PROTEIN SYNTHESIS IN THE PERFUSED RAT LIVER*,‡,§

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Several reports of investigations in which perfused rat livers were used to study plasma protein synthesis (1, 2) as well as other basic hepatic functions (3–5) suggested that these preparations offered many possibilities for the investigation of the factors involved in protein metabolism under well defined experimental conditions.

Consequently, a perfusion apparatus was constructed and preliminary experiments were conducted to test the disappearance rate of P^{32}-labelled chromic phosphate in colloidal form from the perfusate. The results of a typical experiment, together with the bile flow prevailing at the same time are presented.

Attention was then turned to plasma protein synthesis using L-methionine-S^{35} as the tracer amino acid. The results showed that the livers were capable of rapidly incorporating this amino acid into protein which appeared in the perfusate. Regardless of the initial experimental conditions employed, however, all the specific activity curves for the protein in the perfusate approached a plateau at the end of 4 hours. Similar results have been obtained by others (1). This phenomenon could be due to either the loss of synthetic ability by the liver, or to the depletion of substrate, or to a decrease in the specific activity of the labelled amino acid. The first possibility appeared unlikely, since it was found in confirmation of other work (1) that when the incorporation was proceeding at a reduced rate the addition of more labelled amino acid resulted in an immediate increase. Consequently, studies were designed to ascertain whether the cause of this plateau was due to the rapid dilution

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‡ We are indebted to the United States Atomic Energy Commission, Oak Ridge, for supplies of S^{35}, C^{14}, and P^{32} used in these studies.

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of the labelling agent by similar non-labelled material present in or produced by turnover of protein in the system during the experiment.

Methionine-S^{35} is hardly adaptable to this purpose because of the difficulty of determining the specific activities of small quantities of the free amino acid. Therefore, we turned to the use of L-lysine and L-histidine-C^{14} prepared by microbiological methods (6). With these amino acids it is possible to make determinations of the specific radioactivities of carboxyl carbon of the free amino acid by using the appropriate specific decarboxylases (7, 8).

The evidence obtained from studies with these two amino acids indicates that the specific activity of both lysine and histidine changes markedly during the perfusion period. If the data for plasma protein synthesis are corrected for this decrease in the specific activity, it is found that the rate of incorporation of the tracer is approximately linear for 4 hours, and that the rate observed agrees with *in vivo* measurements of plasma protein turnover.

EXPERIMENTAL

(*a*) *General Methods.*—A perfusion-aeration apparatus similar to the one described by Miller *et al.* (1) was employed.

All liver donors were male rats of the Long-Evans strain, and in so far as possible the blood donors were large rats of the same sex. None were fasted. The surgical technique used in cannulating the hepatic vessels and bile duct, as well as the general experimental procedures followed during the perfusion, has been described by others (1, 3). Details of the studies reported here are summarized in Table I, and are typical of all the experiments carried out.

Prior to the addition of radioactive materials the liver was allowed to "equilibrate" for at least 30 minutes after starting the perfusion. All studies were conducted during the "steady phase" of blood flow (5). Zero time is the point when the labelled material was added to the reservoir.

(*b*) *Preparation of Isotopic Compounds.*—Colloidal chromic phosphate labelled with P^{32} was prepared according to the method of Dobson and Jones (9).[1]

The isotopically labelled amino acids used in these studies were synthesized biologically. L-methionine-S^{35} was separated by column chromatography from hydrolysates of the yeast *Torulopsis utilis* grown with traces of $S^{35}O_4^{=}$ (10, 11); L-lysine-C^{14} and L-histidine-C^{14} were similarly isolated as the monohydrochlorides from the bacterium *Rhodospirillum rubrum* grown in the presence of $C^{14}O_2$ (6).

The two latter amino acids were purified for use by recrystallization as the monohydrochlorides with the aid of carrier. When tested with the solvent system butanol, water, acetic acid (10:5:2) on Whatman No. 1 filter paper, no ninhydrin positive or radioactive material was present on the paper elsewhere than at the locus of the particular amino acid.

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[1] The authors wish to express their thanks to Dr. E. L. Dobson and Dr. G. F. Warner for a generous supply of this compound, as well as for valuable assistance in conducting these studies.

TABLE I

Experimental Conditions during Perfusions of Livers of Rats

Experiment	Tracer and quantity		Liver donor		Perfusion fluid				Plasma protein‡
			Body weight	Liver weight	Whole blood*	Amino acid	Glucose	Hematocrit reading	
		μM	gm.	gm.	ml.	mg.	mg.	per cent	gm. per cent
PRL 7§	$CrP^{32}O_4$		398	16.6	71	M, 100‖	300	17	
12	Met	20.3¶	300	12.9	63	C, 100**	300	19	
13	"	40.6	290	10.7	73	"	"	20	
14	"	"	340	12.4	71	"	"	20	
15	"	" ‡‡	290	10.2	60	"	"	20	
32§§	Lys	76.7‖‖	322	12.3	200	—	200	41	7.0
33	Lys	"	320	12.9	195	C, 184¶¶	200	43	8.3
36	His	" ***	300	9.1	180	— —	200	48	
38	His	"	290	12.4	160	— —	200	49	
34	$NaHC^{14}O_3$‡‡‡		304	11.5	190	— —	200	41	8.0

* In all experiments rat blood was used with Krebs-Ringer phosphate as diluent in PRL-7 to 15. None was used thereafter.

‡ Plasma protein was determined by the method of Gornall and coworkers (15).

§ In this experiment the rate of loss of the colloidal chromic phosphate-P^{32} from the perfusate and bile flow were studied. For the rest of the experiments the incorporation of the tracer into the plasma protein was the main variable under investigation.

‖ M, Merck essential amino acid mixture. The approximate composition of this mixture according to the manufacturer's assay is given by Miller and coworkers (1). The authors are indebted to Dr. R. A. Peterman of Merck and Co., Rahway, New Jersey, for a generous supply of this material.

¶ L-Methionine-S^{35} with a specific activity of 2.09×10^5 C.P.M. per μM.

** C, A complete amino acid mixture based on the analysis of plasma and liver protein, and containing the L-isomers of 18 amino acids in the following percentages: arginine hydrochloride 6, histidine hydrochloride 2, lysine hydrochloride 8, tyrosine 4, tryptophan 2, phenylalanine 5, methionine 5, threonine 5, serine 8, leucine 8, isoleucine 4, valine 6, glutamic acid 14, aspartic acid 7, glycine 5, alanine 5, proline 5, hydroxyproline 1.

‡‡ A second addition of labeled substrate, 40.6 μM, was made after 4 hours.

§§ 1.0 mg. of terramycin hydrochloride added to the perfusate in this and succeeding experiments.

‖‖ L-lysine-C^{14} (generally labelled in chain) with a specific activity of 4.45×10^4 C.P.M. per μM.

¶¶ Amino acid mixture C without lysine.

*** L-histidine-C^{14} (generally labelled in all carbons) with a specific activity of 1.81×10^4 C.P.M. per μM.

‡‡‡ The lysine used in this study was non-radioactive. The $NaHC^{14}O_3$ was added in 8 equal doses of 3.50 μM each at 30 minute intervals. The activity of the bicarbonate was 6.54×10^6 C.P.M. per μM.

(*c*) *Decarboxylation of Lysine and Histidine.*—Determination of the specific activity of the carboxyl carbon of free lysine-C^{14} or histidine-C^{14} was carried out with the aid of specific decarboxylases. The L-lysine decarboxylase was prepared from *Bacterium cadaveris*[2] (strain 6578) according to the method of Zittle and Eldred (7); L-histidine decarboxylase was obtained from *Clostridium perfringens*[2] (strain 6785) as described by Epps (8).

Aliquots of plasma were treated with 40 per cent trichloroacetic acid (TCA) so that the final concentration of the acid was 10 per cent. Liver samples were thoroughly homogenized in water before addition of the TCA. After stirring, the protein coagulum was removed by centrifugation. The TCA in the supernates was extracted in a Soxhlet apparatus with ether so that the final pH was about 5; the aqueous residue was taken to dryness with gentle heating and vacuum desiccation overnight. The dried samples were dissolved in 2.5 ml. of buffer (M/5 phosphate buffer, pH 6.0 for the lysine decarboxylase; M/5 acetate buffer, pH 4.5 for the histidine decarboxylase). After solution was complete, 2.0 ml. of the buffer containing the sample was pipetted into the reaction chamber of a double-side arm Warburg vessel. One arm contained the bacterial powder suspended in 0.5 ml. of the same buffer; the other side arm was empty, but the gas vent capillary above it was charged with 0.1 ml. of 50 per cent CO_2-free NaOH and capped with a rubber policeman. The gas vent was well greased so that the NaOH was completely sealed off from the vessel (and from the air).

After an equilibration period with shaking in a constant temperature bath (30°), the bacterial suspension was tipped into the reaction chamber, and readings of the CO_2 evolved were taken every 5 minutes until gas evolution was complete. The mass of the amino acid in the flask was calculated from the corrected total volume of CO_2 liberated. Blanks for the bacterial powders alone were zero, and suitable control experiments carried out with a mixture of 17 L-amino acids indicated that the enzyme preparations used in these studies were specific for the particular amino acid.

The CO_2 liberated by the enzyme was collected without unsealing the flask by rotating the gas vent while compressing the rubber policeman to introduce the NaOH into the side arm. With the policeman still compressed to avoid drawing any of the gas out of the flask, the gas vent was again rotated to seal the flask. After shaking in the bath for 30 minutes longer to ensure complete absorption of the gas, the side arm containing the NaOH and the gas vent were rapidly and thoroughly washed with CO_2-free distilled water and the combined washes were collected in a test tube containing sufficient carrier to give a total mass of 20 mg. of $BaCO_3$ in every sample after addition of excess $BaCl_2$. The mass of $BaCO_3$ contributed by the samples was negligible compared to the total mass of the compound derived from the carrier.

After precipitation the $BaCO_3$ was collected with suction on tared Whatman Number 42 filter paper, the filter papers were thoroughly dried with heat and suction, reweighed, and counted (12).

(*d*) *Preparation of Samples and Radioactivity Measurement.*—Assay for chromic phosphate was carried out by plating aliquots of the whole perfusate on aluminum foil. The samples were dried, wrapped in cellophane, and the P^{32} was counted by methods

[2] Our thanks are due to Dr. S. S. Elberg for his advice regarding the culture of the organisms.

already described (9). These data have been corrected for background and coincidence, and, where necessary, for residual P^{32} remaining in the perfusate from the preceding chromic phosphate study.

Isotopically labelled plasma and liver proteins were washed as described by Levine and Tarver (13), and sedimented onto tared aluminum discs for counting by the method of Farber, Kit, and Greenberg (14). Control experiments indicated that the washing procedure was effective in removing any unincorporated radioactive material.

The S^{35}-labelled samples were counted using an open gas flow tube. Protein labeled with L-lysine or L-histidine-C^{14} and $BaC^{14}O_3$ samples were counted with a tube having a thin pliofilm window. A stream of ethanol-saturated helium was passed continuously through the tube, since the window was not impervious to air.

All plates were counted for a sufficient time to give values with a standard error of 5 per cent. Empirically determined self-absorption corrections were applied to protein samples labelled with S^{35} and C^{14}; the $BaC^{14}O_3$ plates were corrected to a standard mass of 40 mg. All samples were corrected for background. Primary standard plates made with aliquots of the radioactive amino acids used in the experiments were routinely counted together with the samples from each experiment and served to provide correction factors for the decay of S^{35}-containing samples.

RESULTS AND DISCUSSION

(*a*) *Blood Flow and the Uptake of Colloidal Chromic Phosphate.*—It was observed that blood flow through the liver followed a definite pattern. When diluted blood with a hematocrit reading of 16 to 20 was employed, there was first a rapid rate of flow of about 30 ml. per minute, which in 10 minutes decreased to 15 to 20 ml. per minute. Thereafter, there was a gradual increase in rate to 30 to 55 ml. per minute which was maintained for several hours.

Fig. 1 shows the rapid rate of removal of colloidal chromic phosphate from the perfusing blood in PRL-7. Curve 1 was obtained during the 1st hour of the perfusion; curve 2 during the 2nd hour when the blood flow was artificially reduced to 50 per cent of its initial value; and curve 3 was obtained during the 5th hour after restoration of the circulation rate.

Now it has been shown by Dobson and Jones (9) *in vivo* that the rate at which colloidal chromic phosphate is removed from the circulating blood follows monomolecular reaction kinetics. Therefore, if k is the fraction of the total perfusate cleared of colloid per unit time and $t_{\frac{1}{2}}$ is the time for the concentration of the colloidal material to be reduced to half its initial value, then $k = 0.693/t_{\frac{1}{2}}$. Hence, knowing the total volume of the perfusate, it is possible to calculate the volume cleared per unit time.

In Fig. 1, which shows the results of clearance studies on PRL-7, the rates of removal of colloid correspond to half-lives of 3.1, 7.4, and 1.8 minutes for curves 1, 2, and 3, respectively. From these values the calculated rates of flow are 33, 14, and 56 ml. per minute assuming that *in vitro* as *in vivo*, one

passage through the liver results in complete clearance. It is seen that the 50 per cent artificial reduction in the actual rate of flow caused a similar reduction in the rate of flow as calculated from the rate of *loss* of colloid. In five studies, in addition to the three just dealt with, the blood flow calculated from the rate of removal of the colloid corresponded to the observed flow

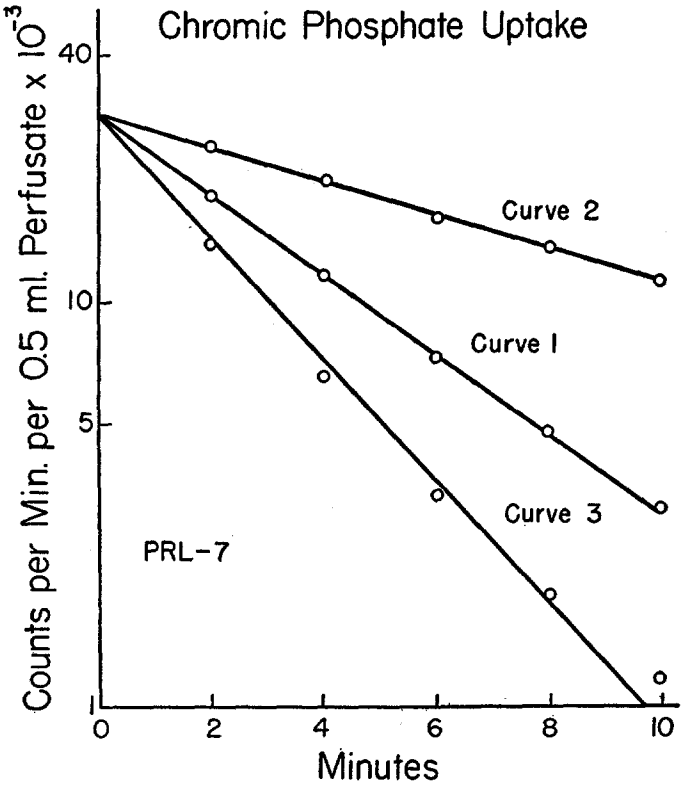

FIG. 1. Rate of removal of colloidal chromic phosphate from the blood perfused through a rat liver (PRL-7). The time at which the studies were conducted is indicated in Fig. 2. Note that the ordinate has a logarithmic scale.

rate. Thus the reticuloendothelial system in the perfused liver is in a normal functional state in so far as the handling of particulate material is concerned.

(*b*) *Bile Flow.*—The rates of bile flow observed in the present experiments agree well with those reported by Brauer and coworkers (3), generally amounting to 4 to 6 ml. in 6 hours. Similar rates were found in two rats with their bile ducts cannulated and maintained under light anesthesia. In several experiments it was observed that under anoxic conditions the bile flow was much reduced. Whether the rather rapid early decrease observed in the rate

of bile flow in PRL-7, shown in Fig. 2, in part resulted from the same cause is not clear.

(*c*) *Incorporation of Labelled Amino Acid into the Plasma Protein.*—A general description of the conditions under which the incorporation studies reported in this communication were conducted is to be found in Table I. In all four experiments in which L-methionine-S^{35} was used as a labelling agent, a complete amino acid mixture of the composition shown in the footnote to Table I as well as glucose, was added to the perfusing rat blood, which was diluted with Krebs-Ringer phosphate. The incorporation of the methionine observed in these experiments is plotted in arbitrary units of specific activity

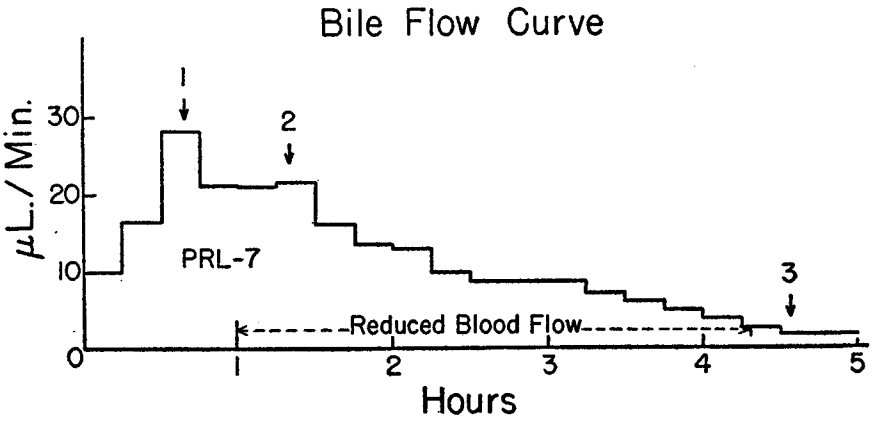

FIG. 2. Rate of flow of bile for 5 hours in PRL-7. Numbered arrows indicate times at which chromic phosphate uptakes were carried out (see curves in Fig. 1).

calculated from the relationship given in the description of Fig. 3. The results of the experiments are comparable because this method of calculation corrects for the difference in the pool size, that is, in the total plasma protein, except there are variable amounts of preformed plasma protein in the livers used.

The data plotted in Fig. 3 show that there was a greater incorporation of radioactivity in PRL-13, 14, and 15 than in PRL-12. This, no doubt, is due to the fact that the amount of methionine added to the perfusion fluid in the former three experiments was twice that used in the last mentioned, so that the amino acid concentration was approximately double. Otherwise, the curves are essentially similar in all cases, all showing a considerable reduction in the rate of increase in specific activity with time after the first 2 hours of perfusion. When a second dose of methionine was added to the perfusing fluid (PRL-15) there was an immediate increase in rate of incorporation of radioactivity.

When L-lysine-C^{14} and L-histidine were used in experiments of the same type but using whole blood as the perfusion fluid, then, either with or without the addition of the complete amino acid mixture (less the amino acid used

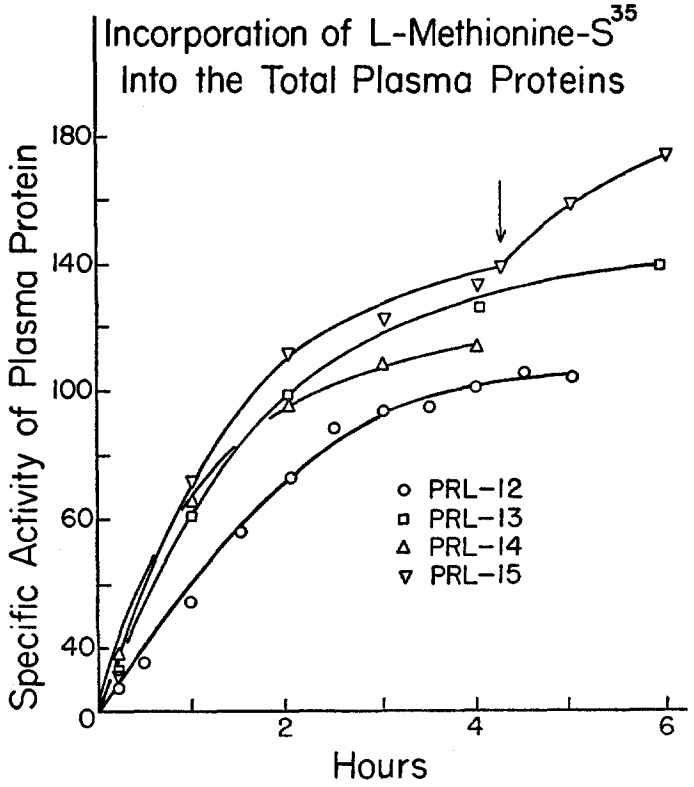

FIG. 3. Changes in specific activity (SA) of plasma proteins with time during the perfusion of rat livers with L-methionine-S^{35}. The arrow on the curve for PRL-15 indicates where extra substrate was added. The specific activity in these experiments is calculated from the relationship:

$$SA = \frac{\text{C.P.M. in protein}}{\text{mg. protein}} \times \frac{\mu\text{M methionine}}{\text{C.P.M. in methionine}} \times \text{B.V.} \times 10^6.$$

B.V. = volume of blood used in the perfusion, C.P.M. = radioactivity in counts per minute. The micromoles methionine refer to the amount of methionine used in the experiments, the denominator of the fraction to the activity in this amount.

for labelling), there was generally a similar decrease in the rate of increase in specific activity with time. These data are shown in the dotted curves of Figs. 5 and 6. The data used in plotting these curves have not been corrected

for differences in blood volume since this was approximately the same in all four experiments (see description of calculation below Fig. 5).

This type of fall off in rate of incorporation of radioactivity with time observed with methionine, lysine, and histidine, in these experiments and the stimulation of incorporation which results when a fresh dose of amino acid is added to the perfusion fluid were previously observed by Miller and coworkers (1). The stimulating effect of fresh labelled material shows that the livers maintain their capacity to incorporate amino acid at a more or less normal rate for 4 to 6 hours.

(*d*). *Specific Activity of the Free Amino Acid in the Perfusate.*—In experiment PRL-32, using lysine generally labelled with C^{14}, two specific activity values were determined on the original material, that of the total carbon (column C, 4.45×10^4 C.P.M. per micromole), and that of the carboxyl carbon (column B, 4.18×10^3 C.P.M. per micromole). Ten minutes after the addition of the lysine to the perfusion fluid, which contained no other added lysine but only that preformed in the blood used, the specific activity of the carboxyl carbon was found to be 1.39×10^3. If it is assumed that the 10 minute interval suffices only for complete mixing with blood lysine and that in this time the tissue lysine is not exchanged with that in the fluid to any significant extent, it is possible to calculate the lysine concentration in the perfusing fluid by simply applying the dilution principle.

$$\text{Total blood lysine after 10 minutes} = \frac{76.7 \times 4.18}{1.39} = 231 \text{ micromoles.}$$

In which the amount of lysine added = 76.7 micromoles.

By difference, lysine in perfusing fluid (200 ml.) = 154 micromoles
= 11 mg. per cent.

According to the literature (17–19) the lysine content of rat blood determined by microbiological methods varies from 3.5 to 14 mg. per cent depending on the dietary treatment of the animals. Hence, the value found in PRL-32 is such as might be expected to exist.

When the specific activity of the free lysine and histidine in the perfusing fluid was followed at intervals during the period of perfusion the results shown in Fig. 4 were obtained. These results are plotted by relating all the specific activities to those found at 10 minutes (0.166 hour) which are assigned the value of 100. The data show that in the 4 hour period of the experiments there is a fall in specific activity to between 25 and 35 per cent of its 10 minute value. Unfortunately the results are not sufficiently accurate to show whether the fall is linear in nature or whether it occurs more rapidly at the beginning, as with PRL-32, since the volumes of carbon dioxide evolved in the decarboxylation were such as to give very low readings with the conventional Warburg apparatus used. At any rate it is clear that there is a very significant

reduction in specific activity of these two amino acids with time. With methionine-S^{35} it has, so far, been impossible to obtain similar data. However, the results with lysine and histidine have been confirmed by determining these amino acids by chromatographic methods, and it has been found also

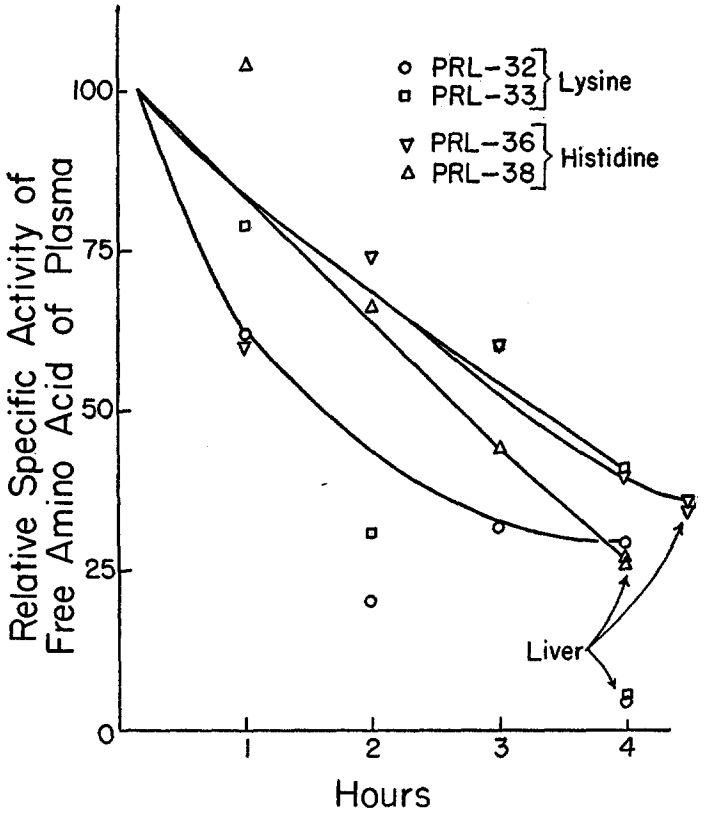

FIG. 4. Changes in the specific activities of the free lysine and histidine in the blood plasma during perfusion. The specific activity of the amino acids in the fluids 0.166 hour after addition of the labelled material is taken as 100. All other values are calculated as indicated in the footnotes to Table II (footnotes B, C, and D).

that there is no significant reduction in the concentration of these amino acids during the 4 hour period.

(*e*) *Correction of Incorporation Data.*—By using these relative specific activity (RSA) data it is possible to correct the incorporation data so that the results are expressed as if the incorporation had continued with the amino acid at its initial specific activity (10 minute value). An example of the method of calculation is shown in the footnotes to Table II. The apparent incorpora-

tion of lysine (micromoles per gram of protein) after different intervals is shown in column E, and from this the increment in each hour is determined (column F). The increment per hour is then corrected by multiplying by the ratio of the initial relative specific activity (RSA) of the free lysine (100)

TABLE II

Lysine—Changes in Specific Activity and Incorporation during a Perfusion Experiment (PRL-32)

Time	Radioactivity in free lysine			Radioactivity in plasma protein			
A	B	C	D	E	F	G	H
0	4.18	4.45		0.0			
0.166	1.39	1.48		0.047			
0.5		(1.21)	82	0.084			
1.0	0.86	0.92		0.268	0.268	0.98	0.32
1.5		(0.77)	52	0.503			
2.0	0.29	0.31		0.76	0.49	2.86	0.88
2.5		(0.56)	38	0.93			
3.0	0.44	0.47		0.99	0.22	1.76	0.50
3.5		(0.44)	30	1.13			
4.0	0.40	0.43		1.16	0.17	1.75	0.43

A, hours from the time the lysine was added to the perfusion fluid.

B, specific activity of the free lysine carboxyl carbon as determined by the decarboxylase method (C.P.M. per μM $\times 10^{-3}$). The zero time value is that for the original material.

C, specific activity of the free lysine (all carbon atoms) (C.P.M. per μM $\times 10^{-4}$). The zero time value is that for the original material; other values are calculated by proportion from the corresponding values in column B. The midpoint values for each hour, shown in parentheses, are calculated from column D and the 10 minute value (1.48).

D, relative specific activity of lysine taken from Fig. 4. The value at 0.166 hour is taken as 100; other values are read from the curve at the midpoints of each hour.

E, apparent incorporation of lysine into the total plasma protein in terms of micromoles of lysine per gram of protein; *i.e.*, C.P.M. per gm. protein/C.P.M. per μM lysine as added (4.45×10^4).

F, apparent incorporation of lysine per hour obtained by subtraction of appropriate values in column E.

G, values in column F corrected by using the actual midpoint values for the specific activities of the free lysine given in parentheses in column C; *i.e.*, column E $\times$ 4.45/column C.

H, values in column F corrected by dividing by the relative specific activity of the lysine given in column D $\times$ 100.

to the RSA values in the middle of the hour. The RSA values read from Fig. 4 are shown in column D of Table II and the corrected increments in incorporation are to be found in column H. The summed values obtained from the latter column appear as the solid curve for PRL-32 in Fig. 5. Similar calculations lead to the data for the solid curves for PRL-33 (Fig. 5) and for PRL-36 and 38 (Fig. 6).

Except in the case of PRL-36, these corrected values for the incorporation of radioactivity as lysine and histidine fail to show the pronounced plateau effects seen in the uncorrected data. Therefore, it is clear that the plateau effect is not due to fall in synthetic ability nor is it due to depletion of sub-

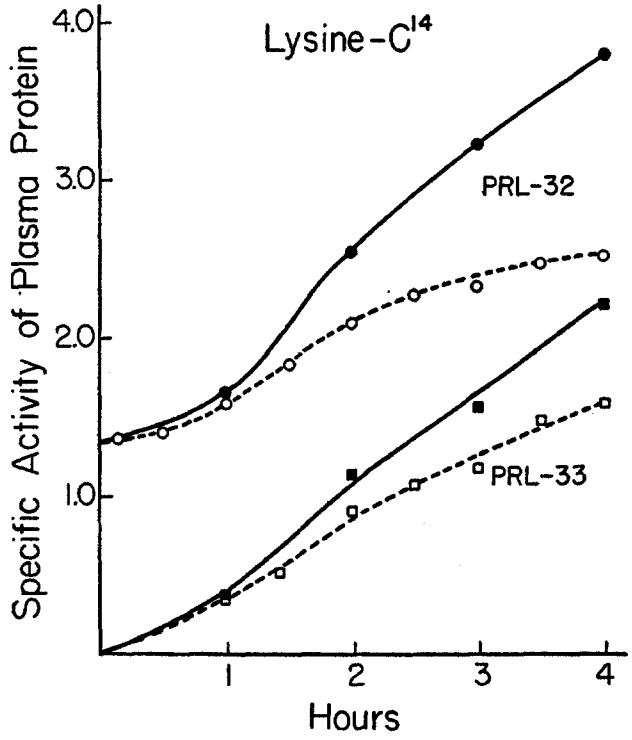

FIG. 5. Changes in specific activity of plasma proteins during perfusion with lysine-C^{14}. Dotted curves, specific activity values (apparent incorporation) in terms of micromoles of lysine per gram of protein (see column and footnote E, Table II). Solid curves, specific activity values corrected to compensate for the change in the specific activity of the free lysine by dividing by the relative specific activity of the free amino acid calculated as column D, Table II. For experiment PRL-32 the values plotted are summations of the individual hourly values given in column H. Note that for convenience both curves for PRL-32 have been displaced upward on the vertical axis approximately 1.3 units.

strate; rather it is due to the change in specific activity of the precursor amino acid with time.

From Figs. 5 and 6 there appears to be a lag in the incorporation of lysine and histidine during the 1st hour, but with methionine (Fig. 3) no such phenomenon is observed. This lag may be due to the fact that there is a pre-

cursor of albumin being formed in the system as suggested by the work of Peters (20), or due to a lag in release of protein into the circulation as suggested by Green and Anker (21), or again it may be due to the time required for equilibration of the radioactive amino acid between the blood and the liver cells—the site of synthesis. Considering the behavior of the specific activity of the free lysine and the histidine in the perfusing fluid, the latter

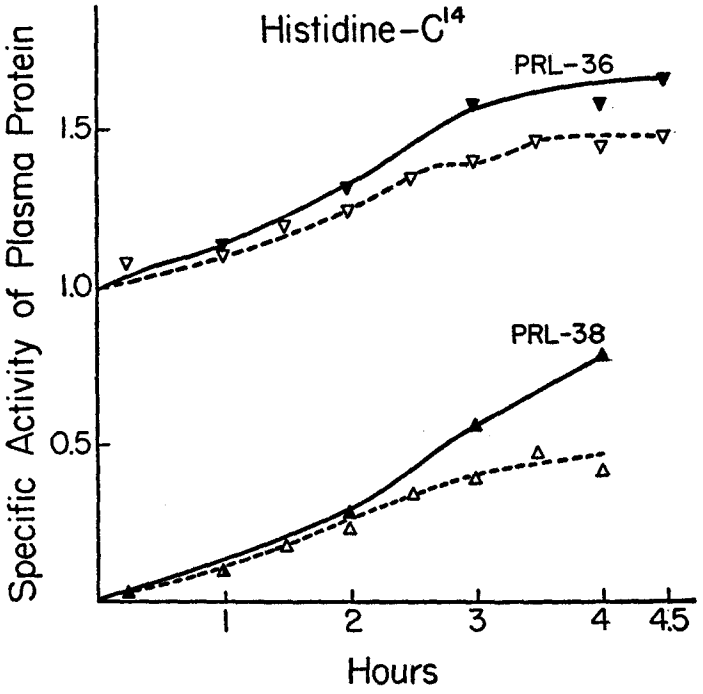

FIG. 6. Changes in specific activity of plasma proteins during perfusion with histidine-C^{14}. See notes on Fig. 5. All values for PRL-36 are displaced 1 unit upward on the vertical axis.

is a quite probable explanation of the phenomenon. On this basis the absence of a lag period when methionine is used may be due to a more rapid intracellular equilibration of methionine as compared with that of the two basic amino acids, perhaps because there is less free methionine in the liver cells than of the other two amino acids (17, 19, 22).

It should be noted that the addition of the complete amino acid mixture (for composition see footnote **, table I) did not appear to have any stimulating effect on the synthesis. Apparent stimulating effects of amino acids on incorporation reported by others (1) are probably due to the fact that the total lysine in the system was simultaneously increased. This would cause a

decrease in the rate of fall in the specific activity of the labelling agent due to the increased pool size.

(*f*) *Absolute Rates of Incorporation—Rates of Protein Synthesis.*—In the previous section consideration was given to the data for the incorporation of radioactivity from lysine and histidine as corrected by using the relative

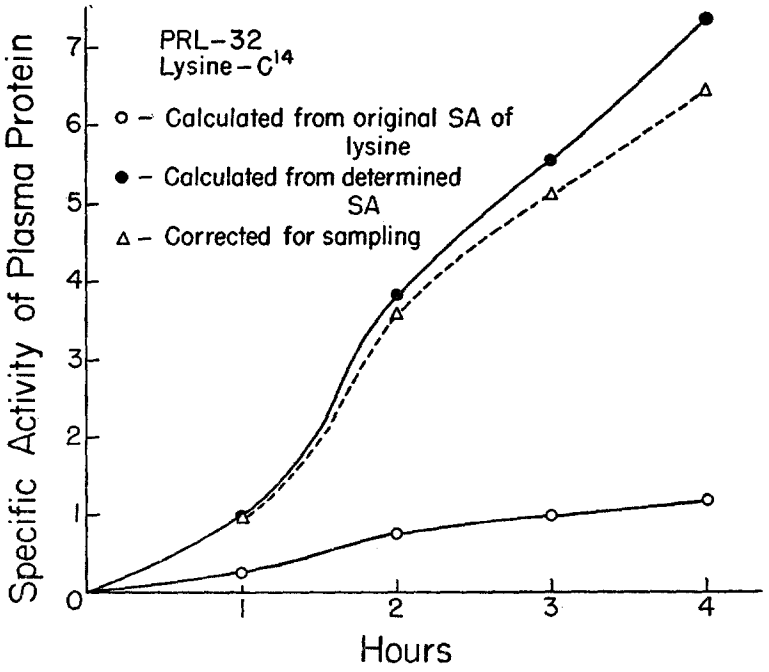

FIG. 7. Effect of change in specific activity of the free lysine and of removing samples of blood during the experiment on the calculated values of the specific activities of the plasma proteins. Lower solid curve, values of column E, Table II. Upper solid curve, summated values from column G, Table II. Middle broken curve, as upper solid curve but values corrected to compensate for the samples of the perfusing blood removed.

specific activity of the free amino acids. It is also possible to calculate the actual moles of lysine incorporated using the specific activity data. From this data the rates of plasma protein synthesis may be determined if it is assumed that the uptake of lysine represents the net synthesis of protein.

In Table II, column F, are shown the apparent incorporation rates of lysine in micromoles per hour for the 4 individual hours of the experiment, obtained by dividing the incorporated counts by the original specific activity of the lysine (4.45×10^4 C.P.M. per micromole). If instead of the original specific activity the actual specific activity for the midpoint of each period, given in

column C, is employed as the denominator, then the "true" incorporation rates for the lysine are obtained. These are shown for each hour in column G. The summated data from this column are plotted in the upper solid curve of Fig. 7. The lower solid curve is from the uncorrected data (column E).

At 4 hours the value for the total incorporation is 7.35 micromoles of lysine, whereas the uncorrected value is 1.16. Thus the corrected value for the rate is six times the uncorrected. It is necessary to make one further correction in the data, to take into account the decrease in the pool size due to the removal of samples from the perfusing blood during the period of the experiment. The values corrected for this factor are shown in the broken curve of Fig. 7, the 4 hour value in this case being 6.44.

Since, the rate of synthesis proceeds more slowly in the 1st hour, the rate of protein synthesis in PRL-32 may be calculated from the average of the values for the 3 following hours, using the values corrected for sampling (2.65, 1.50, 1.31, average 1.82 micromoles lysine per gram of plasma protein per hour) as follows:—

(1.) *Pool size.*—Total plasma protein in the perfusate:

Volume of blood, 200 ml.; plasma, 59 per cent by volume; plasma protein, 7.0 per cent

$$\text{Total plasma protein} = 200 \times \frac{59}{100} \times \frac{7}{100} = 8.3 \text{ gm.}$$

(2.) *Protein synthesis.*—Incorporation of lysine-C^{14}:

Incorporation, 1.82 μM/gm./hr.; total incorporated, 1.82 $\times$ 8.3 = 15.1 μM/hr.; lysine content of plasma protein (16), 0.48 μM/mg.; weight of rat, 322 gm.; protein synthesized, 15.1/0.48 $\equiv$ 31.4 mg./hr. $\equiv$ 750 mg./day $\equiv$ 234 mg./100 gm. rat/day.

If the maximum value for the incorporation (2.65) is used in the calculation rather than the average (1.82), then the rate of synthesis turns out to be 341 mg. per 100 gm. of rat per day rather than 234. Since it was found at the end of the experiment that the specific activity of the free lysine in the liver was somewhat less than in the perfusion fluid, these rates may need further correction. In addition, the estimated pool size is in error by the amount of preformed albumin in the liver. Both of the factors mentioned would presumably make the calculated rates of synthesis lower than the actual. However, if albumin is released more rapidly from the liver under the conditions of the experiments then the calculated rates of synthesis may be too high.

In spite of these possible errors in the determination the value of 234 mg. of plasma protein synthesized per 100 gm. of rat per day is in good agreement with the value for total plasma protein turnover in the intact rat (23). Depending on the protein content of the diet the turnover varied from 170 to 245 mg. per 100 gm. of rat per day.

(*g*) *Lysine Specific Activity Data.*—In handling the data it has been assumed that during the course of the experiments the specific activity of the total carbon in the lysine (and histidine) does not change relative to the carboxyl carbon. This assumption appears to be valid because no reversible breakdown of the carbon skeletons of these amino acids has been demonstrated, and, in particular, the decarboxylation is not a reversible process. This is experimentally demonstrated in PRL-34 in which labelled bicarbonate was added to the perfusate. Although the perfusion proceeded satisfactorily, no incorporation into either the protein or free lysine was demonstrable. Hence, all the incorporation into the protein must be due to unchanged lysine and no significant amount to recarboxylated lysine fragments or to carbon dioxide fixation into other amino acids. No doubt carbon dioxide fixation occurs in these experiments, but it is not detectable under the conditions employed.

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SUMMARY

1. When the rat livers are perfused under the conditions of these experiments with rat blood diluted with saline, the livers remain capable of removing colloidal chromic phosphate normally for 4 hours or more; that is, the reticuloendothelial system continues to function normally.

2. Good rates of bile flow continue, generally for 4 hours.

3. The livers incorporate radioactivity from the amino acids methionine, lysine, and histidine at rapid rates for 1 or 2 hours. Thereafter the rates fall.

4. The specific activity of the free lysine and histidine in the perfusate falls rapidly during the experiments (to 25 or 35 per cent of its original value at 10 minutes).

5. The fall in rate of incorporation of radioactivity is attributable to the fall in amino acid specific activity.

6. Addition of a complete amino acid mixture to the perfusate does not appear to have any stimulatory effect on incorporation of radioactivity from labelled amino acids.

7. With lysine, on the assumption that incorporation is due to new protein formation, there is a rate of synthesis equivalent to 230 mg. of plasma protein per 100 gm. of rat per day. This result is in agreement with turnover data obtained from rats *in vivo*.

8. The results emphasize once again the importance of precursor specific activity in the interpretation of metabolic experiments with labelled amino acids.

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