Plasma Albumin Catabolism in Tissue Slices.* (25678)

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Plasma albumin, alpha and beta globulins are known to be synthesized in the liver(1) but the site of breakdown of these plasma proteins is not clearly established. *In vivo* experiments(2) suggest that liver is the main site of albumin degradation, but only limited breakdown of plasma albumin has been demonstrated in isolated perfused rat liver(3,4). Kidney also has been implicated as a site of plasma protein breakdown(5). Roberts and Kelley report extensive catabolism of all plasma proteins by rat liver slices(6). Since this was the only available report of such breakdown, we reinvestigated catabolism of plasma proteins by slices of liver and other organs.

Methods. Animals. Rats of Slonaker-Addis strain weighing about 200 g, and Californian rabbits were used. *Albumin I^{131}.* Plasma albumin was iodinated by procedure of Staub(7). To obtain a preparation of uniform halflife iodoalbumins were "screened" according to McFarlane(8). Homologous iodoalbumin was injected into rats or rabbits, and after 3-4 days animals were bled. Following dialysis against 0.9 NaCl solution to remove non protein I^{131} this screened material was used as radioactive substrate. The iodoalbumin preparations are described in greater detail elsewhere(9). Human iodoalbumin (RISA)® was purchased from Abbott Lab. *Incubation.* Uniform tissue slices of approximately 0.5 mm thickness were prepared with McIlvain-Buddle Slicer(10). Duplicate samples of rinsed and blotted slices were incubated in 20 ml heavy wall graduated glass tubes, that fit a homogenizer pestle. 250 mg of slices were incubated in 2 ml of various media (bicarbonate buffer, phosphate buffer, and serum) at 38° for 3 hours in presence of labelled plasma protein in shaking water bath. The gas phase was either pure oxygen or a mixture of 95% O_2 + 5% CO_2. At end of incubation period, slices were homogenized in the tubes, trichloracetic acid to yield final concentration of 5% was added and the mixture brought to 7 ml volume. Tubes were centrifuged and the clear supernatant used for assay. I^{131} activity was assayed in a scintillation well counter in conjunction with a single channel analyser. In our system one microcurie of I^{131} assayed at approximately 300,000 cts/min, with background count of about 10 cts/min. The extent of plasma protein breakdown was calculated from the difference between activity of trichloracetic acid extract present initially and at end of incubation. Total activity in vessel was obtained from assay of iodoalbumin, and in experiments where iodoalbumin was preinjected (see below), by assaying the trichloracetic acid residue. Proper corrections for counting geometry were applied as needed. *Precision of evaluation of lower limit of breakdown.* The lower limit of non protein I^{131} formation that can be measured depends on a) initial activity of trichloracetic soluble (non protein) fraction and b) background count rate. Unscreened iodoalbumin preparations had about 1% of activity soluble in trichloracetic acid, and screened preparations between 0.1 - 0.4%. In our conditions 10% increase over these initial blanks could be determined. Thus minimal breakdown rates of the order of 0.1% of unscreened preparations and about 0.02% of screened preparations could be estimated with an error of ± 50% or less. Total activity/vessel was usually well above 100,000 counts/minute with unscreened preparations. Thus minimal fractional breakdown rates, discussed above, were well above background count rate. On the other hand in experiments with preinjected albumin (see below) total activities/vessel ranged from several hundred to several thousand counts/minute. In these experiments minimal breakdown rate that can be determined is limited by the value of background count rate (about 10 cts/min) and fractional

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TABLE I. Breakdown of Screened and Unscreened Homologous Exogenous Iodoalbumin by Liver Slices and Homogenates.

Preparation	Incubation medium	Iodoalbumin breakdown (%) Not screened	Screened
	Rat		
Slices	Serum	2.0	.03
	Bicarbonate	2.8	.08
	Phosphate	2.4	.00
Homogenate	Serum	2.4	.04
	Bicarbonate	3.4	.10
	Phosphate	3.3	.07
	Rabbit		
Slices	Serum	.07	.00
	Bicarbonate	.04	.00
	Phosphate	.02	.00
Homogenate	Serum	.04	.00
	Bicarbonate	.03	.00
	Phosphate	.03	.00

300 mg slices and 0.5 ml whole homogenate (1:1 in 0.25 M sucrose) incubated with 2 ml medium for 3 hr at 39°. Results are avg of duplicate flasks. Serum dialysed for 48 hr against bicarbonate buffer. Gas phase: 5% CO_2 - 95% O_2 in serum and Krebs-bicarbonate buffer, 100% O_2 in Krebs phosphate buffer. Albumin screened 3 days for rat, 4 days for rabbit. Volume of iodoalbumins 0.2 ml in serum, dialysed against saline. Activity/flask in cpm: Rat, 260,000 unscreened, 32,000 screened; rabbit, 140,000 unscreened, 91,000 screened.

breakdown rates of less than 0.5-3% could not be evaluated reliably.

Results. Tissue and organ slices from rabbits and rats were incubated with their homologous plasma proteins as well as with human iodoalbumin. Slices were prepared from liver, kidney, spleen, lung, heart, small intestine, diaphragm, skeletal muscle and skin. A few experiments were performed with liver homogenates. Twenty experiments were performed employing tissues from about 80 animals. Since all experiments were essentially negative, and very limited or no plasma albumin breakdown was detected, only a few typical experiments are presented.

Exogenous screened and unscreened iodoalbumin. Table I represents experiments on incubation of liver slices and liver homogenates with screened or unscreened homologous iodoalbumin. With unscreened iodoalbumin there was breakdown by rat liver, but very little by rabbit liver preparations. On the other hand there was no significant breakdown in either case with screened iodoalbumin. The results were not markedly affected by composition of medium or by protein concentration, which was 10-20 times higher in serum than in buffers. It is unlikely that liberation of I^{131} of unscreened iodoalbumin by rat liver, independent of albumin concentration, represents true albumin breakdown. It appears then, that screened iodoalbumin is not degraded *in vitro* by rat or rabbit liver. McFarlane(8) first pointed out the distinction in catabolism of screened and unscreened iodoalbumin. This difference is due to presence in unscreened iodoalbumin of fractions of much shorter halflife than the bulk of labeled protein. A marked difference in catabolism of the 2 types of preparations has been reported in perfused liver(3,4).

Extravascular iodoalbumin breakdown. The absence of breakdown of added iodoprotein by tissue slices might be due to inability of exogenous albumin to penetrate into the slice. To determine whether extravascular albumin is broken down in slices, animals were injected with iodoalbumin and after several days tissue slices prepared from organs of these animals. Iodoalbumin content of tissue was presumably due in part to presence of residual blood and in part to extravascular exchangeable albumin. This later fraction may be considered as representing endogenous albumin.

Rats were injected with human iodoalbumin and sacrificed 2-3 days later. Rabbits were injected with homologous iodoalbumin and used 1-2 weeks later. Results are summarized in Tables II and III. In rats, 20-30% of activity in slices was in the non-protein fraction, but in rabbits non-protein I^{131} constituted less than 5% of total activity. From the specific activity of plasma albumin, albumin content of viscera was estimated to range from 1-3 mg/g, and of muscle and skin about 0.5 mg/g. Heterologous injected iodoalbumin is broken down as fast(2) or faster than the homologous compound. From Table II it appears that up to 2% of albumin present in viscera was broken down. This represents a *maximal* breakdown of about 0.01 $\pm$ 0.01 mg/g in liver and 0.03 $\pm$ 0.03 mg/g in lung in the 3 hour period. This amount of breakdown is of doubtful significance and it is not certain whether any breakdown oc-

TABLE II. Breakdown of Preinjected Human Iodoalbumin by Rat Tissue Slices.

Tissue	Approx. albumin content, mg/g	Exp. 1 % non-protein I^{131} Initial	Exp. 1 % non-protein I^{131} Final	Exp. 2 % non-protein I^{131} Initial	Exp. 2 % non-protein I^{131} Final	Exp. 3 % non-protein I^{131} Initial	Exp. 3 % non-protein I^{131} Final	Avg increase in non-protein, %
Liver	.8	17	19	31	34	37	38	2
Kidney	1.3	32	35	32	30	49	46	−1
Lung	3.2	9	10	6	7	9	9	−1
Spleen	1.1	27	32	30	29			2
Muscle	.5	10	10	10	11	11	10	0

Rats were inj. with about 100 μc of commercial human iodoalbumin (RISA) and sacrificed 2 to 3 days later. 250 mg slices in 2 ml bicarbonate buffer. 95% O_2 - 5% CO_2, 37°, 3 hr. Results are avg of duplicate flasks. Total activity/flask from about 300 (muscle), to 2000 (lung) cpm.

curred at all. Similar results were obtained with homologous rat iodoalbumin. In slices of rabbit viscera the increase in non-protein activity after 3 hour incubation ranged from 0.5-1.5% of total activity. There was no increase in muscle (Table II). Maximal breakdown is of the same order as in rat. Again, the values are of doubtful significance, since such low rates of breakdown cannot be measured reliably by our methods.

S^{35} labeled plasma proteins. Catabolism of S^{35} biosynthetically labeled rat and rabbit plasma albumin and globulin by a variety of tissue slices was also studied. The only tissue that broke down S^{35} labeled plasma proteins at an appreciable rate was intestinal mucosa. This breakdown is likely to be due to presence of digestive enzymes in this tissue. As results with other organs were similar to those obtained with iodoalbumin and essentially negative, the data are not presented here in detail. Also a small amount of S^{35} amino acids liberated from albumin breakdown may be reutilized for protein synthesis. Reutilization of liberated I^{131} on the other hand, does not occur.

Discussion. *In vivo* experiments indicate that liver is a major site of plasma albumin breakdown (2). Our results, in which slices of small intestine were the only tissue to catabolize exogenous plasma protein seem to be inconsistent with *in vivo* results. Failure to observe significant breakdown in organ slices is not likely to be due to lack of penetration into the slice. When iodoalbumin was injected so that tissue extra-vascular albumin became labeled, no significant breakdown was detected (Tables II and III). Breakdown of screened iodoprotein also did not occur in liver homogenates.

Minimal value for plasma albumin turnover in the rat is estimated to be about 2.5 mg/hr/

TABLE III. Breakdown of Preinjected Iodoalbumin by Rabbit Tissue.

Tissue	Approx. albumin content, mg/g	Days after inj. of albumin: 7 % non-protein Initial	7 % non-protein Final	14 % non-protein Initial	14 % non-protein Final	Avg increase in non-protein, %
Liver	.8	2.4	2.9	2.8	3.4	.5
Kidney	3.5	3.6	4.2	3.0	2.9	.4
Lung	3.0	1.7	3.2	2.7	3.0	.9
Spleen	1.6			2.8	3.3	.5
Small intestine	1.2	2.6	3.8	1.4	1.7	.8
Heart	2.8			1.3	1.3	
Diaphragm	.7			1.6	1.4	−.2
Liver homogenate		2.2	2.4	2.4	2.7	.2
Kidney "		3.1	3.3			.2

0.5 g rabbit tissue slices (spleen 0.25 g) incubated with 3 ml Krebs Ringer bicarbonate buffer. Gas phase: 95% O_2 - 5% CO_2, incubated at 37° for 3 hr. Homogenate 1:1 in 0.25 M sucrose, 1 ml incubated with 2 ml buffer. Results are avg of duplicate flasks. Total counts ranged from 1400 cpm vessel (diaphragm) to about 7000 (lung, kidney) cpm.

100 g body weight.* Assuming that ⅔ of the breakdown occurs in rat liver, we obtain a breakdown rate of 0.4 mg/hr/g liver. In rabbit the corresponding breakdown would be 1.0 mg/hr 100 g body and 0.15 mg/hr/g liver (1). Breakdown rates of this order could be readily detected in our experiments since the lower limit of methods used would reveal breakdown of at least 0.01 mg/hr of exogenous albumin/g of tissue.

The only detailed report of plasma protein breakdown in liver slices appears to be that by Roberts and Kelley(6). They found that when plasma proteins, labeled biosynthetically with phenylalanine 3-C^{14}, were incubated with liver slices, incorporation of C^{14} into CO_2, glucose, and glycogen occurred. Their paper reveals certain inconsistencies in results. Rate of albumin breakdown, as well as production of respiratory CO_2 implied by their results seems unphysiologically high. Thus, a conservative estimate of albumin breakdown, computed from their data, is 11 mg/hr/g liver.† Even this low estimate is of a different order of magnitude than that occurring *in vivo*. The presence of a contaminant of high specific activity in their plasma protein preparations may account for such results.

In biosynthesis of plasma proteins from S^{35} and C^{14} precursors, non-protein radioactive compounds, tightly bound to protein may be formed. Also non-protein compounds, absorbed on protein may be labeled during iodination with I^{131}. In short term experiments, as with slices or organ perfusions, liberation of small amounts of activity may erroneously indicate appreciable degradation rates of plasma proteins. Since this activity may be derived from bound non-protein compounds great caution in interpretation of such results is required.

Recently abstracts reporting plasma albumin breakdown by liver slices(11), and liver mitochondria have appeared(12). Since the preliminary reports lack adequate experimental detail, these findings cannot be evaluated. Armstrong and Tarver(13) on the other hand, could not detect $C^{14}O_2$ formation from C^{14} labeled plasma albumin by rat liver slices.

To demonstrate *in vitro* breakdown, special conditions, as yet unknown, may be required. Therefore no final conclusions on the role of tissues in plasma protein catabolism can be derived from negative results in tissue slice experiments as obtained above.

Summary. I^{131} labeled human albumin and screened and unscreened iodinated rat and rabbit albumins were incubated with tissue slices, prepared from rat and rabbit liver, kidney, spleen, lung, heart, muscle, and skin. No breakdown was observed in rat and rabbit liver homogenate. When iodoalbumins were preinjected into animals prior to preparation

* Taking plasma volume as 4% of body weight, plasma albumin concentration as 30 mg/ml, total body albumin pool 2.5 as large as plasma pool, and a turnover time of rat plasma albumin of 120 hours (about 80 hour halflife) we get $\frac{30 \times 4 \times 2.5}{120} = 2.5$ mg/hr/100 g rat. Liver is assumed to constitute 4% of body weight. In rabbit albumin content is about the same but turnover time is taken as 300 hours.

† Assuming that 25% of carbon 3 of phenylalanine liberated from protein is catabolised to CO_2, and using Roberts *et al.*(6) data in Table II (100 mg slices, 10 mg of albumin, 2¼ hr incubation, 5.7% $C^{14}O_2$) we calculate that $\frac{5.7 \times 10 \times 100}{100 \times 2.25 \times 25 \times 0.10} =$ 11 mg/g/hr were catabolised by liver slices. The assumption of 25% yield of CO_2 from carbon 3 of phenylalanine is likely to be far too high and the albumin breakdown much higher than the minimal value computed here. We found that when 3-C^{14} phenylalanine was incubated with liver slices the main water soluble metabolic products were unidentified phenolic acids and appreciable amounts of activity were incorporated into tissue proteins. CO_2 yield was low, less than 10% of catabolised C^{14}. Assuming also that CO_2 yield from all carbons of protein is the same as from carbon 3 of phenylalanine and taking albumin to consist of 55% of C and using Roberts data from his Table II we obtain $\frac{5.7 \times 10 \times 0.55}{100 \times 2.25 \times 10 \times 0.014} = 100$ μmoles of respired CO_2/g/hr. No doubt however, additional CO_2 is derived from endogenous substrate, and also the average CO_2 yield from protein carbon is much higher than that from phenylalanine. Thus this calculation certainly gives a minimal value and the true value as calculated from Roberts $C^{14}O_2$ yields should be much higher. Roberts himself determined directly respiratory CO_2 production by his liver slices, and the maximal value observed by him was 69 μmoles of CO_2/g/hr.

of slices, to introduce iodoalbumin into the extravascular exchangeable pool of various organs, significant breakdown did not occur. Breakdown of exogenous S^{35} biosynthetically labeled homologous albumin and globulin occurred only in slices of small intestine.

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Incorporation of P^{32} into the O-Phosphodiester of L-Serine and Ethanolamine in the Turtle.* (25679)

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Roberts and Lowe(1) reported presence of O-phosphodiester of L-serine and ethanolamine ([2-aminoethyl]-[L-2-amino-2-carboxyethyl]-phosphoric acid) in turtle tissue. This compound (SEP) also has been found in tissues of snake and alligator. SEP and the DL-serine phosphodiester have been synthesized (2). A similar compound and a phosphagen thereof have been found in the earthworm in which the 2-aminoethyl group is replaced by 2-guanidoethyl or phosphoguanidoethyl group (3). The purpose of our study was to obtain information on biosynthesis of SEP in turtle tissue. A preliminary report of this work has been made(4).

Results. Comparison of isolated and synthetic SEP. Mobilities of isolated SEP(1) and the synthetic compound(2)† were compared in 4 solvent systems by descending chromatography: (a) phenol saturated with water; (b) methyl Cellosolve-water (9:1); (c) acetone-acetic acid-water (4:2:2); and (d) n-butanol-acetic acid-water (8:20:20). The compounds were visualized by spraying with ninhydrin. The results are shown in Fig. 1. In each solvent the compound on the left was the isolated material, that on the right the synthetic material, and in the middle a mixture of the two. Approximately 200 γ of each were used. In each case the natural and synthetic materials showed identical mobilities.

Incorporation of P^{32} orthophosphate into SEP. After drilling holes through plastron above the heart with a trephine, approximately 1 mc of P^{32} orthophosphate in isotonic saline was injected into the ventricle of each of 5 unanesthetized turtles (*Pseudemys elegans*), which were sacrificed at 9, 26, 48, 76 and 99 hours, respectively, after injections. The holes were covered with adhesive tape after injection to prevent contamination and leakage of body fluids. The various organs were removed and homogenized in 10 volumes of 80% ethyl alcohol. After centrifugation the clear supernatant fluids were evaporated to dryness under stream of nitrogen. Residues were taken up in 50% alcohol so that 0.1 ml of the mixture was equivalent to 100 mg of original fresh weight of tissue, and 0.1 ml aliquots were spotted on paper for chromatography. Two-dimensional chromatograms were run routinely in phenol-water and

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