Amino Acid Metabolism and the Biosynthesis of Prothrombin in the Perfused Rat Liver[1] (34432)

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The basic technique of rat liver perfusion developed by Miller *et al.* (1, 2) has been modified in this laboratory to permit the study of the action of vitamin K on the *de novo* biosynthesis of prothrombin and related clotting factors in rat liver. A combination isotope labeling and immunochemical precipitation of prothrombin has been employed to demonstrate *in vitro* biosynthesis of this coagulation protein (3). Other laboratories have also demonstrated the release or synthesis of prothrombin and other vitamin K-dependent clotting factors in isolated, perfused rat livers (4–7). During the course of our studies of the effect of vitamin K on prothrombin biosynthesis, the metabolism of different amino acids in the isolated perfused rat liver varied to a great extent. Thus the mode of addition of radioactive amino acid to the isolated liver during perfusion is very important for calculation of rates of biosynthesis since the concentration of amino acids in the perfusate, and presumably in the liver, vary greatly during a 6-hr perfusion. The present communication reports the changes in concentration of different amino acids in the isolated perfused rat livers under these conditions.

Materials and Methods. Normal male rats of the Edward Doisy colony at St. Louis University, originally of the Wistar strain, ranging in body weight from 200–300 g, were used. Warfarin in doses of 3 mg/100 g of body weight was administered to the rats 48 and 24 hr prior to the isolation of the livers. The technique of liver perfusion was that of Miller *et al.* (1). Glucose was added at 300 mg/100 ml; heparin, 10 units/ml; and terramycin, 10 mg/100 ml. The composition of amino acid mixture (μmole/ml of perfusate) was: L-aspartic acid, 0.739; L-threonine, 0.376; L-serine, 0.457; L-glutamine, 0.470; L-proline, 0.236; glycine, 0.671; L-alanine 0.736; L-valine, 0.396; L-methionine, 0.075; isoleucine, 0.274; leucine, 0.191; L-phenylalanine, 0.266; L-lysine, 0.821; L-histidine, 0.124; L-arginine, 0.579; and L-tryptophan, 0.094. Fifty μCi of L-leucine-1-^{14}C (51 mCi/mmole) was added to the perfusate at zero time. Warfarin in concentration of 10 μg/ml was added to the perfusate to antagonize the endogenous synthesis of clotting factors. Prothrombin was measured by the method of Hjort *et al.* (8). Prothrombin radioactivity was determined by precipitation with specific antibody (9). Vitamin K_1 was added to the perfusate 2 hr after zero time in certain perfusions. Perfusion was terminated at 6 hr. Samples of perfusate were taken at zero time and at 2-hr intervals for the assays of amino acids, prothrombin, and radioactivity as follows. The particulate matter in the perfusate samples was first removed by centrifugation at 6000*g* for 10 min. Protein was precipitated by adding 0.2 ml of a 21% aqueous solution of sulfosalicylic acid to each ml of the perfusate. The precipitates were then removed by filtering through Millipore filters. One or 0.5 ml of such deproteinated perfusate was applied to each column of a Beckman model 120 amino acid analyzer for ion exchange chromatography (10). Norleucine was added to the analyzed sample as an internal standard. A split stream was developed to approximate a 1:1 ratio. Fractions of effluent were collected in minute intervals (av volume 0.62 ml/fraction). One-tenth ml

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TABLE I. Change in Concentration of Amino Acids in the Perfusate of an Isolated Rat Liver Observed Over a 6 hr Period.

Amino acid	(μmoles/ml of perfusate); perfusion (hr)			
	0	2	4	6
Aspartic acid	0.778	0.341	0.086	0.025
Threonine	0.334	0.224	0.160	0.183
Serine	0.666	0.334	0.322	0.392
Glutamic acid	0.046	0.305	0.430	0.461
Proline	0.219	0.093	0.046	0.042
Glycine	0.650	0.239	0.116	0.101
Alanine	0.705	0.461	0.323	0.336
Valine	0.322	0.426	0.583	0.898
Methionine	0.049	0.022	0.011	0.011
Isoleucine	0.218	0.282	0.393	0.614
Leucine	0.135	0.278	0.473	0.797
Tyrosine	—	0.029	0.026	0.053
Phenylalanine	0.160	0.022	0.026	0.035
Lysine	0.461	0.348	0.273	0.119
Histidine	0.064	0.020	0.040	0.037
Ammonia	0.424	0.336	0.411	0.342
Arginine	0.361	0.113	0.045	0.022

of each fraction was added to 10 ml of Bray's solution (11) for the determination of radioactivity in a Packard scintillation spectrometer.

Results. The changes in the levels of different amino acids in the perfusate from isolated rat livers studied over 6 hr are presented in Table I. It is evident that different amino acids had a different metabolism during the 6-hr perfusion period. The amino acids that increased were glutamic acid, valine, isoleucine, and leucine. The amino acids which decreased were aspartic acid, proline, glycine, alanine, methionine, phenylalanine, lysine, and arginine. Threonine and serine decreased considerably during the first 2-hr period but stayed relatively constant during the last 4-hr period. In general the changes in all the amino acids were greater during the first 2-hr period. After that, the rate of change decreased. Vitamin K had no significant effect on the overall amino acid metabolism of the isolated perfused rat liver nor did it affect the rate of uptake of leucine-1-^{14}C as shown in Table II. It was shown furthermore from study of the split stream, that practically all the radioactivity present

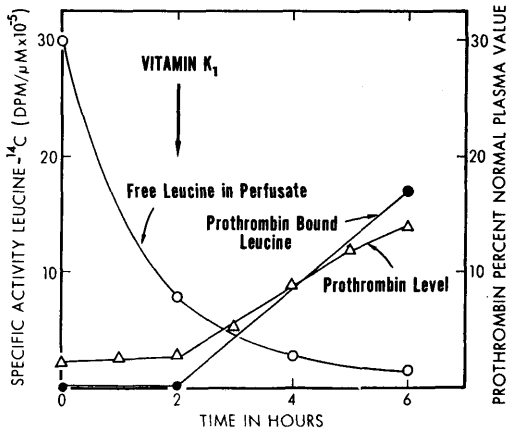

FIG. 1. Change in the specific activity of free and protrombin-bound L-leucine-1-^{14}C and total prothrombin during 6 hr of perfusion of an isolated rat liver. Vitamin K_1 (Aquamephyton) in a dose of 5 mg was added to the perfusate at 2 hr.

in the perfusate samples was recovered as leucine.

Changes in the specific radioactivity of the free and prothrombin-bound leucine in the perfusate with time are presented in Fig. 1. The addition of vitamin K_1 (5 mg) to provide a concentration of about 25 μg/ml of perfusate at 2 hr resulted in a prompt increase in both total and radioactive prothrombin. The parallel changes in both total assayable and radioactive prothrombin suggest that vitamin K stimulates *de novo* biosynthesis of this coagulation protein.

Discussion. It is evident that the uptake, release, and metabolism of individual amino acids by the isolated rat liver is highly variable. For example, leucine concentrations in-

TABLE II. Changes in Radioactivity of L-Leucine-1-^{14}C in Rat Liver Perfusate at Various Times.[a]

Time of perfusion (hr)	(dpm $\times$ 10^{-5}/ml of perfusate)	
	Addition of vitamin K_1 (5 mg/liver)	No addition of vitamin K_1
0	4.2	3.7
2	2.2	1.6
4	1.4	1.2
6	1.1	1.1

[a] 50 μCi of L-leucine-1-^{14}C were added to a total of 180 ml of perfusate at zero time.

creased 5–6 fold whereas aspartic acid fell to 5% of its initial value. Phenylalanine fell to 10% of its initial value in 2 hr and remained low. Tyrosine, which was not added initially, remained low throughout the 6-hr perfusion. Whether these changes parallel those in the hepatocyte remains doubtful. Prothrombin levels rose after the addition of vitamin K fairly linearly for 4 hr despite the low and conceivably limiting concentration of certain amino acids in the perfusate. The degree of enrichment of prothrombin-bound leucine, furthermore, calculated from the amount of prothrombin formed and its amino acid composition (12), suggests that the intrahepatic amino acid pool from which protein synthesis occurred is not in instantaneous isotope equilibrium with that of the perfusate. Nonetheless, the flux of amino acids in the perfused liver suggests that attempts to control them by graded infusions may result in a more stable environment in which to measure protein synthesis. This also applies to isotope enrichment of a given amino acid in order to measure specific rates of protein synthesis. In the experiments reported, the specific activity of leucine decreased to 4% of its initial value at the end of 6 hr. Experiments in progress suggest that constant infusion of both labeled and unlabeled amino acids improves the conditions for quantitating rates of specific protein synthesis by the isolated perfused liver.

Summary. The amino acid composition of the perfusate of an isolated rat liver varies markedly, and highly individually for given amino acids, over a 6-hr period. Glutamic acid, valine, isoleucine, and leucine concentrations increased whereas the concentrations of aspartic acid, proline, glycine, alanine, methionine, phenylalanine, lysine, and arginine decreased. In livers bathed in warfarin (10 μg/ml), vitamin K_1 added at 2 hr in a concentration of 25 μg/ml caused a prompt and linear increase in prothrombin. The specific activity of L-leucine-1-^{14}C added to the perfusate at zero time declined exponentially to 4% of initial values as the concentration of total leucine increased 5-fold. The prothrombin synthesized under these conditions was highly radioactive, suggesting that vitamin K stimulates *de novo* synthesis of this coagulation protein.

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