Utilization of Labeled Proteins in Synthesis of Tissue Proteins.* (24859)

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Numerous studies indicate that proteins administered parenterally can supply body protein needs over long periods of time(1-7). Howland and Hawkins(5) and Whipple and Madden(6) observed a positive nitrogen balance and no significant nitrogen loss in urine when plasma proteins were given to dogs intravenously, and increased excretion of nitrogen when plasma was given orally. These results were presented as evidence that injected plasma proteins are more completely utilized by the body than are orally administered proteins, and that injected plasma proteins can be utilized for synthesis of tissue proteins without prior breakdown to their constituent amino acids. Other workers(7-10), however, suggest that proteins administered intravenously diffuse into the lymphatics, and are then slowly metabolized, breaking down gradually and probably completely to amino acids. Yuile *et al.*(11) studied metabolism of C^{14}-lysine-labeled albumin and globulin administered intravenously to dogs. They found that transfer of radiocarbon from plasma to tissue proteins is accompanied by very small loss of radioactivity in urine and expired air; there was no evidence of complete breakdown to amino acids suggesting that plasma proteins are utilized in body economy as such, or after only partial catabolism within the cell. The following report presents data suggesting that isotopically-labeled proteins enter ascites cells *in vitro*, are utilized for synthesis of cell proteins, and do not appear to be completely broken down to their constituent amino acids.

Methods. Ehrlich mouse ascites carcinoma cells were obtained as described(12) and resuspended in 5 volumes of solution (Incubation Media) having following composition: 0.02 M potassium phosphate buffer, pH 7.8; 0.035 M potassium bicarbonate; 0.025 M potassium chloride; 0.004 M magnesium chloride; and 3 mg of heparin/100 ml. Two ml aliquots of the cell suspension were incubated with radioactive proteins or an equivalent amount of radioactivity as free amino acids for varying periods to 6 hours. At end of incubation the cells were recovered by centrifugation, washed twice with cold Incubation Media, precipitated with trichloroacetic acid and total acid-soluble and protein fractions isolated(13,14). In experiments in which the cellular fractions of ascites cells were prepared by differential centrifugation (15-17) following incubation, total acid-soluble and protein fractions were obtained from individual cellular fractions by similar procedure. Determinations of radioactivity were carried out by direct plating method(17). Ascites cell and plasma proteins were labeled *in vivo* by single intraperitoneal injections of 1 mg of DL-leucine-2-C^{14} (specific activity = 4.5 x 10^6 cpm/mg) or 4 mg S^{35}-L-methionine (specific activity = 6 x 10^6 cpm/mg), into tumor bearing mice. Forty-eight hours after injection of isotopic amino acid, the ascites tumor was removed and cells and plasma separated by centrifugation. Plasma proteins were dialyzed overnight against running water at 4°, then for 4 hours against 10 volumes of Incubation Media. An insoluble residue, formed during dialysis, was removed by centrifugation and discarded. The labeled ascites cells were lysed in distilled water(17), centrifuged at 144,000 x g for 2 hours, and the supernant dialyzed as described above.

Results. In vitro incorporation of radiocarbon from plasma proteins labeled with C^{14}-leucine or S^{35}-methionine, and from corresponding free amino acids is presented in Fig. 1. Approximately 16,000 cpm of C^{14}-leucine (1A) or C^{14}-plasma proteins (8 mg, 1B) and 40,000 cpm of S^{35}-methionine (1C) or S^{35}-plasma proteins (6 mg, 1D) were incubated with ascites cells for 1, 2, 4 and 6 hr in total volume of 3 ml. Transfer of C^{14} or S^{35} from plasma proteins to cell proteins occurred without accumulation of significant levels of acid-

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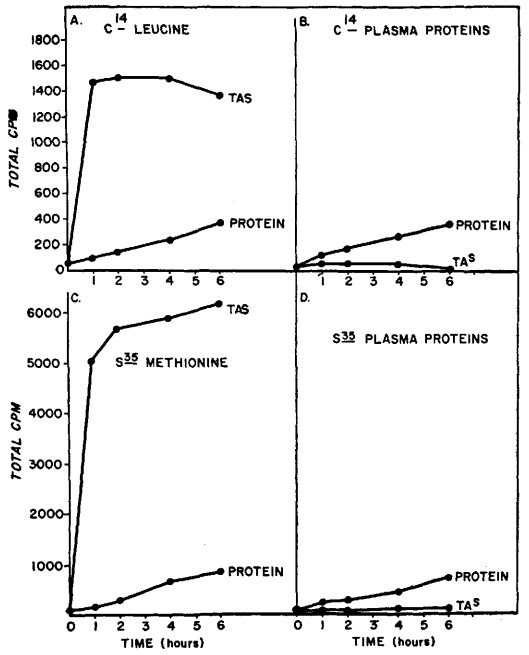

FIG. 1. Incorporation of radiocarbon into ascites cell protein and acid soluble fractions. Cells were incubated with C^{14}-leucine (A), C^{14}-labeled ascites plasma proteins (B), S^{35}-methionine (C) and S^{35}-labeled ascites plasma proteins as described in text.

soluble radioactivity whereas in experiments with free amino acids, markedly higher levels of acid-soluble radioactivity occurred concomitantly with incorporation into proteins.

Plasma proteins (140,000 cpm, 20 mg) and in a separate experiment cell proteins (180,000 cpm, 15 mg) previously labeled *in vivo* with S^{35}-methionine, were incubated with ascites cells for 1 hour in total volume of 5 ml. Following incubation, the cells were recovered by centrifugation, lysed in distilled water and separated into nuclear, mitochondrial, microsomal and supernatant fractions(17). A similar experiment was carried out with S^{35}-L-methionine (150,000 cpm). The results are summarized in Table I. Radioactivity was recovered in proteins of most fractions from cells incubated with labeled plasma proteins; no radioactivity was detected in acid-soluble fractions. Incubation with S^{35}-cell proteins obtained from the cellular supernatant of labeled ascites cells revealed significantly higher levels of radioactivity in proteins of cell fractions isolated, as compared to plasma protein incubations. Radioactivity was also detected in the acid-soluble fraction from nuclei, mitochondria and supernatant but not from microsomes; in most cases, however, protein-bound radioactivity was significantly greater than acid-soluble radioactivity. It should be pointed out that the nuclear fraction is probably contaminated to some extent with whole cells not lysed by this procedure.

Significant incorporation of radiosulfur from S^{35}-L-methionine into the proteins of various cellular fractions occurred during incubation period; non-protein radioactivity, however was higher in all fractions.

The small amounts of radioactivity found in acid-soluble fraction prepared from the incubating media after removal of ascites cells by centrifugation (Table I), may have been due to adsorbed free amino acids not removed by dialysis during preparation of labeled proteins or to incomplete precipitation of some labeled proteins used as substrates.

TABLE I. Incorporation of Radioactivity from S^{35}-Labeled Proteins and S^{35}-L-Methionine into Proteins of Ascites Cell Fractions.

	Total radioactivity					
	Plasma proteins		Cell proteins		L-methionine	
	t_o	1 hr	t_o	1 hr	t_o	1 hr
Total acid-soluble						
Nuclei	0	0	0	135	897	4,433
Mitochondria	0	0	0	291	0	125
Microsomes	0	0	0	0	0	1,320
Supernatant	0	0	162	548	4,218	34,080
Incubation media	3640	3948	3456	2183	146,000	106,000
Proteins						
Nuclei (8 mg)	78	350	104	4586	18	902
Mitochondria (1 ")	0	1	8	104	6	42
Microsomes (5 ")	26	41	19	2889	16	241
Supernatant (11 ")	198	1133	59	2931	5	1,078

Low levels of radioactivity recovered in acid-soluble fractions following incubation with labeled cell proteins suggest that these proteins may have been degraded to some extent to low-molecular weight products either as the result of catheptic hydrolysis or of metabolic degradation. Incubations with isotopic free amino acids led to accumulation of considerable amounts of free amino acids within the cell during incorporation into proteins; however, incubations with isotopic proteins led to labeling of proteins without significant accumulation of intracellular free amino acids. These results suggest that ascites cells can utilize proteins for synthesis of tissue proteins by pathways which do not involve complete breakdown to free amino acids or low molecular weight peptides. The observation that protein of cellular fractions of ascites cells contains radioactivity following incubation with labeled proteins, would indicate that the proteins used as substrates enter the cell and that protein labeling is not due to adsorption on cell surface as might be interpreted from studies with intact cells (Fig. 1). The failure of Fillerup *et al.*(18) to observe penetration and oxidation of C^{14}-labeled albumin by ascites cells may have been due to low levels of radioactivity used in their incubation.

It is interesting that incubations with soluble proteins obtained by high speed centrifugation of lysed radioactive ascites cells lead to a significantly greater labeling of cell proteins than with labeled ascitic plasma proteins.

Summary. Ehrlich ascites cells were incubated *in vitro* with radioactive amino acids or radioactive ascites proteins prepared biosynthetically. Incubations with free amino acids resulted in accumulation of large amounts of radioactivity in the acid-soluble fraction and in incorporation of radioactivity into the proteins. Incubations with labeled proteins resulted in labeling of cell proteins without significant accumulation of radioactivity in the acid-soluble fraction. Radioactive proteins were isolated from various cell fractions of ascites cells following incubation with labeled free amino acids or proteins. These results suggest that proteins enter ascites cells where they are utilized for synthesis of cellular proteins; free amino acids do not appear to be intermediates in this process.

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