Transvascular Passage of Albumin-I^{131} in Skin of Mice.* (31531)

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The ground substance of connective tissue is usually treated as an amorphous monophasic system. It is usually assumed that interstitial water becomes coextensive with the ground substance water. Published data on the rate of exchange of labeled albumin-I^{131} between vascular and extravascular spaces indicate that most observers view this exchange as occurring into a monophasic system(1-4). However some type of physical-chemical interaction must occur with the meeting of the "streams" representing the extravascular filtrate, pericellular environment and the products of the fibroblasts and of other cells, all of different composition.

From thermodynamic considerations, Joseph *et al*(5) hypothesized the existence of a two phase system—a "colloid-rich water-poor" phase and a "water-rich colloid-poor" phase. Subsequent studies by this group supported this concept(6,7). The data of Berson *et al* (8) suggest that extravascular space in the human might be considered to be composed of 2 compartments. Recently Huggins *et al*(9) observed that labeled albumin-I^{131} in the dog disappears from the plasma first at a rapid rate which then changes to a slower rate after 8 hours. Mixing is complete between vascular and extravascular albumin-I^{131} in approximately 3 days. The data suggested that the extravascular space has at least 2 compartments.

This laboratory has become concerned with the possibility that hyaluronic acid may be one of the factors which influence the quantity of extravascular albumin-I^{131} in the skin of mice(10,11). A study was undertaken of the clearance of albumin-I^{131} from the blood stream. It was observed that the dynamics of the appearance of labeled albumin-I^{131} in the skin was consistent with the concept of the existence of a biphasic system in the ground substance.

Methods. Male mice weighing 28 ($\pm$1.5) g were injected into the tail vein with 0.1 μc of human serum albumin-I^{131}. They were permitted food and water *ad lib* in individual plastic cages. At various time intervals the animals were killed by cervical fracture and a sample of serum was obtained. The pelt was removed and treated as previously described to determine the quantity of albumin-I^{131} present in the skin(11).

Results. A circulation time of 4 minutes was assumed. The c.p.m. in the serum and in the skin are shown in Table I. Calculations were made according to the procedure of Sterling(1). The K values, representing turnover rate as a fraction of the albumin-I^{131} pool turned over per hour were derived from the equation of exponential decay, $Y = Y_o e - Kt$. The linear equations $\log Y = Kt + b$ were obtained by the method of least squares.

Serum. Radioactivity decreased rapidly from the serum to 53.6% of the 4-minute value in 2 hours ($K_1 = -.1380$). Radioactivity then decreased more slowly to 23.8% in approximately 13 hours ($K_2 = -.0319$) when equilibrium was reached ($K_3 = -.0132$). The albumin-I^{131} pool was 100.3 mg with a half time of albumin-I^{131} turnover ($t\frac{1}{2}$) of 22.8 hours.

Skin. Radioactivity in the skin increased rapidly for 2 hours ($K_1 = 0.1960$) then at a slower rate for approximately 6½ hours ($K_2 = 0.0464$) when equilibrium was reached $K_3 = -.0120$). The $t\frac{1}{2}$ was 25.0 hours, not strictly in agreement with the whole-body value. The extravascular pool of albumin-I^{131} in the skin of mice of this weight was previously shown to be 8.70 mg(11).

Discussion. The data indicate clearly that albumin-I^{131} leaves the blood stream at 3 exponential rates and that the extravascular space might be considered to be composed of

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TABLE I. Albumin-I^{131} in Serum and Skin of Mice.

		Serum			Skin		
Time(t)	No.	c.p.m.* in .02 ml	Phase	% 4 min value	c.p.m.	Phase	c.p.m. (corrected)†
4 min	18	16921 ± 510‡	Y_1	100.0	10100 ± 450	Y_1	
30 "	11	13757 ± 321		81.3	19144 ± 759		10933
1 hr	21	12939 ± 419		76.4	23489 ± 710		15773
1.5 "	19	10143 ± 396		59.9	23914 ± 932		17854
2 "	21	9082 ± 288	Y_2 →	53.6	28331 ± 1236	Y_2 →	22917
3 "	9	8396 ± 528		49.6	29724 ± 1526		24714
4 "	22	8179 ± 232		48.3	35236 ± 1623		30358
5 "	9	7167 ± 331		42.3	35951 ± 1333		31679
6 "	36	6505 ± 160		38.4	28404 ± 1082	Y_3 →	34526
8 "	14	6095 ± 240		36.0	38049 ± 1166		34413
13 "	21	4034 ± 200	Y_3 →	23.8	31453 ± 1756		29050
18 "	11	3633 ± 238		21.4	27458 ± 1160		25297
24 "	12	3104 ± 150		18.3	22786 ± 986		20938
36 "	10	2104 ± 154		12.4	16669 ± 1191		15417
48 "	13	1445 ± 80		8.5	11814 ± 609		10956
60 "	8	1011 ± 72		5.9	8472 ± 358		7876
72 "	18	712 ± 34		4.2	6334 ± 175		5910

* c.p.m. = counts per min.
† Corrected for entrapped serum based on 4 min value.
‡ Standard error of mean.
→ value corresponding to time when a new rate becomes apparent.

Serum	Skin
$\log Y_1 = -.1308t + 2.0001$	$\log Y_1 = .1960t + .0298$
$\log Y_2 = -.0319t + 1.7941$	$\log Y_2 = .0464t + 4.2723$
$\log Y_3 = -.0132t + 1.5644$	$\log Y_3 = -.0120t + 4.6179$

2 compartments. This is confirmed by the pattern of appearance of albumin-I^{131} in the skin. The data are consistent with the biphasic system in ground substance proposed by Joseph *et al*(5). This laboratory has been investigating the possibility that the quantity of extravascular albumin in the skin might be influenced by the quantity of hyaluronic acid present by virtue of the molecular sieving action of the latter(12,13). The data now suggest that extravascular albumin is distributed in 2 phases in the skin; one which it enters somewhat more rapidly than it leaves the blood stream and another which it enters at approximately ¼ the rate of the first. The former phase corresponds to the "water-rich colloid-poor" phase of Joseph *et al*(5) which relates to rapidly flowing interstitial-lymphatic water; the latter is the "water-poor colloid-rich" phase containing the hyaluronic acid. The second transport system is analogous to a column of polysaccharide gel grains with solvent flowing through the interstices(14). This is what occurs in gel filtration.

The quantity of albumin in each phase was determined as follows: The total extravascular phase contained 8.70 mg of plasma albumin. The K_3 phase of the skin curve extrapolated to t = 0 yielded 41,480 c.p.m. The 2-hour value also extrapolated to t = 0, allowing for metabolic decay, yielded 24,490 c.p.m. Thus 59.0% (5.13 mg) was associated with the rapidly moving phase and 41.0% (3.67 mg) with the slower moving phase. Corresponding calculations for whole-body albumin yielded 100.3 mg total albumin pool; 36.8 mg circulating albumin, 43.0 mg in the rapidly moving extravascular phase, and 20.5 mg in the slow moving phase. The latter constitutes 32.0% of the extravascular albumin compared with 41.0% in the skin.

The t½ was 22.8 hours for the total organism and 25.0 hours in the skin. This discrepancy is related to differences in the K_3 values (−.0132 and −.0120, respectively), the reasons for which are not apparent. Since human albumin-I^{131} was injected the data probably have no bearing on endogenous albumin metabolism. It is also not clear why metabolic equilibrium was apparently reached in approximately 6½ hours in the skin but not until 13 hours according to the serum clearance curve.

Summary. The clearance of intravenously

injected human serum albumin-I^{131} from the plasma of mice and its appearance in the extravascular space in the skin was studied. The data demonstrate the presence of at least 2 phases in the skin in which the turnover rate for one is approximately 4 times greater than the other. The albumin in the slower moving phase is thought to be present in the space encompassed by the domain of hyaluronic acid.

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Amino Acid Utilization by Isolated Adipose Tissue of Meal-Fed Rats. (31532)

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Restricting food consumption to a single daily meal (meal-eating) induces marked adaptive changes in the laboratory rat. Liver and adipose tissue of meal-fed animals has a greater lipogenic capacity than does similar tissue from *ad libitum*-fed rats (nibblers)(1-3). The influence of meal-feeding on protein and amino acid metabolism has received little attention. It has been demonstrated that adipose tissue of meal-fed rats can oxidize and incorporate into lipid significantly more aspartate, glutamate and leucine carbon than tissue of nibbling control rats(4-6). Cohn *et al*(7) have reported that meal-fed rats excrete more ^{15}N from a dose of ^{15}N-labeled protein than do nibbling rats and, also, that the activity of hepatic arginine synthetase was enhanced by meal-feeding. Earlier studies had shown that meal-fed rats excrete more nitrogen in the urine than do nibbling controls (8). These data would suggest that protein catabolism is increased in meal-fed animals because of a limited ability to handle ingested amino acids during the short meal period. The available data do not, however, yield information concerning the metabolism of amino acids by specific tissues in meal-fed rats. Because of the important role of adipose tissue in the response to meal-feeding, the influence of meal-feeding on the ability of this tissue to utilize amino acids *in vitro* has been investigated.

Methods. Male Holtzman rats weighing approximately 200 g were housed in stainless steel cages having raised wire floors in a temperature and humidity controlled room (70° F and 50% relative humidity). The rats were divided into 2 groups, one of which was allowed access to food from 8-10 AM only (meal-eaters) and the other was fed *ad libitum*. Purina rat chow served as the diet and was ground to a powder in order to enable measurement of food consumption. Food consumption and body weight were determined weekly. The animals were maintained on these feeding regimens for 7 weeks, a period

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