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Plasma Protein VI. Catabolism *in vitro*.* (25505)

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It is apparent from numerous studies on turnover that serum albumin is being synthesized and degraded at a rapid rate in the mammal. The data of Cohen and coworkers (1) indicate that degradation occurs in the rabbit at 48 mg/hour. The present work was designed to determine the site of breakdown in the rat by carrying out *in vitro* studies with tissue slices and homogenates incubated with albumin which had been randomly labeled with C^{14}. Studies of this nature have already been carried out by Roberts and Kelley(2), who labeled rat albumin by administering phenylalanine-3-C^{14} to donor rats and then measured catabolism of labeled albumin in tissue slices from livers of the same species. They reported that albumin was rapidly broken down, a result that we have been unable to repeat. Katz and coworkers (unpublished) have likewise been unable to confirm results of Roberts and Kelley, using iodinated and S^{35}-labeled serum albumin. Other workers investigated the breakdown of albumin in liver either *in vivo* or by using perfusion technic (with or without reticuloendothelial blockade with india ink). Results of such experiments are not in good accord. Miller and coworkers (3) reported considerable breakdown of plasma protein by the perfused rat liver, whereas Gordon(4) found that perfused rat liver broke down albumin at a rate of only 10 to 15% of that calculated for the intact animal from turnover data. However, it has been reported that partial hepatectomy in rats (5) and mice(6) results in decrease in turnover rate of albumin. Following reticuloendothelial blockade various workers have reported conflicting results(6,7,8).

Methods. Slices or homogenates of rat tissues were incubated at 37° in buffer with plasma protein or albumin randomly labeled with C^{14}, made by feeding donor animals with labeled *Rhodospirillum rubrum*(9,10). Serum albumin was separated by salt fractionation. The method used was based on observations of Butler and Montgomery(11), and involved precipitation of globulins with 4 vol. 2.4 *M* phosphate buffer at pH 6.5. The solution was allowed to stand at room temperature for 24

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TABLE I. Serum Albumin Breakdown *In Vitro*.

System	Tissue used, mg	Labeled substrate,* mg	SA† × 10^{-3}	Incubation time, hr	Activity $BaCO_3$‡	Recorded TCA soluble, %
Rat liver slices						
Bicarb.§	300	A (15)	4	3.5	None	None
Phosph.‖	100, 160	A (10)	4	2, 2.5	"	"
Rat tissue homogenate						
Liver-bicarb.	200–750¶	P (1-15)	2.4	3.5–5	"	"
"	600	A (9)	4	5	"	"
Kidney-phosph.	500	A (10)	4	2.5	"	"
Spleen- "	600	A (10)	4	2.5	"	6.2
Ascites- " **	500–600	A (10)	4	2.5–5.5	"	None
Liver- "	700	AH (5)	(21)††	2.5	+	−30‡‡

* A, serum albumin (randomly labeled with C^{14} from *Rhodospirillum rubrum* grown in $C^{14}O_2$ containing medium); P, total plasma protein labeled as A; AH, acid hydrolysate of A (5 mg).

† SA, specific activity in terms of cpm/mg protein.

‡ Activity recorded from CO_2 in ionization chamber, generally from 60 mg of $BaCO_3$. When standard samples of $C^{14}O_2$ (100 dpm) were passed through chamber, activity registered was very much above background, showing that apparatus was functioning at normal efficiency.

§ Bicarbonate buffer at pH 7.4 (Cohen(13)).

‖ Krebs-Ringer phosphate buffer at pH 7.4 (Cohen(13)).

¶ 5 incubation experiments.

** Ehrlich ascites tumor cells from C^3H mouse; wt of cells estimated from packed cell vol.

†† Total activity added to incubation mixture (cpm).

‡‡ Since all activity added was TCA-soluble, activity disappeared from medium due to catabolism of amino acids to $C^{14}O_2$.

hr. Then the albumin solution was filtered off, subjected to thorough dialysis and lyophilized. Its purity was evaluated by electrophoresis on paper. Total plasma protein in some experiments was obtained by thorough dialysis of C^{14}-labeled blood plasma. Radioactivity in solid samples was determined by sedimenting samples on aluminum planchets followed by counting with G.M. counter in conventional manner. However, to increase sensitivity, CO_2 produced from labeled albumin or other substrate was introduced into the chamber of a vibrating reed electrometer and its activity measured by rate of charge method(12). By this method the sensitivity for detecting radioactivity was increased by a factor of 5 above that observed with a G.M. tube with a thin window. Activity is reported as cmp or dpm (disintegrations minute), depending on whether the G.M. counter or Electrometer was used in determination of radioactivity. Tissue slices or homogenates were incubated under conditions given in the Table, in the presence of 1% CO_2 in 99% oxygen. When tissue slices were used, they were added to 2 ml of labeled albumin solution made up in either bicarbonate or phosphate buffer (see footnotes, Table I). Tissue homogenates were made up to contain the amount of tissue used in 2 ml of buffer. To this was added the albumin dissolved in 2 ml of the same buffer. Two to 4 liters of gas mixture were passed slowly through the incubation flask, and into a pair of traps containing NaOH solution. In all experiments a water trap (conc. H_2SO_4) and ionization chamber (250 ml) were placed between incubation flask and NaOH traps, to monitor the activity directly. However, in no case, except with the hydrolysate of albumin, was sufficient radioactivity present to make rate of charge differ significantly from background rate. When the period of incubation was over, sulfuric acid was tipped into the incubation mixture to drive out any residual CO_2. The CO_2 trapped in the NaOH solution was precipitated as $BaCO_3$. CO_2, generated from weighed amounts of the precipitate, was introduced into the evacuated ionization chamber for determination of its activity. Contents of the flask used for incubation, were treated with sufficient trichloroacetic acid (TCA) to precipitate all the protein. After centrifuging the protein, aliquots of the supernatant were dried on polyethylene planchets, and activity was determined with a G.M. counter. The difference between activity found after incuba-

tion and that found at zero time is recorded in last column of Table. The amount, expressed as per cent of activity added, is taken to represent the fraction of the protein broken down to amino acids and soluble peptides.

Results of these experiments which were nearly all negative are reported in Table I. There was no significant production of $C^{14}O_2$ from labeled albumin or plasma protein, although when albumin was hydrolyzed, some of the amino acid carbon was catabolized to $C^{14}O_2$ (last line of Table). The only significant breakdown of labeled albumin to give TCA soluble products was found with spleen homogenate incubated at pH 7.4.

Gordon(4) reported that perfused liver from rats, degraded albumin at 1 mg/hour. Hence, assuming analogous behavior of liver slices, 160 mg of liver slices in 2.5 hours should have degraded 27 μg of albumin, or 108 cpm should have been released. In the ionization chamber this 108 cpm is equivalent to approximately 540 dpm. One-tenth of this activity should have easily been detected. Therefore, it is necessary to conclude, either that albumin does not penetrate the tissue slice with sufficient rapidity so that breakdown is demonstrable in 2.5 hours, or that breakdown of albumin depends on its concentration, and that this was not sufficiently high in these experiments. However, this should hardly have been the case in experiments involving homogenates. If the first situation prevails it is not clear why albumin was not broken down in the homogenates, unless breakdown requires some type of structural integrity.

It is difficult to explain why our results are in such great contrast with those of Roberts and Kelley(2). Even though a different type of labeling was employed by these workers, it is not apparent why an albumin labeled with phenylalanine-tyrosine-3-C^{14} should behave differently from albumin labeled in any other way *provided the albumin has not become modified in some way during its preparation.* It might be suggested that phenylalanine-tyrosine-3-C^{14} is reincorporated into newly synthesized albumin in liver slices to a greater extent than are the randomly labeled C^{14}-amino acids. Such a phenomenon has been shown to occur in the intact rabbit by Penn and coworkers(14). However, if reincorporation of essential amino acids does occur to a considerable extent in tissue slice experiments, the question arises as to why methionine-cystine-S^{35} was not reincorporated in the investigations of Katz and Sellers(3). Thus all that remains is to suggest that at least part of the albumin used by Roberts and Kelley had suffered a denaturation, rendering it more vulnerable to breakdown.

In view of the lack of breakdown of albumin in our work, and the low rate of breakdown observed in liver perfusion experiments of Gordon(4), as compared with the high rate anticipated from turnover data, we are investigating other possible sites of serum albumin catabolism.

Summary. 1. Rat serum albumin randomly labeled with C^{14} amino acids was prepared. 2. Neither this serum albumin, nor dialyzed whole plasma similarly labeled, was broken down to any significant extent by any of the tissue slice or homogenate systems tested, although it was anticipated that this should have been demonstrable from breakdown rates calculated from turnover data and from liver perfusion data. 3. It is suggested that either serum albumin breakdown depends on its concentration, the concentration being too low in our experiments; or that albumin is broken down at some other site in the animal.

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Passive Transfer of Resistance to Tuberculosis Through Use of Monocytes.* (25506)

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Numerous attempts have been made to elucidate the mechanism of acquired resistance to tuberculosis. In particular, the possible role of cellular changes in acquired resistance has received considerable attention. Initial experiments by Lurie(1) indicated that rate of multiplication of tubercle bacilli was reduced in monocytes obtained from immunized animals when compared to monocytes obtained from nonimmunized animals. These early experiments were performed with a technic which employed the anterior chamber of eyes of nonimmunized rabbits as culture medium. Rich(2) however, using tissue culture technics, was unable to demonstrate inhibition of multiplication of tubercle bacilli in leukocytes obtained from immunized animals. Recent experiments utilizing improved tissue culture methods also resulted in divergent findings. Suter(3) reported significant inhibition of multiplication of tubercle bacilli in monocytes obtained from immunized guinea pigs when compared to multiplication in monocytes obtained from normal guinea pigs. He also found that serum obtained from immunized animals had no influence on results. Mackaness(4) on the other hand, reported no difference in this respect between monocytes from normal and immunized rabbits. Fong *et al.* (5) indicated that effective resistance to virulent tubercle bacilli by immune monocytes required continuous presence of immune serum. Lastly, Berthrong and Hamilton(6) recently reported that monocytes from immune guinea pigs limited intracellular multiplication of virulent tubercle bacilli. A direct resolution of the role of cells in acquired resistance to tuberculosis would involve the demonstration of transfer of resistance to tuberculosis to normal animals through use of monocytes from immunized animals. The present paper deals with experiments demonstrating this transfer.

Materials and methods. Mice of Strong A strain were used. Animals weighing initially 18-22 g were housed in separate metal cages in groups of 10 and were fed food and water *ad lib.* Mice were challenged by administration of 1 mg wet weight of H37Rv strain of *Mycobacterium tuberculosis* var. *hominis,* For this purpose, 3 week old surface pellicle cultures were harvested from modified Proskauer and Beck medium and standardized, using methods previously described(7). Immunized animals employed as donors for passive transfer experiments were given 1 mg wet weight of BCG‡ intravenously 3 weeks before sacrifice. These mice were immunized at appropriate consecutive intervals so that in each instance of passive transfer, donor preparations were available of the same age and as nearly alike as possible. A summary of ma-

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