Metabolic Interrelationships among Amino Acids Studied with Isotopic Nitrogen

STIG E. G. ÅQVIST

Biochemical Department, Karolinska Institutet, Stockholm, Sweden

Isotope investigations in this laboratory concerning the formation of proteins and nucleic acid bases in rat liver have been performed with N^{15} -labeled glycine. Experiments were also carried out to test the relationship of other N^{15} -labeled amino acids to purine and pyrimidine synthesis, thus allowing, at the same time, possibilities for a study of interrelationships between the different amino acids involved in protein synthesis. The present paper deals with these different relationships among amino acids, as far as can be judged from a study in which the nitrogen of the amino acids has been labeled with N^{15} . The utilization of amino acid N^{15} for the formation of nucleic acid bases will form the subject of another publication ¹.

EXPERIMENTAL

The N^{15} -labeled amino acids used in this investigation were isolated from biologically marked protein by the use of Dowex 50, according to the technique described by Åqvist ². The amino acids were administered to adult albino rats fed on a diet of bread, milk, oats and carrots. The animals were allowed to eat *ad libitum* throughout the experiment. In most of the experiments two 200-gram rats were used for each amino acid tested, and the amount of amino acid administered was on the order of 100 mg per 100 g rat weight. The exact amount of each amino acid, together with its content of isotope, is presented with the table in each case. The amino acid was dissolved in 4–16 ml of water, depending on the solubility of the substance in question. This volume was divided into four doses, which were injected subcutaneously at two-hour intervals. Two hours after the last injection the animals were killed by a blow on the head and bled. The total period of exposure to isotope was therefore eight hours.

The livers were taken out and blotted to remove blood. They were cut into small pieces into ethyl alcohol, and then ground in a Turmix blender and dried with alcohol and ether. The dried tissue was fragmented in absolute alcohol by means of mechanical vibration, as described by Hammarsten ³. The remaining lipid material was extracted by

boiling with 3 : 1 alcohol-ether for two hours and the preparation was then dried again. Nucleic acids were isolated by the method of Hammarsten³ and the residue was twice treated with hot trichloroacetic acid solution, according to Schneider⁴, to insure complete removal of nucleotides. The total remaining material was taken for the liver protein and used for the studies reported in this paper. This liver protein was dried and hydrolyzed, and the individual amino acids isolated by the use of starch chromatography, according to Åqvist². The incorporation of isotope from the injected amino acid into each amino acid of the total liver protein was determined in each experiment by estimating the content of N^{15} by means of a mass spectrometer.

RESULTS AND DISCUSSION

Glutamic acid

N^{15} -labeled glutamic acid was injected in the form of the neutralized hydrochloride. The amount of amino acid injected and its content of N^{15} are indicated with Table 1. The isotope values found in each amino acid isolated from the liver protein are shown in this table, where the figures for excess of N^{15} are expressed as calculated on the basis of 100 % N^{15} in the injected compound. The amino acids isolated are listed in the table in the order in which they emerge from the starch column. The figure for threonine includes that for a lysine compound. The existence of this compound in the threonine fraction has been demonstrated and discussed in previous work².

As might be expected, the highest content of N^{15} in the amino acids isolated is found in glutamic acid itself, but the isotope has also been distributed, in somewhat lesser amounts, to almost all the other compounds listed. Of the other amino acids, the ones showing greatest incorporation of N^{15} from injected glutamic acid are alanine and then aspartic acid. The leucines, serine and arginine are next, and the excesses of N^{15} in the remaining compounds are

Table 1. Incorporation of N^{15} from glutamic acid into liver protein amino acids. Two 200-gram rats, 350 mg glutamic acid each, 17.2 % excess N^{15} .

Insoluble humin	Soluble humin	Leuc. + isoleuc.	Leucine	Isoleucine	Phenylalanine	Val., meth., tyr.	Valine + meth.	Valine	Methionine	Tyrosine	Proline	Alanine	Glutamic acid	Threonine	Aspartic acid	Serine	Glycine	Ammonia	Arginine	Lysine	Histidine
.26*	.24	.75	.58		.26		.45	.38	.91	.37	.23	1.39	1.88	.05	.94	.86	.36	.43	.63	.08	.04

* All figures in Tables 1—9 are given as atom per cent excess N^{15} calculated on the basis of 100 % N^{15} in the administered compound.

example, from both injected glutamic acid and ammonium salt the incorporation of N^{15} into alanine is about 80 % of that into glutamic acid, and the figure for aspartic acid about 50—60 % of the glutamic acid value.

These similarities in ratios and in pattern of distribution of N^{15} seemed significant, especially when it became apparent, as shown below, that these similarities also appeared in the incorporation of N^{15} from other injected amino acids. Next to the injected amino acid, glutamic acid has always been found to have the greatest excess of N^{15} and the excesses in the other amino acids have usually shown the same general ratio to glutamic acid in each experiment. This suggested the idea that amino acid interconversions, at least with respect to nitrogen, might follow a common pathway under our experimental conditions, and that glutamic acid might occupy a central position in this metabolism. To clarify these relationships, the figures for excesses of N^{15} in the various amino acids were recalculated for each experiment on the basis of the excess in glutamic acid as 1.00. These recalculated values are shown in Table 10. The similarity, in the different experiments, in the ratios between the figures for the various amino acids can be clearly seen from this table. Since the amounts of labeled amino acids available were insufficient to allow the use of large enough numbers of rats to eliminate biological variations, direct figures could not be comparable from one experiment to another, and no attempt was made, therefore, to administer the same molar amount of each amino acid. Recalculating the figures on the same basis, as is done for Table 10, makes it possible to compare the figures from different experiments, even though the amounts of injected amino acid were unequal and the extents to which the amino acids were utilized in metabolism were different.

From Table 10, the hypothesis of a common pathway of amino acid metabolism appears to be justified by the similarity in the ratios between the figures. The few cases in which the values are significantly higher than expected from the general pattern can be explained by direct interconversions between the injected amino acids and these specific compounds, usually interconversions which are already well-known. The similarity of ratios from one experiment to another would, of course, be apparent no matter what amino acid was used as a basis of calculation. However, the predominant incorporation of N^{15} into glutamic acid from all amino acids seems to justify using this amino acid as a basis of calculation and postulating a central position for it in the metabolism. Thus the hypothesis suggests itself that in metabolism the amino acids distribute their nitrogen to other amino acids by way of glutamic acid, except for certain direct conversions.

Even injected ammonia may distribute its nitrogen by first forming the α -amino group of glutamic acid, as indicated by the similarity in the two

experiments in the ratios between glutamic acid and the other amino acids. This does not explain the high N^{15} content in the ammonia isolated from liver protein, but this could be attributed to direct contribution of injected ammonia to the amide nitrogen of dicarboxylic amino acids, which is the source of most of the ammonia from protein. The high figure for N^{15} in arginine from injected ammonia may well be due to the contribution of the urea moiety of the arginine molecule.

Aspartic acid

The solution of aspartic acid was neutralized by the addition of sodium bicarbonate and injected in the usual manner. Table 3 shows the figures for the incorporation of N^{15} from the administered compound into the other amino acids.

Table 3. Incorporation of N^{15} from aspartic acid into liver protein amino acids. Two 200-gram rats, 350 mg aspartic acid each, 8.1 % excess N^{15} .

Insoluble humin	Soluble humin	Leuc. + isoleuc.	Leucine	Isoleucine	Phenylalanine	Val., meth., tyr.	Val. + meth.	Valine	Methionine	Tyrosine	Proline	Alanine	Glutamic acid	Threonine	Aspartic acid	Serine	Glycine	Ammonia	Arginine	Lysine	Histidine
.49	.83	.66	1.51	.36		.52		.18	.51	.39	1.70	2.54	.03	1.36	.55	.52	.79	.82	.21	.34	

The most striking thing about the figures in this experiment is that the highest excess of N^{15} is not found in the injected amino acid, but in glutamic acid; both glutamic acid and alanine have higher values than does aspartic acid itself. The ratios between glutamic acid and these other two amino acids are almost the same as in the first two experiments with glutamic acid and ammonia, and in fact follow the general pattern illustrated by Table 10. It would seem that the metabolic activity of aspartic acid is a rapid and complete equilibration in terms of the common pattern of nitrogen distribution.

The other amino acids also bear the same general relation to glutamic acid that is indicated in Table 10. The excess of N^{15} in isoleucine seems high, but it is hard to know whether or not the difference from the general pattern is significant.

A higher excess of N^{15} in glutamic acid than in aspartic acid, from administered aspartic acid, has also been observed by Wu and Rittenberg⁵. However, their suggestion that the metabolic behavior of aspartic acid is comparable

to that of ammonia does not seem to be supported by the present figures. In the experiment with injected ammonia, the figure for amide nitrogen was found to be higher than both those for glutamic acid and aspartic acid, but in the aspartic acid experiment the N^{15} content of the amide nitrogen is lower than either the glutamic acid or the aspartic acid figure, indicating that the path of aspartic acid metabolism can not be only by way of ammonia.

Alanine

The figures for N^{15} in the amino acids isolated after injection of labeled alanine are shown in Table 4. Both this table and Table 10 show that the

Table 4. Incorporation of N^{15} from alanine into liver protein amino acids. Two 200-gram rats, 200 mg alanine each, 17.0 % excess N^{15} .

Insoluble humin	Soluble humin	Leuc. + isoleuc.	Leucine	Isoleucine	Phenylalanine	Val., meth., tyr.	Val. + meth.	Valine	Methionine	Tyrosine	Proline	Alanine	Glutamic acid	Threonine	Aspartic acid	Serine	Glycine	Ammonia	Arginine	Lysine	Histidine
.32	.53		1.07	1.10	.24	.90	.96	.80		.27	.43	2.76	2.13	.01	1.21	.63	.58	.73	.92	.12	.09

distribution of N^{15} from labeled alanine follows the same general pattern already pointed out. As in the other experiments (except that with aspartic acid) the highest excess of N^{15} is found in the injected amino acid itself. However, the figure for the excess in alanine is not much above the usual level for this amino acid in the general pattern, indicating a rather rapid and extensive activity of alanine in nitrogen metabolism. The ratios between the figures for the other amino acids and that for glutamic acid are approximately the same as in the other experiments. The figures for both leucines and for valine are somewhat higher than usual, but perhaps not enough higher to indicate direct conversions.

Proline

As shown in Tables 5 and 10, injected N^{15} -marked proline has distributed its nitrogen to the other amino acids in approximately the same proportion and to about the same extent as have the other administered compounds. Stetten and Schoenheimer⁶ have shown that proline forms glutamic acid directly, by opening of the ring. It was of interest, therefore, to observe

Table 6. Incorporation of N^{15} from threonine, serine and glycine into liver protein amino acids. One 200-gram rat, 230 mg threonine, 17.0 % excess N^{15} . Two 200-gram rats, 200 mg serine each, 17.2 % excess N^{15} . Ten 200-gram rats, 200 mg glycine each, 31.0 % excess N^{15} .

Adminis- tered compound	Insoluble humin	Soluble humin	Leuc. + isoleuc.	Leucine	Isoleucine	Phenylalanine	Val., meth., tyr.	Val. + meth.	Valine	Methionine	Tyrosine	Proline	Alanine	Glutamic acid	Threonine	Aspartic acid	Serine	Glycine	Ammonia	Arginine	Lysine	Histidine	
Threonine	.27	.30		.04	.12	.09	.17	.19	.24		.04	.08	.30	.34	5.76	.28	1.15	.80	.39	.27	.08	.02	
Serine	.32	.19		.16	.13	.12		.17	.11		.31	.11	.62	.71	.74	.47	5.23	2.62	.70	.45	.04	.03	
Glycine	.46	.10	.12			.13	.10						.06	.73	.85	.00	.55	3.98	4.53	.71	.52	.01	.07

experiment the amino acid was administered to a larger group of rats, and from the pooled livers, cell nuclei were separated from cytoplasm. Details of this procedure have been described previously⁹. The liver protein used for the isolation of the labeled amino acids recorded in Table 6 was that from the cytoplasm only. The figures are therefore not really comparable to those in the other amino acid experiments in this paper, but the cytoplasm protein is probably fairly representative of the whole cell protein.

It can be seen from this table that in all three experiments by far the highest excess of N^{15} is found in the injected amino acid itself. All three have contributed N^{15} to the other amino acids, although in the case of threonine the extent of nitrogen transfer is quite low. All these amino acids show the usual pattern when recalculated over glutamic acid, as can be seen from Table 10. This is true even for glycine, although the protein in this experiment is not exactly the same as in the other experiments. The fact that the ratios (Table 10) in the threonine experiment all seem to be somewhat high may be due to a slightly low figure for glutamic acid, since a rather small error could produce such variations.

It is apparent that the values for serine and glycine are high in all three experiments, much higher than could be expected from the common metabolic pattern over glutamic acid. After the injection of labeled glycine, the serine isolated from the protein contains 88 % as much N^{15} as does the isolated glycine itself. Administration of labeled serine produces glycine with an N^{15} content 50 % as high as that in the isolated serine. These results indicate a direct interconversion of glycine and serine, with the equilibrium favoring serine. Both of these conversions have been previously demonstrated in experiments both with carbon isotopes and with N^{15} ¹⁰⁻¹⁴.

In the experiment with marked threonine a high level of N^{15} is found in both serine and glycine, with more in the former. Unfortunately, this experiment is complicated by a slight contamination of the injected threonine with serine. The labeled amino acids injected in all these experiments were isolated by a separation on Dowex 50 ion exchange resin, from which threonine and serine emerge only partly separated from each other. Presumably pure fractions were selected, as indicated by paper chromatography, but in this case slight contamination was detected in a later paper chromatogram². Some highly-labeled serine must, therefore, have been injected together with the threonine, and the high figures for isolated serine must be due partly to this. It is thus rather difficult to draw definite conclusions about the contribution of threonine to either glycine or serine, but certain indications are apparent. If glycine were formed only from serine here, one would expect a glycine to serine ratio of about 0.5, based on the experiment with injected serine. The fact that the glycine to serine ratio is higher than this makes it appear probable that the formation of glycine does not proceed only from injected contaminating serine and that some of the glycine, and in turn serine, is formed by a path which proceeds from threonine to glycine. Such a conversion of threonine to glycine has been reported by Meltzer and Springson¹⁵. The experiment with injected glycine shows no transfer of N^{15} to threonine, indicating that such a reaction is not reversible.

It can be seen that throughout the experiments in this paper threonine has received no N^{15} from any of the amino acids administered. Except for the experiment with injected threonine itself, the only experiment which shows an appreciable concentration of N^{15} in threonine is the one with administered serine. It does not seem probable that this is due to a conversion of serine to threonine, for in other experiments in which serine is highly labeled, as from injected glycine, there is no significant incorporation of N^{15} into threonine. It is more likely that the explanation is a contamination of the injected serine with highly-labeled threonine, as in the reverse case, although no contamination of serine was detected by paper chromatography at any stage. If the figures found from injected threonine itself can be used as reference, the contamination of threonine in injected serine must have been on the order of 10 %. This extent of contamination seems surprisingly high, even in view of the fact that threonine gives a weak ninhydrin reaction on filter paper, but a direct conversion of serine to threonine seems even less likely.

It can be seen in the tables that the ammonia value is slightly higher than usual in all three experiments. This is probably due to a partial destruction of the serine and threonine during hydrolysis, which has been demonstrated by Rees¹⁶. This destruction may result in the formation of labeled ammonia,

which would be found in the ammonia isolated, but the decomposition products may also contribute to some extent to the two humins.

In the experiment with injected threonine, the lysine compound found in the isolated threonine fraction was separated from the threonine on Dowex 50. The figure for N^{15} excess in this fraction, therefore, represents undiluted threonine.

Leucine, isoleucine and valine

Table 7 shows the N^{15} contents in the liver protein amino acids after the administration of labeled leucine, isoleucine and valine. Included in Table 10 are the figures recalculated on the basis of the excess in glutamic acid as 1.00.

Table 7. Incorporation of N^{15} from leucine, isoleucine and valine into liver protein amino acids. Two 200-gram rats, 200 mg leucine each, 17.1 % excess N^{15} . One 200-gram rat, 215 mg isoleucine, 17.4 % excess N^{15} . Two 200-gram rats, 200 mg valine each, 17.5 % excess N^{15} .

Adminis- tered compound	Insoluble humin	Soluble humin	Leuc. + isoleuc.	Leucine	Isoleucine	Phenylalanine	Val., meth., tyr.	Val. + meth.	Valine	Methionine	Tyrosine	Proline	Alanine	Glutamic acid	Threonine	Aspartic acid	Serine	Glycine	Ammonia	Arginine	Lysine	Histidine
Leucine	.41	.44	2.34	3.07	.76	.09			.36	.19	.21		.76	.92	.02	.47	.22	.23	.29	.33	.00	.04
Isoleucine	.41	.64	1.39	.81	2.39	.18	.64	.74	.37	.94	.29	.27	.54	.68	.01	.34	.21	.20	.20	.24	.10	.08
Valine	.38			1.12	.99	.12	2.06		2.43	.35	.15	.17	.70	.83	.00	.45	.29	.24	.40	.33	.03	.06

As usual, the highest excess of N^{15} is found in the injected amino acid in each case. All three acids have contributed high amounts of N^{15} to glutamic acid and to the other amino acids, for the most part in conformance with the general pattern of ratios. The chief significant variation from this pattern is the transfer of nitrogen among these amino acids themselves. The injection of N^{15} -leucine results in a high incorporation of isotope into isoleucine, labeled isoleucine transfers a very large amount of N^{15} to leucine and injected valine produces high labeling in both the leucines. The excesses in valine from both leucines are only slightly higher than the values that could be expected. These results would seem to indicate a direct interconversion between leucine and isoleucine (with the equilibrium favoring leucine) and a conversion of valine to one or both of them (with an equilibrium strongly in favor of the leucines).

It is interesting that such conversions should appear to exist among amino acids that are known to be essential in the diet. However, it must be remembered that since these results have been obtained with N^{15} -labeled amino acids, they give no information about the extent to which the carbon skeleton takes part in such conversions, and may well indicate a shift of only part of the molecule or of just the amino group. Perhaps some doubts could be raised about these figures because of the possible existence of slight contamination of the leucines with each other. They emerge close together from the Dowex 50 isolation (compare threonine and serine), and although they have been found free of contamination when tested with paper chromatography, traces of the other leucine could perhaps have been present in each one. However, the valine definitely could not have contained any contaminating leucines.

The transfer of N^{15} from isoleucine to methionine is also very high and might indicate some direct conversion. It has already been pointed out that all the methionine values are questionable because the amounts isolated have been too low for accurate analysis. The excess here is less doubtful, however, because the figure for the fraction of valine plus methionine is considerably higher than that for valine alone.

Phenylalanine and tyrosine

N^{15} -labeled tyrosine could not be injected subcutaneously in the desired amount, due to its low solubility in water, and was therefore given as a water suspension by stomach tube. The animals received the tracer in one dose and were then killed twelve hours after the feeding. Phenylalanine was injected in the usual way. The N^{15} excesses in the amino acids isolated from the liver

Table 8. Incorporation of N^{15} from phenylalanine and tyrosine into liver protein amino acids. Two 200-gram rats, 200 mg phenylalanine each, 16.4 % excess N^{15} . Two 200-gram rats, 130 mg tyrosine each (by stomach tube), 17.6 % excess N^{15} .

Adminis- tered compound	Insoluble humin	Soluble humin	Leuc. + isoleuc.	Leucine	Isoleucine	Phenylalanine	Val., meth., tyr.	Val. + meth.	Valine	Methionine	Tyrosine	Proline	Alanine	Glutamic acid	Threonine	Aspartic acid	Serine	Glycine	Ammonia	Arginine	Lysine	Histidine
Phenyl- alanine		.16		.16	.25	4.61	.91		.13	.16	3.41	.09	.84	1.11	.02	.56	.13	.11	.19	.45	.33	.10
Tyrosine	.25	.28		.15	.16	1.48	.80	.17	.13		3.34	.11	.55	.78	.01	.42	.12	.15	.18	.30	.05	.06

protein are shown in Table 8. The ratios of the excesses to that of glutamic acid are given in Table 10.

In both experiments the highest excess of N^{15} is found in the injected amino acid and the isotope has been distributed to the other amino acids in line with the general pattern. The variation in experimental conditions for the administration of tyrosine apparently has not affected this pattern. The only variation can be seen in the figures for these two amino acids themselves. A direct conversion of phenylalanine to tyrosine is indicated by the high figure for tyrosine in the phenylalanine experiment; this has been observed previously by Moss and Schoenheimer¹⁷ in isotope experiments. However, the high excess in phenylalanine after feeding of tyrosine would seem to indicate that this conversion is reversible, in contrast to the results in the feeding experiments of Womack and Rose¹⁸.

In neither experiment is there a significantly high excess of N^{15} in alanine, although a direct relation might be expected. However, since alanine bears such a close relationship to glutamic acid, its content of N^{15} may equilibrate rapidly with that of glutamic acid and may therefore not appear to be significantly higher. This is supported by the results in the experiment with injected alanine.

Arginine, lysine and histidine

The three basic amino acids were each injected in the form of the free base. The amounts used are shown with Table 9. Since the labeled amino acids were isolated from biologically marked proteins, the isotope was distributed throughout the molecule, and the figures are overall excesses of N^{15} . In

Table 9. Incorporation of N^{15} from arginine, lysine and histidine into liver protein amino acids. Two 200-gram rats, 200 mg arginine each, 4.2 % excess N^{15} . Two 200-gram rats, 200 mg lysine each, 4.1 % excess N^{15} . Two 200-gram rats, 200 mg histidine each, 4.1 % excess N^{15} .

Adminis- tered compound	Insoluble humin	Soluble humin	Leuc. + isoleuc.	Leucine	Isoleucine	Phenylalanine	Val., meth., tyr.	Val. + meth.	Valine	Methionine	Tyrosine	Proline	Alanine	Glutamic acid	Threonine	Aspartic acid	Serine	Glycine	Ammonia	Arginine	Lysine	Histidine
Arginine	.28	.22	.34			.52	.46	.39			.48	.87	.94	1.63	.10	1.09	.30	.06	1.09	4.76	.63	.25
Lysine	.27	.00	.53			.27	.13					.23	.50	.98	.13	.79	.34	.11	.22	.40	4.25	.16
Histidine	.21	.24	.26			.49	.11					.30	1.60	1.53	.11	1.33	.41	.53	.78	1.32	.30	5.41

Table 10. Levels of N^{15} in liver protein amino acids, calculated on the basis of the excess in glutamic acid as 1.00.

Adminis- tered compound	Insoluble humin	Soluble humin	Leuc. + isoleuc.	Leucine	Isoleucine	Phenylalanine	Val., meth., tyr.	Val. + meth.	Valine	Methionine	Tyrosine	Proline	Alanine	Glutamic acid	Threonine	Aspartic acid	Serine	Glycine	Ammonia	Arginine	Lysine	Histidine
Glut. a.	.14 .13	.40	.31		.14		.24	.20	.48	.20	.12	.74	1.00		.03	.50	.46	.19	.23	.34	.04	.02
Ammonia	.11 .13	.14			.13	.08	.14				.24	.05	.85	1.00	.04	.65	.12	.16	1.49	.66	.01	.02
Asp. a.	.19	.33	.26	.59	.14		.20		.07	.20	.15	.67	1.00		.01	.53	.22	.20	.31	.32	.08	.13
Alanine	.15 .25		.50	.52	.11	.42	.45	.38		.13	.20	1.30	1.00		.01	.57	.30	.27	.35	.43	.06	.04
Proline	.14 .16		.19	.21	.06	.21	.24	.20		.10	4.27	.78	1.00		.01	.61	.19	.13	.24	.50	.07	.03
Threonine	.76 .85		.11	.33	.25	.49	.54	.66		.11	.24	.89	1.00	16.85	.82	3.36	2.34	1.09	.77	.22	.05	
Serine	.45 .27	.25	.23	.18	.17		.24	.16		.44	.15	.88	1.00	1.05	.67	7.41	3.72	.99	.63	.06	.04	
Glycine	.54 .12	.14			.15	.11					.07	.86	1.00		.03	.65	4.69	5.34	.83	.62	.02	.08
Leucine	.45 .48	2.56	3.36	.83	.10		.40	.21	.23		.83	1.00			.02	.52	.24	.25	.32	.36	.00	.05
Isoleuc.	.61 .95	2.05	1.19	3.52	.27	.94	1.09	.55	1.38	.43	.39	.80	1.00		.02	.51	.30	.29	.29	.35	.15	.11
Valine	.46		1.34	1.18	.14	2.47		2.92	.42	.18	.20	.84	1.00			.54	.35	.28	.49	.39	.04	.07
Phenylal.	.15		.14	.23	4.14	.82		.12	.15	3.06	.08	.76	1.00		.02	.50	.12	.10	.17	.40	.03	.09
Tyrosine	.31 .35		.19	.21	1.89	1.02	.22	.17		4.26	.14	.71	1.00		.02	.54	.16	.19	.23	.38	.07	.08
Arginine	.17 .14	.21			.32	.28	.24			.29	.53	.57	1.00		.06	.67	.19	.04	.67	2.91	.39	.15
Lysine	.28	.54			.27	.13					.23	.51	1.00		.14	.80	.34	.11	.22	.40	4.32	.16
Histidine	.14 .16	.17			.32	.01					.20	1.04	1.00		.07	.87	.27	.35	.51	.86	.19	3.53

the experiment with injected lysine, the lysine compound found in the threonine fraction was separated from threonine (as in the experiment with administered threonine), so that the figure for threonine does not include the highly labeled lysine compound.

Tables 9 and 10 show approximately the same pattern for N^{15} distribution from the basic amino acids as from the other acids. Some differences can be seen, but the fact that the N^{15} is distributed throughout the molecule of the injected compound could be expected to produce some variations. Also it must be remembered that there was a low excess of N^{15} in the injected compound, so that the direct figures were quite low and were multiplied by a factor of about 22 to obtain the figures based on 100 % N^{15} . In view of this, many of the differences, such as can be seen in figures for alanine, aspartic acid and ammonia are probably not significant. Of course, significantly high excesses are found in the injected amino acid in each experiment. It is possible that the figure for proline from injected arginine may be high due to a conversion of ornithine to proline, as reported by Stetten and Schoenheimer⁶. The actual difference observed, however, is small.

From the present data it is impossible to tell whether the N^{15} found in the isolated compounds is derived from the α -amino groups of the injected compounds or from labeled nitrogen from other parts of the molecules. In the work of Weissman and Schoenheimer¹⁹ with lysine marked with N^{15} in the α -position, the N^{15} was found to be distributed to the other amino acids tested, and when γ -labeled histidine was used as a tracer by Tesar and Rittenberg²⁰, this nitrogen was likewise distributed among the various amino acids investigated. On the basis of these studies it might be assumed that nitrogen from any part of the basic amino acids could take part in transfer reactions. There is no way of knowing which nitrogen atoms account for the transfer of N^{15} observed in our experiments with these amino acids, but all three have distributed their nitrogen to the same extent as have any of the other amino acids. However, as pointed out earlier, neither lysine nor histidine ever received any significant amount of N^{15} from any of the amino acids injected.

GENERAL DISCUSSION

Previous workers^{5, 6, 10, 13-15, 19-24} have found N^{15} to be incorporated into almost all amino acids from labeled ammonia and from every N^{15} -marked amino acid thus far tested, indicating extensive exchange of the amino group in metabolism. This is confirmed by the present investigation, in which fifteen different amino acids and ammonia, labeled with N^{15} , have been administered separately to rats, and the content of isotope determined in all amino acids isolated from total liver protein. As can be seen in the tables already presented, each of the administered compounds has distributed its nitrogen to all the amino acids isolated except threonine, lysine and histidine.

When the tables are compared with each other, similarities can be observed in the figures. The highest excess of N^{15} is always found in the injected compound (except in the case of aspartic acid), but in the figures for N^{15} transferred to the other amino acids a high value is always observed for glutamic acid. Moreover, the excesses of N^{15} in the other amino acids show a fairly constant relationship to each other. This can be seen more clearly in Table 10, in which the figures for N^{15} excess have been recalculated for each experiment on the basis of the N^{15} excess in glutamic acid as 1.00. This recalculation eliminates certain variations between experiments and makes the figures more comparable. Since it has been necessary to use so few animals for each experiment, differences in the transfer of N^{15} from the injected amino acids could be due partly to biological variation between animals in the rate of amino acid metabolism. Such variation is eliminated by setting the figures for all experi-

ments equivalent to a common level of nitrogen transfer. Variation due to differences in the amount of amino acid injected is also rendered negligible.

When the figures for different experiments are recalculated on a common basis in this fashion, and compared as in Table 10, the similarities in ratios are quite apparent, and a picture can be constructed of a general pattern for nitrogen distribution in amino acids. This indicates that amino acid metabolism, as judged by nitrogen exchange, may follow a common pathway. The simplest explanation for this is that, from each injected compound, the path of nitrogen transfer proceeds over one compound in central position, which then distributes the nitrogen to other amino acids. Such a compound must, of course, show a higher incorporation of isotope than the compounds to which it subsequently donates nitrogen. Glutamic acid seems a likely possibility for this central compound because of its high incorporation of N^{15} in all experiments. It might thus be implied that amino acid nitrogen transfer proceeds in general by some common metabolic pathway over glutamic acid. The figures in several experiments which are significantly higher than the levels found in the general pattern have then been assumed to indicate specific direct pathways between the injected compound and these particular amino acids. Some of these direct conversions have been observed previously.

The choice of glutamic acid as the compound in central position may seem arbitrary. The constancy of ratios among N^{15} levels in the different amino acids would naturally be apparent no matter what amino acid was taken as a basis of calculation. Even if glutamic acid does show a very high level of isotope, we can not say whether this is a result of a rapid, primary uptake of N^{15} by this amino acid, or whether it merely signifies that labeled glutamic acid is incorporated into protein more rapidly than are the other amino acids. Another explanation could be that total liver protein is made up of various individual proteins, in which the summary of amino acid compositions and rates of turnover favors the importance of glutamic acid (*i.e.*, the protein containing most glutamic acid may also have the highest turnover rate). These factors could affect not only glutamic acid but all the isolated labeled compounds. Their contribution could be clarified by carrying out this sort of study on each of several individual proteins. If the same pattern was found in all cases, one might conclude that it represented a general pattern of intermediary metabolism and not merely reflected protein compositions and turnovers.

However, the present experiments do offer some direct evidence for the correctness of the theory which places glutamic acid in a central metabolic position. This exists in the results of the experiment with administered proline. As already pointed out, the pattern of ratios in this experiment does not differ

significantly from that in any other, in spite of the existence of a known direct conversion of this amino acid to glutamic acid⁶. This supports the hypothesis that the metabolic transfer of nitrogen from each amino acid proceeds to glutamic acid, and that the distribution of isotope to the other amino acids depends on the level in glutamic acid.

In such theories about intermediary metabolism of amino acids nothing has been stated about the mechanisms involved. Glutamic acid has generally been recognized to maintain an important position in metabolism, especially with respect to transamination reactions. The extensive literature on this subject has been ably reviewed by Braunstein²⁵. In connection with this, it is of interest to observe that alanine and aspartic acid also show high figures for incorporation of N¹⁵ in all of these experiments and close correlation with the glutamic acid value. It is probable that the similarity in the levels of N¹⁵ in these three amino acids is a reflection of their close equilibrium with respect to nitrogen exchange, due to the known transamination reactions between them. However, very little is definitely known about similar reactions between glutamic acid and the other amino acids which could explain the amounts of N¹⁵ found in them. Perhaps the most logical mechanism to be expected would be a transfer of nitrogen from the injected amino acid to α -keto-glutaric acid and from the resulting glutamic acid to the respective keto acids of the others. However, from the present data it can not be decided whether or not the reactions proceed in this way, nor, if they do, whether the mechanism involved is as direct as that implied in the usual conception of transamination.

It is equally unjustified to postulate mechanisms for the specific conversions believed to be indicated by variations from the general pattern. Here again it must be remembered that all the figures for levels of N¹⁵ are subject to other variables than uptake from the injected compound, and may therefore be misleading, but it is highly probable that these large differences do really indicate direct pathways. These conversions have already been pointed out; some have been observed earlier but others have not been reported previously. It must be kept in mind that, even for these direct pathways, the figures do not indicate whether or not the carbon skeletons of the amino acids take part, but merely the extent of nitrogen transfer in each case.

The observation in these experiments that no significant amount of N¹⁵ is incorporated into lysine confirms previous work²¹⁻²⁴ with several administered labeled amino acids. Similarly, no significant uptake of N¹⁵ into histidine has been found here from any of the administered compounds, although it has been reported earlier that histidine incorporated a small amount of N¹⁵ in the α -position after the feeding of labeled ammonia²⁶. Other workers

have found no transfer of isotope to threonine from N^{15} -marked glycine¹³ or leucine¹⁵. The present results show this to be true for threonine no matter what amino acid is administered. It would then appear that none of these three amino acids can receive nitrogen from the common metabolic transfers, and that they may therefore be essential even with respect to the amino group of the molecule. However, all three of these acids have distributed nitrogen in line with the general pattern, so that processes of transfer apparently do occur. We can not say which nitrogen atoms of lysine or histidine have participated in these reactions. The transfer from threonine is less extensive than from any other amino acid.

It would be valuable if one could use the figures from these experiments as a quantitative index of the extent of metabolic activity of these amino acids. Unfortunately, however, one can not do this with certainty, because various factors influence the figures and it is almost impossible to distinguish between the effects of different ones. The levels of N^{15} found in the amino acids isolated are affected by the rate of incorporation of the different amino acids into protein and by the protein compositions and turnovers, as has already been pointed out. In addition, the isotope levels must be influenced by the amounts of amino acid present in free state in the animal at the time of isotope administration, which would dilute the injected labeled compounds. However, it might be assumed that this amount of free amino acid would be negligible in each case, in comparison with the large amount of amino acid injected, and variations between amino acids as a result of this may be insignificant. The most important variable which we can not know and control is the fact that all figures for isotope level reflect the extent of both the production of the amino acid, as indicated by its incorporation of nitrogen, and the breakdown or utilization, as evidenced by its transfer of nitrogen. Within the limits of variations due to incorporation rates, the level of N^{15} in the amino acids in the general pattern are an index of the extent to which the amino acids receive nitrogen. The best indication of the extent to which the amino acids transfer nitrogen is a comparison of the direct figures for the amount of N^{15} contributed by each of them to one particular amino acid, for example glutamic acid, which shows an appreciable incorporation of isotope in all experiments. The validity of this comparison is somewhat questionable due to the fact that the amounts of amino acids injected have been different. In both these comparisons small differences can not be considered decisive, and one can, at best, draw conclusions only from the extremes, since both transfer and incorporation of nitrogen proceed simultaneously and one can not determine to what extent each has been operative. The amino acids which transfer most nitrogen to glutamic acid are aspartic acid and alanine, indi-

cating a high extent of deamination of these amino acids in metabolism. The comparatively low figures for N^{15} remaining in the injected compound in the experiments with the administration of these amino acids may also reflect this, as well as an extensive production of amino acid. The production of new molecules dilutes the isotope level and extensive breakdown of the amino acid, as in these cases, makes this dilution effect more evident. This dilution by newly formed molecules is absent for the amino acids threonine, lysine and histidine, which have been shown not to incorporate nitrogen; the metabolism of these three amino acids seems therefore to be limited to transfer of nitrogen. A high figure for N^{15} remaining in the administered compound could therefore be expected in the experiments with these amino acids. Injected threonine can be seen to maintain a very high excess of N^{15} , in agreement with this expectation. Since the incorporation of isotope into glutamic acid from injected threonine is extremely low, it would appear that threonine is also poorly deaminated; both transfer and incorporation of nitrogen are low for this amino acid. Unfortunately, the figures for lysine and histidine are complicated by the nitrogen in the molecule other than the α -amino group, and by the fact that the molecule is labeled with N^{15} throughout. It is therefore impossible to draw such definite conclusions from them. However, we can perhaps cite aspartic acid and alanine, on the one hand, and threonine, on the other, as the extremes in extent of metabolic activity (both formation and breakdown) among the amino acids studied here.

SUMMARY

Figures are presented for the N^{15} uptake into eighteen different compounds (fifteen amino acids, ammonia, and soluble and insoluble humin) isolated from normal total liver protein after the administration of each of fifteen different amino acids and ammonia, labeled with N^{15} . In each case the isotope has been found to be distributed to all of the compounds isolated except threonine, lysine and histidine. Similarities in all the experiments in the ratios of N^{15} contents of the different compounds are discussed as being indicative of a common pathway of amino acid metabolism which always results in approximately the same pattern of nitrogen distribution. Evidence is presented and discussed for a central position of glutamic acid in this common metabolic transfer of nitrogen. Figures which are significantly higher than the usual levels in the general pattern are considered as pointing to specific conversions between the administered tracer and these particular compounds. On the basis of this evidence the direct pathways believed to be indicated by these figures are a reversible interconversion of glycine and serine, a non-

reversible transformation of threonine to glycine, a reversible interconversion of phenylalanine and tyrosine, a reversible interconversion between leucine and isoleucine and a probably reversible pathway between valine and either or both of the leucines. The figures in these experiments are also considered to be in agreement with a direct transformation of proline to glutamic acid, although the evidence is of a somewhat different nature.

REFERENCES

1. Hammarsten, E., and Åqvist, S. Unpublished.
2. Åqvist, S. E. G. *Acta Chem. Scand.* **5** (1951) 1031.
3. Hammarsten, E. *Acta Med. Scand., Suppl.* **196** (1947) 634.
4. Schneider, W. C. *J. Biol. Chem.* **161** (1945) 293.
5. Wu, H., and Rittenberg, D. *J. Biol. Chem.* **179** (1949) 847.
6. Stetten, M. R., and Schoenheimer, R. *J. Biol. Chem.* **153** (1944) 113.
7. Eliasson, N. A., Hammarsten, E., Reichard, P., Åqvist, S., Thorell, B., and Ehrensvärd, G. *Acta Chem. Scand.* **5** (1951) 431.
8. Åqvist, S. E. G. *Acta Chem. Scand.* **5** (1951) 1065.
9. Bergstrand, A., Eliasson, N. A., Hammarsten, E., Norberg, B., Reichard, P., and v. Ubisch, H. *Cold Spring Harbor Symp. Quant. Biol.* **13** (1948) 22.
10. Ratner, S., Rittenberg, D., Keston, A. S., and Schoenheimer, R. *J. Biol. Chem.* **134** (1940) 665.
11. Sakami, W., *J. Biol. Chem.* **178** (1949) 519.
12. Goldsworthy, P. D., Winnick, T., and Greenberg, D. M. *J. Biol. Chem.* **180** (1949) 341.
13. Elliot, D. F., and Neuberger, A. *Biochem. J.* **45** (1949) xiii.
14. Shemin, D. *J. Biol. Chem.* **162** (1946) 297.
15. Meltzer, H. L., and Springson, D. B. *Fed. Proc.* **9** (1950) 204.
16. Rees, M. W. *Biochem. J.* **40** (1946) 632.
17. Moss, A. R., and Schoenheimer, R. *J. Biol. Chem.* **135** (1940) 415.
18. Womack, M., and Rose, W. C. *J. Biol. Chem.* **107** (1934) 449.
19. Weissman, N., and Schoenheimer, R. *J. Biol. Chem.* **140** (1941) 779.
20. Tesar, C., and Rittenberg, D. *J. Biol. Chem.* **170** (1947) 35.
21. Foster, G. L., Schoenheimer, R., and Rittenberg, D. *J. Biol. Chem.* **127** (1939) 319.
22. Schoenheimer, R., Ratner, S., and Rittenberg, D. *J. Biol. Chem.* **127** (1939) 333.
23. Rittenberg, D., Schoenheimer, R., and Keston, A. S. *J. Biol. Chem.* **128** (1939) 603.
24. Schoenheimer, R., Ratner, S., and Rittenberg, D. *J. Biol. Chem.* **130** (1939) 703.
25. Braunstein, A. E. *Advances in Protein Chem.* **3** (1947) 1.
26. Schoenheimer, R., Rittenberg, D., and Keston, A. S. *J. Biol. Chem.* **127** (1939) 385.

Received July 5, 1951.