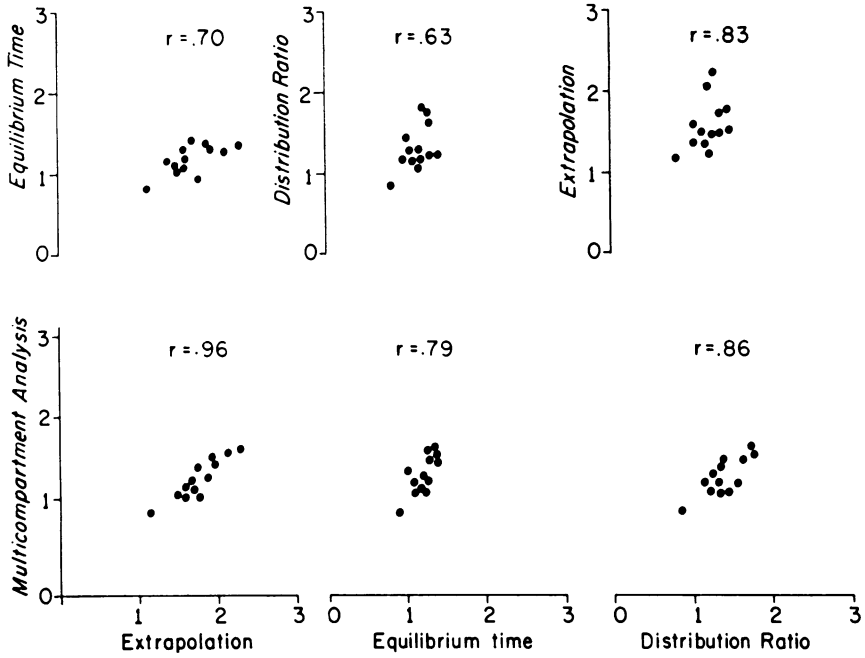

FIG. 16. SCATTER DIAGRAMS OF THE RELATIONSHIPS BETWEEN THE RATIOS OF THE EXTRAVASCULAR (EV) TO INTRAVASCULAR (IV) ALBUMIN MASSES CALCULATED BY EACH OF THE FOUR METHODS FOR THE 13 DIFFERENT SUBJECTS.

DISCUSSION

General assumptions

The general assumptions necessary for mathematical analysis and interpretation of the results of this and similar types of studies, discussed by others (4, 8, 9, 31, 32), are in brief: *1*) The metabolism of I^{131}-albumin is identical with that of unlabeled native albumin. *2*) The subjects are in a steady metabolic state during the study. *3*) After liberation from the albumin molecule, I^{131} is quantitatively excreted in the urine. In some model systems (methods I, II, and III), excretion must proceed without significant delay.

In addition to these general assumptions, more specific requirements must be met to allow detailed mathematical analysis. These more specific assumptions will be commented upon in the following section.

Each of the three general assumptions can be partially justified for the experiments herein presented, as follows.

Assumption 1. Major efforts were taken to prevent denaturation of the albumin during preparation and labeling. Cohn fractionation was chosen as the method for isolating the albumin because it yields a homogeneous product that has been demonstrated to be biologically acceptable (*2*). The chromatographic behavior of ethanol-precipitated albumin has been found similar to that of albumin prepared by Porath column separation (33), a method which consistently yields albumin of excellent physiological properties (*7*, 8, 11, 15, 16, 21). Because of its possible denaturing effect (34–36), heat treatment after separation of the albumin was specifically avoided. The albumin was lightly iodinated by the best method available to this laboratory, and the levels of radiation to which the albumin was exposed were well below those known to cause radiation damage to protein (33, 37, 38). Both testing by physicochemical methods and appraisal of *in vivo* behavior were used to assess the degree to which isolation and iodination had denatured the albumin. Dialysis, electrophoresis, ultracentrifugation, and DEAE cellulose column chromatography gave evidence of an excellent product from the physicochemical standpoint. Variation in specific activity of the albumin eluted from the DEAE column suggested, however, that all molecules were not equally labeled (Figure 1). Literal interpretation of those data is, however, prevented by the fact that immediately after radioiodination, the labeled albumin was diluted with nonlabeled carrier albumin of a different lot (prepared by the same fractionation method but heat treated).

The indices of *in vivo* behavior employed in these studies were the apparent biological half-life of albumin, determined from the plasma radioactivity curve, and its catabolic rate as reflected by urinary excretion of radioactivity. After "distribution equilibrium," the random destruction of labeled albumin is manifested by a gradual exponential decline in plasma activity. This decline, however, does not precisely reflect albumin catabolism because continuous re-entry of albumin of higher specific activity from extravascular compartments constantly diminishes the negative slope of the plasma activity curve (8, 9, 16, 21). Thus the biological half-life reflects not only degradation, but also, to a lesser extent, distribution. Pearson, Vetter and Veall (39) have described a method for deriving the half-life independent of the effect of distribution. Although theoretically sound, the method incorporates the hazard of determining a rate constant for urinary excretion early in the study when it is most apt to be spuriously high. If the early rate of I^{131} excretion is not increased due to rapid catabolism of denatured I^{131}-albumin, and the initial portion of the plasma activity curve is sufficiently accurate, this method would have merit. The mean biological half-life of 14.8 days observed in the subjects of the present studies, although acceptable as reflecting a reasonably good radioalbumin preparation, nevertheless is shorter than some half-lives recently reported in normal subjects by other investigators (2, 3, 6, 9–16, 40) (individual half-lives of 14 to 25.0 days).

The degree of urinary excretion of the radioalbumin label during the first few days after intravenous administration provides an index of biological denaturation. Our subjects excreted from 5.37 to 9.28 per cent of the injected radioactivity within the first 24 hours and from 4.26 to 8.23 per cent the second day (Table I). Berson, Yalow, Schreiber and Post (2) reported that 4.3 and 3.5 per cent were excreted during the first and second days, respectively, in one normal subject, and from 3 to 4.5 per cent has been found to be ex-

creted during the first day by those testing McFarlane's product (5, 9, 16, 41). Steinfeld and colleagues (19) found that from 9 to 19 per cent of the administered radioactivity was excreted within the first 48 hours after administration of their best material.

Once initial bodily equilibrium of the injected radioalbumin has been established, the urinary excretory rate can be derived in terms of urinary activity, expressed as a fraction of plasma activity, extravascular activity, or total body activity. Such expressions are correct only if albumin is catabolized in the compartment designated in the denominator of the fraction. Since iodide^{131} distribution is rapid (42), and urinary excretion is prompt, urinary radioactivity can be expected to parallel closely the radioactivity level in the compartment in which I^{131}-albumin degradation occurs. Although there is evidence that the site of albumin synthesis is contiguous, or in rapid exchange, with the plasma space (43), the principal sites of degradation have not been determined. Evidence of different types has been presented to implicate variously the liver, kidney, or gastrointestinal lumen (44–52) as major sites of albumin catabolism. However, it is now known that the catabolic sites of radioiodinated albumin are partially determined by the degree of biological denaturation during preparation (35, 47).

Since the urinary radioactivity curves of the present studies most closely paralleled the respective plasma radioactivity curves (Figure 6), it appears that catabolism of the particular radioalbumin preparation used herein did indeed occur at a site contiguous, or in very rapid I^{131} exchange, with the plasma compartment; other workers have made similar conclusions (8, 20, 21). With this decision, a constant daily per cent urinary excretion of the plasma radioactivity should occur if the general assumptions 2 and 3 hold. Were these terms completely valid, one very reasonable explanation for a progressive decrease in U/P values is biological nonhomogeneity. The mean U/P values for all subjects (Figure 11) did show a definite slight decrease with respect to time. These points are further discussed in a later section.

From all of this evidence it is concluded that, despite physicochemical excellence, this I^{131}-albumin preparation was probably slightly denatured during isolation or radioiodination, or both.

Ability to prepare a biologically nondenatured I^{131}-albumin is still an important problem (12, 15, 19). Biological purification of a prepared material (47, 53, 54) is rarely, if ever, practical for the human subject, although the accomplishment of this has been reported in one instance (20). Additional biological methods for detecting an approximate degree of denaturation of specific preparations have been devised (35, 45).

Assumption 2. All subjects continued normal daily activities but maintained as steady metabolic states as seemed possible, short of complete metabolic balance conditions. Serial weights and laboratory studies varied minimally. Proteinuria was not present. One subject developed an anal fissure with intermittently guaiac-positive stools, but fecal excretion was less than an acceptable maximum equivalent of 2 per cent (2) of the urinary activity for the corresponding day. There was no evidence of gastrointestinal bleeding in the other subjects. The fact that in 16 of the 20 studies, plasma activity approximated a constant fraction of the calculated total body activity after equilibrium (2) was further evidence that no major deviations from the steady state occurred. Alterations in this fraction would be induced by changes in the volume or mass of distribution, or by unmeasured losses of radioactivity. Inadequate urine collections in Subject 7 probably accounted for the change in this fraction. However, in Subjects 4A, 2A, and 2B, the changes could not be attributed to unmeasured urine losses; therefore, a slight deviation from the steady state was suspected.

Assumption 3. Maximal urinary radioactivity occurred on the day of isotope administration in all but four of these studies, suggesting a minimal lag in excretion of the I^{131} after liberation from the protein molecule. It is known that approximately 84 per cent of intravenously administered NaI^{131} is excreted within 24 hours in subjects taking excess unlabeled iodide (2). Further, recovery of radioactive iodide in athyroid individuals (55), or in the presence of a thyroid blocking agent (20), is complete within 3 days, indicating that there is no incorporation of liberated I^{131} into newly synthesized protein. Nevertheless, suggestions of a bodily radioiodine-trapping mechanism were reported by Lewallen and associates

(20). Accordingly, those authors added an extra compartment in their multicompartmental models to accommodate these observations. However, in the studies herein reported, 95.7 per cent of the injected radioactivity was excreted at infinite time, according to the calculation methods of Lewallen and co-workers; these are depicted for the present studies in Figure 12 and summarized in Table II. Since the most trivial cause of deviation from 100 per cent is incomplete urinary collection, it seems doubtful that there is any significant trapping in the present series, even considering that the calculations are based upon extrapolations. Similarly, these data on cumulative excretion provide no evidence for other important iodide excretory pathways, such as perspiration (56).

Model configurations, albumin distribution and flow

Since albumin is distributed in a vast number of body compartments, current mathematical approaches to the kinetics of distribution require oversimplification of this complex system. Many of these approaches have incorporated undesirable suppositions. All of the analytic methods require the assumption that the albumin molecules are distributed rapidly and evenly within each assumed compartment. The validity of this has not yet been conclusively demonstrated. However, there is reasonable evidence to suggest that albumin is mixed rapidly within and distributed equally throughout the main portions of the plasma space of normal subjects (57); although mixing and distribution within tissue capillaries of individual organs undoubtedly vary in efficiency, averages for the total body indicate general rapidity. Furthermore, labeled proteins and large dextran molecules have been shown to be distributed equally throughout some extravascular compartments within hours after intravenous administration (58–62). Although albumin may possibly be distributed widely within cells of many organs (63), no specific comment can be made as to the uniformity of this distribution. A succinct review of the specific assumptions necessary for each of the four major mathematical approaches employed herein has been provided by Freeman and Matthews (9). These are here reviewed briefly:

I. *The extrapolation method* assumes that subsequent to an early time of bodily equilibration, the mean specific activity of the extravascular pools equals at all times that in the plasma space. The fact that the calculation of the specific activity of the extravascular compartments from the data of Table I demonstrated it to be consistently higher after equilibrium than for the plasma (Figure 7) points out the limitations of this method [see also discussion by Lewallen and associates (20)]. Nevertheless, as is evident from Table III, in normal adult subjects the method appears to yield values similar to those obtained from analysis of a three-compartment model, wherein such an assumption is not made. In pathological conditions, this assumption would seem more dubious when the catabolic rate is increased, if there were renal or intestinal leak of labeled protein (64–68), or if there is altered capillary permeability (69).

II. *The equilibrium time method* is relatively simple, and requires a study period of less than a week. The degradation compartments are assumed to have the same average specific activity as plasma. There must be no lag in urinary excretion of the metabolized albumin label. This method can be very troublesome in that it necessitates accurate estimation of the equilibrium time from the extravascular activity plot where the curvature is so gradual that it is very difficult to select accurately the point of maximal radioactivity (Figures 4 and 7). In addition, the slight increases in urinary excretion rates usually found during the early days, because of rapid catabolism of denatured albumin, lend risk to any calculations from urinary data pertaining to this period.

III. *The activity distribution ratio method* likewise assumes that the plasma and degradation compartments have the same specific activities. However, it allows that the specific activity of the extravascular albumin remains higher than that in the plasma after equilibrium, a concept inferred by Figure 7. For accuracy, frequent timed urine collections throughout the study period are imperative. A major advantage is that calculations are made from data that are independent of the effect of excessive early degradation resulting from use of slightly denatured albumin.

IV. *Multicompartmental analysis.* With this type of approach, the behavior of the labeled

species is observed in several anatomical localities in the body as a function of time. Studies in the experimental animal (59, 70) support the concept of multiple extravascular volumes of distribution, in that labeled albumin of various extravascular sites exchanges with plasma at different rates. The location of the physiological extravascular compartments has not yet been precisely determined in man, although there is evidence (71, 72) that the viscera comprise a volume of rapid exchange, and that skin and muscle participate in relatively slow exchange. Whether certain of the extravascular volumes exchange directly with each other has not been determined.

It is assumed that the system is in a stationary state with respect to the properties studied and that linear relationships exist between the flows of radioactivity and the gradients of specific activity. If sampling were made in all compartments, then it would be possible to characterize the system uniquely, in terms of compartmental content and intercompartmental flows of the substance under investigation. Since the assumption of discontinuities of transfer rates, i.e., the compartmentalization, is often questionable, and since the number of compartments in biological systems usually greatly exceeds the number of sampling localities, it is probably not possible to characterize such systems uniquely. Thus, multicompartmental analysis of biological systems can lead to approximations only. In most instances, the degree of approximation is not known, so that the results are of questionable validity, but in other instances, limited but useful information can be obtained by using some of the principles of the approach. Berman and Schoenfeld (73) have recently given a comprehensive discussion of the general character of multicompartmental analysis and also developed a new method for characterizing systems with insufficient data. This method may be of future value, but seems to suffer from the disadvantage that the physical requirement of nonnegative values of the flow constants may only be approximately realized in biological systems because of relatively large errors of the observations. Further discussion on the general problems of the use of mathematical models for interpretation of these types of experiments is provided by Cornfield, Steinfeld and Greenhouse (32).

It became readily apparent that, for all the mathematical methods, accurate measurement of plasma volume is crucial, because upon it depend all values of total plasma radioactivity. The mean I^{131}-albumin plasma volumes for large groups of normal subjects have been established (74, 75), and the error of the method determined (76). However, with two exceptions (74, 77), there is a paucity of published data regarding the variability of serial plasma volumes determined with labeled albumin in normal subjects over long intervals. Care was taken in this study to avoid the recognizable errors (78, 79).

Discussion of results

One possible explanation for the slight negative slope of the U/P ratio is the presence of denatured I^{131}-albumin molecules. Some of the published data graphs of previous investigators have likewise shown such slight negative slopes (7, 9, 16, 21). It seems impossible to estimate the total degree of denaturation, or to determine whether this is in the form of a slight denaturation of all albumin molecules or is a series of populations having varying degrees of denaturation. Recently reported (35, 45) biological tests of I^{131}-albumin denaturation should be of assistance in this connection.

The calculations of the cumulative urinary excretion of radioactivity at infinite time provide little evidence of a substantial I^{131} "permanent" bodily trap (20) or nonurinary excretion route. Why this difference exists between the data presented here and those of Lewallen and associates (20) is unclear. Such a calculation probably provides a useful check upon the completeness of the urinary collections and the sufficiency of blockage of thyroidal uptake of I^{131}.

The representative models shown in Figure 9 are all compatible with the plasma data, in spite of the marked differences in physiological significance.

Model 6 is of especial interest because of previous work with it by Freeman and Matthews (9) and by Cohen, Freeman and McFarlane (15). With respect to the catabolic rate for albumin, our data (Table III) are very similar, giving a range in all subjects of 164 to 236 mg per kg per day as compared to Cohen's 136 to 257 mg per kg per day (15). With respect to pool sizes, the slowly exchanging extravascular pool (pool 3 of model

6 in Figure 9) was found from our data to be slightly larger, and the rapidly exchanging EV pool slightly smaller (Figure 15).

The principal sites of albumin catabolism are not precisely known. It has been recognized that degradation does occur in several bodily tissues (44–47, 49, 50) and that the relative amount degraded in each location may be influenced by denaturation (35, 47). Recent studies indicate that an appreciable amount of the body's daily degradation occurs in the gastrointestinal lumen after leakage in digestive secretions (48, 51, 67, 80); after intraluminal breakdown, the I^{131} freed from labeled albumin should be promptly absorbed into the plasma space. The generated urinary curves from the models, which allow catabolism to occur within or in rapid exchange with the plasma compartments, are in much better agreement with the experimental data (Figure 10) than are those from models wherein catabolism occurs primarily in extravascular spaces. The consistent discrepancy between the experimental and generated curves of Figure 10 is compatible with the fact that the U/P values very slowly decrease with time (Figure 11).

SUMMARY

1. The behavior of radioiodinated albumin was investigated in 20 studies of 13 different normal young human males by means of an ethanol-fractionated, lightly radioiodinated albumin preparation, not subjected to heat treatment. This preparation was characterized by numerous physicochemical methods, showing generally excellent homogeneity.

2. The apparent biological half-life of this I^{131}-albumin in these subjects, as determined from the plasma radioactivity disappearance curve, ranged from 12.7 to 18.2 days, with a mean of 14.8 days.

3. The ratio of the radioactivity in urine and plasma (U/P), expressed as a fraction of injected dose, showed a significant slight decrease with respect to time.

4. The calculated fraction of the radioactivity recovered in the urine at infinite time averaged 95.7 per cent of the administered amount.

5. The amount of albumin catabolized daily averaged 16 to 18 g, as calculated by several methods.

6. Distribution of exchangeable albumin between the extravascular and intravascular compartments of the body was examined in detail by each of four general mathematical methods—I) extrapolation, II) equilibrium time, III) activity distribution ratio, and IV) multicompartmental system analysis—for a number of three-compartment models.

7. The extravascular to intravascular (EV–IV) albumin mass ratios, calculated by the four methods, provided means ranging from 1.17 to 1.76. A statistical comparison of these data is presented.

8. The theoretical assumptions, advantages, and disadvantages of each of the four general methods of calculation are discussed.

9. The compatibility of fit of the experimental data to a number of multicompartmental models for describing albumin metabolism was tested. All models examined were based upon compatibility with the observed plasma data. The nearest compatibility with both the observed plasma and urinary data was found in only those models wherein catabolism occurs in, or in rapid exchange with, the plasma compartment.

10. The results are discussed with respect to possible biological nonhomogeneity of the labeled albumin preparation employed.

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APPENDIX

Corrections for sampling. *1*) The volume of plasma removed in each blood sample is calculated by the equation: milliliters of blood removed per sample × (100 − hematocrit)/100 = milliliters of plasma removed per sample.

2) The milliliters of plasma removed per sample are then multiplied by the counts per minute per milliliter of plasma to calculate the total radioactivity removed in that sample.

3) Since each deficit is ultimately shared by the total volume of I^{131}-albumin distribution, the total radioactivity removed in each sample is divided by the total volume of distribution (2.5 × plasma volume, assuming an EV-IV albumin mass ratio of 1.5:1) to estimate the radioactivity deficit per milliliter. For the purposes of these corrections, it is assumed that this dilution is complete within 24 hours instead of the observed 48 to 96 hours.

4) To correct for the catabolism which the I^{131}-albumin in the sample would have undergone had it remained in the body, the deficit in radioactivity was calculated as follows: $A_n = A_i e^{-\lambda(n-i)}$ where A_i = radioactivity (cpm per ml) removed by a given sample on day i, A_n = corrected for

catabolism to day n. Day i is equal to or greater than day 0 (day of isotope administration) but less than day n. $\lambda = 0.693/t_{\frac{1}{2}}$ albumin (first approximation).

5) The total accumulative deficit D_n on day n is the sum of the individual deficits, $A_0 + A_1 + A_2 \ldots A_{(n-1)}$, each resulting from radioactivity removed on days 0, 1, 2 . . . $(n - 1)$ respectively, and each corrected for catabolism to day n:

$$D_n = \sum_{i=0}^{n-1} A_i e^{-\lambda(n-i)}$$

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