Amino Acid Interrelationships during Growth, Studied with N¹⁵-Labeled Glycine in Regenerating Rat Liver

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Previous studies have been made in this laboratory on various metabolic interrelationships of amino acids in normal rat liver, as indicated by experiments with N¹⁵-labeled amino acids¹. It was therefore of interest to investigate certain of these relationships in regenerating rat liver, in order to compare this with the normal tissue and perhaps gain some insight into the changes in amino acid metabolism occurring during accelerated growth and protein synthesis. Such a study has been carried out in conjunction with an investigation of the relationship between formation of protein and of nucleic acid bases in regenerating liver. The experiments were made with N¹⁵-labeled glycine as tracer. The results of the comparisons of nucleic acid and protein syntheses have already been published²; the present paper deals with the relationships indicated by the isotope distribution in the amino acids isolated from the liver protein. The proteins from cell nuclei and cytoplasm have been studied separately.

EXPERIMENTAL

The experimental procedures were those described in the previous publication². Partial hepatectomy was performed on albino rats weighing 180–200 g. The technique used for hepatectomy has been reported earlier³. Throughout the experiments the animals were fed on a diet of milk, bread and oats, and were allowed to eat *ad libitum*. The animals were allowed to live for different periods of time after hepatectomy and the total time from operation to death was termed the regeneration stage; the stages chosen were 11, 26, 32, 56 and 170 hours. The labeled glycine (31 % excess N¹⁵) was injected subcutaneously at a level of 100 mg per 100 g body weight (weight at time of operation). The tracer was divided into four doses which were given at two-hour intervals over the last eight hours of the regeneration time. The animals were killed two hours after the last injection of isotope.

Table 1. Incorporation of N^{15} from injected glycine into amino acids of liver cytoplasm protein during regeneration.

Regeneration stage *	Insoluble humin	Soluble humin	Leuc. + isoleuc.	Phenylalanine	Val., meth., tyr.	Val. + meth.	Valine	Methionine	Tyrosine	Proline	Alanine	Glutamic acid	Threonine	Aspartic acid	Serine	Glycine	Ammonia	Arginine	Lysine	Histidine	Cystine	Total hydrolys.	
11 hours	**	.13	.15	.07	.20	.12			.44	.09	.39	.46	.04	.28	5.20	5.93	1.09	.29	.13	.06	1.24	.73	
26 »		.38	.10	.18	.08	.22	.17	.24	.47	.10	.66	.74	.03	.49	7.92	8.14	1.41	.63	.10	.07		1.19	
32 »		.61	.21	.15	.10	.23	.14		.53	.12	.73	.92	.02	.68	6.38	7.68	1.12	.74	.14			1.17	
56 »		.57	.13	.22	.11	.22	.16	.24	.55	.55	.13	1.23	1.00	.03	.87	8.56	9.66	1.55	.90	.17	.06	1.73	1.38
170 »		.40	.12	.16	.09	.17	.15	.18	.51	.30	.13	.68	.82	.65	4.71	5.15	1.07	.69	.05	.03	1.24	.71	
Normal		.46	.10	.12	.13	.10				.06	.73	.85	.00	.55	3.97	4.53	.71	.52	.01	.07		.75	

* Regeneration stage is total hours from partial hepatectomy to death.

** Figures are expressed as % excess N^{15} , calculated on the basis of 100 % N^{15} in the administered glycine.

The livers from all rats in each regeneration stage were pooled, and the cell nuclei were separated from cytoplasm in citric acid according to the technique described earlier ³. The tissue from both sources was treated as in previous experiments ² to remove lipid material and to extract polynucleotides (method of Hammarsten ⁴, followed by extraction with hot trichloroacetic acid after Schneider ⁵). The protein was taken as the final dry, TCA-insoluble residue. This was hydrolyzed in acid and the amino acids separated by starch chromatography and analyzed for N^{15} . Details of these procedures have already been reported by Åqvist ⁶.

RESULTS

Table 1 shows the figures for excesses of N^{15} found in the compounds isolated from the cytoplasm protein. Results are given for normal, non-regenerating liver and for liver at the different stages of regeneration. Values are included for fifteen different amino acids, ammonia and soluble and insoluble humin. The figures for the N^{15} excesses in the total hydrolysates of the proteins are also given. The amino acids are listed in the order in which they emerge from the starch column. All figures are expressed on the basis of 100 % N^{15} in the injected glycine.

In the case of the protein from cell nuclei only glycine and serine were analyzed for N^{15} . The figures are presented in Table 2 for the same regeneration stages. The excesses in the total protein hydrolysates are also given. The figures are expressed in the same way as for Table 1.

The figures in Table 1 show that the highest level of N^{15} is found in the isolated glycine in both normal and regenerating liver, as would be expected

Table 2. Incorporation of N^{15} from injected glycine into amino acids of liver nuclei protein during regeneration.

Regeneration stage *	Serine	Glycine	Total hydrolysate
11 hours	**	5.62	.76
26 "	9.29	10.18	1.40
32 "	8.17	8.21	1.19
56 "	7.91	9.01	1.30
170 "	4.30	4.86	.71
Normal			.74

* Regeneration stage is total hours from partial hepatectomy to death.

** Figures are expressed as % excess N^{15} , calculated on the basis of 100 % N^{15} in the administered glycine.

from glycine as precursor. A very high value is also found in serine in each case. High values can also be seen in ammonia, especially in the regenerating liver. The cystine values which are included are also high. Unfortunately, the amounts of cystine isolated by these procedures were always too small to allow any really exact determination of N^{15} content, and the values can not be considered quantitatively accurate. However, a significant content of N^{15} in cystine does seem to be indicated by these consistently high figures.

From Table 1 it can be seen that except for glycine, serine and ammonia the amino acids of the cytoplasm protein show levels of N^{15} which are in general lower for 11-hours regeneration than for non-regenerating liver, although a few are not significantly different. Most of the figures increase from 11 to 26 hours of regeneration, but some are still below the normal levels at 26 hours. The figures increase further with longer regeneration time and seem to approach a common maximum at 56 hours. They decrease again at the last regeneration stage, in which they approach the levels in non-regenerating liver. The figures for glycine, serine and ammonia are somewhat different, because in addition to the 56-hour maximum they show another lower peak at 26 hours and a drop at the intermediate point of 32 hours. This pattern can also be seen in the excesses in the total protein hydrolysates, indicating a predominant influence by these compounds on the N^{15} level in the total protein.

For the cell nuclei protein, only values for glycine, serine and total protein hydrolysate are given. They are very much the same as those from the cytoplasm protein, except that the maximum at 26 hours is higher than that at

56 hours. The same intervening drop at 32 hours is observed, and the figures for both 11 hours and 170 hours are low. The same pattern is seen in both amino acids and in the total hydrolysate. There are no values for glycine and serine from nuclear protein of non-regenerating liver but the figure for the total hydrolysate is the same as that of cytoplasm and very close to the 11-hour and 170-hour figures in both cases.

In the previous investigation on metabolic interrelationships among amino acids in non-regenerating liver², there were indications of a common pattern of nitrogen distribution no matter what labeled amino acid was administered. A high incorporation of isotope into glutamic acid was always observed, and the ratios of levels of N¹⁵ in the different amino acids were very much the same in each case. A central position of glutamic acid in this common metabolism was postulated, and certain specific conversions were indicated by large increases in the levels of N¹⁵ above the usual ones shown in the general pattern. The general similarities of ratios and the large differences indicating direct metabolic pathways were made clearer in that study by recalculating the figures found in each experiment on the basis of the excess in glutamic acid as 1.00. The same thing is done for the figures in these experiments; the results are shown in Table 3.

The figures for non-regenerating liver in Table 3 have been presented in earlier work¹, along with other amino acid experiments, and are typical of the general pattern of nitrogen distribution found from all administered amino acids, except for the high values in glycine, serine and ammonia. These values would seem to indicate a direct conversion of glycine to serine, and a direct

Table 3. Levels of N¹⁵ in amino acids of liver cytoplasm protein, calculated on the basis of the excess in glutamic acid as 1.00.

Regeneration stage *	Insoluble humin	Soluble humin	Leuc. + isoleuc.	Phenylalanine	Val., meth., tyr.	Val. + meth.	Valine	Methionine	Tyrosine	Proline	Alanine	Glutamic acid	Threonine	Aspartic acid	Serine	Glycine	Ammonia	Arginine	Lysine	Histidine	Cystine
11 hours	.28	.32	.16	.42	.25			.95	.20	.84	1.00	.08	.61	11.22	12.80	2.35	.63	.29	.13	2.68	
26 »	.52	.14	.24	.11	.30		.23	.33	.64	.14	.89	1.00	.04	.66	10.68	10.97	1.89	.85	.13	.09	
32 »	.66	.23	.17	.11	.25	.16		.57	.13	.79	1.00	.02	.73	6.93	8.34	1.21	.80	.15			
56 »	.57	.13	.22	.11	.22	.16	.24	.55	.55	.13	1.23	1.00	.03	.87	8.56	9.66	1.55	.90	.17	.06	1.73
170 »	.50	.15	.20	.10	.21	.18	.22	.62	.37	.15	.83	1.00		.80	5.76	6.29	1.31	.84	.06	.04	1.51
Normal	.54	.12	.14	.15	.11					.07	.86	1.00	.03	.65	4.69	5.34	.83	.62	.02	.08	

* Regeneration stage is total hours from partial hepatectomy to death.

contribution of either or both of them to ammonia. In non-regenerating liver the conversion of glycine to serine results in an N^{15} level in isolated serine which is about 88 % of that in the isolated glycine. This ratio of serine to glycine varies somewhat throughout the regeneration stages. The ratio is very nearly the same as this at the first and at the last two regeneration stages for both cytoplasm and nuclei protein, but there are variations in the ratio around 26 and 32 hours of regeneration. (Cytoplasm protein — — 11 hours—0.88, 26—0.97, 32—0.83, 56—0.88, 170—0.92; nuclei protein — — 26—0.91, 32—0.995, 56—0.88, 170—0.88.)

In Table 3 one can compare the relative distribution of N^{15} at each regeneration point with that for non-regenerating liver. It can be seen that the pattern of distribution is much the same in all cases and is very close to the general pattern observed earlier¹. Of the compounds other than glycine, serine, ammonia and cystine, the highest levels of N^{15} are found in glutamic acid, alanine, aspartic acid, arginine and tyrosine, and insignificant amounts of N^{15} are seen in threonine and histidine. All of these levels are in line with the general pattern except possibly those in tyrosine. The figures for lysine in regenerating liver also seem a bit high, for lysine, like threonine and histidine, has not been found to incorporate any significant amount of isotope from any administered labeled amino acid in non-regenerating liver. It is hard to say whether or not this increase in lysine is significant and is a result of a difference due to regeneration.

However, in all the figures larger variations can be seen than have been observed in the previous experiments. These seem especially large in the ratios for glycine, serine and ammonia, but can also be seen even in such cases as aspartic acid and alanine which have earlier been found to have the most constant ratios. The figure for alanine at 56 hours regeneration is particularly high, but the ratios are in general somewhat high in this group and may be partly a result of too low a figure for glutamic acid.

DISCUSSION

The figures presented here for cytoplasm protein from non-regenerating liver have also been included and discussed in the previous study on N^{15} distribution in the same compounds isolated from normal liver after the administration of various amino acids¹. The very high value for serine in all these experiments with injected glycine confirms previous reports of a direct conversion of glycine to serine⁷⁻¹⁰, as has also been discussed in the previous paper. The high figures for ammonia are probably due to a rather extensive decomposition of highly-labeled serine during acid hydrolysis^{1, 11}. The values

are much higher in the regenerating liver than in normal liver, but they parallel the levels of N^{15} in serine. It is probable, therefore, that these figures represent an artifact more than a metabolic transfer of nitrogen. The highest cystine value also corresponds to the glycine-serine maximum. It has already been stated that these values are somewhat doubtful, quantitatively, but the high figures may well be explained by a conversion of serine to cystine, as discussed by Stetten ¹².

A variable but high incorporation of N^{15} into glutamic acid is seen for all the stages of regenerating liver as well as for normal liver, and, except for the cases of special contributions already discussed, the ratios of the other amino acids to glutamic acid for the most part follow the general pattern previously observed ¹. This can be seen more clearly from Table 3, where variations in the extent to which glycine transfers nitrogen to glutamic acid are eliminated. Since the conditions in regenerating liver are those of extensive protein synthesis, variations in this metabolic distribution of nitrogen from glutamic acid to the other compounds might be expected, but there do not seem to be appreciable changes.

From the figures in Table 3 for level of N^{15} in glycine, it can be seen that the amount of nitrogen retained by glycine, with respect to transfer to glutamic acid, is greatest at the first regeneration stage, appreciably above the value for non-regenerating liver, and that there is a continuous decrease in this level with increasing hours of regeneration, approaching the normal figure at 170 hours. The direct figures in Table 1 show that the incorporation of nitrogen into glutamic acid falls below normal at 11 hours and then rises to reach a maximum above the normal level at 56 hours. The isolated glycine, meanwhile, shows immediately a sharp increase in N^{15} level, above that of non-regenerating liver, which reaches a peak at 26 hours, dips and then rises to a maximum, also at 56 hours. The utilization of the injected compound, *i.e.*, its transfer of nitrogen to glutamic acid, is apparently lower during early stages of regeneration. The nitrogen transfer in liver seems to be somewhat inhibited just after hepatectomy, although the incorporation of glycine into protein proceeds at once, as the formation of new protein begins.

The various factors in non-regenerating liver affecting the N^{15} levels in the isolated amino acids, other than uptake from the administered compound, have been quite extensively discussed in the previous study ¹. Among these are the differences in rate of incorporation of the respective amino acids into protein, and the summary of amino acid compositions and turnover rates of the different proteins which make up total liver protein (*i.e.*, glutamic acid may have a high level of N^{15} because it is incorporated into protein faster, or because some individual proteins in the total liver protein which have a high

turnover rate also have a high content of glutamic acid). Such factors may become even more important under the conditions of rapid protein formation in regenerating liver. Another important variable can be found in the differences in extent both of utilization of the amino acid (nitrogen transfer) and of its formation (incorporation of nitrogen). An extensive production of new molecules of the amino acid serves to dilute the isotope content and a rapid breakdown or utilization of the amino acid makes this dilution effect more evident. Thus the very high levels in glycine during early stages of regeneration could be due partly to the decreased utilization to form glutamic acid. However, the N^{15} level in the glycine in free state has been shown not to be appreciably affected by such variations. A separate experiment, in which free glycine existing in the liver tissue has been isolated and analyzed for N^{15} , has shown very small variations in the isotope level at different regeneration stages. It is likely, therefore, that this rise in level of N^{15} in the glycine in protein reflects mostly an increased incorporation of glycine during the formation of new protein. Some increase of this sort can be seen in the figures for all the amino acids, which reach a common maximum at 56 hours. The effect on the other amino acids is less than on glycine (and on the compounds formed directly from glycine) because the others depend on the N^{15} level in glutamic acid, which shows a later and much smaller increase. The figures for serine and ammonia parallel those for glycine, and in Table 3 the ratios for these materials can be seen to drop with increasing regeneration time, just as do those for glycine. A similar tendency can be seen in tyrosine, but it is hard to say whether this indicates a closer relation of tyrosine to glycine or serine than heretofore suspected, or whether the high value at 11-hour regeneration is merely in error.

The factors of protein compositions and turnovers may help to explain the double peak observed in the figures for glycine, serine and ammonia. These factors may be accentuated, not only by the rate of formation of protein, but by the possibility that the different individual proteins may start to regenerate at different times and/or show maxima of synthesis or turnover at different periods of time after hepatectomy. The peak at 26 hours may be the maximum for a different protein (or proteins) than the one represented by the 56-hour peak. The total cell nuclei protein may contain a larger proportion of the first protein(s) and so show a higher maximum at 26 hours, in contrast to the total cytoplasm protein, which may be richer in the second protein(s), which has its peak at 56 hours. The figures for glutamic acid and for the other amino acids may not show this double peak because the intervening dip is obscured by the increase in the extent of transfer of nitrogen from glycine to glutamic acid (and thence to the other amino acids). This

contribution of different proteins could affect any of the amino acid figures and may account for some of the variations observed at these 26- and 32-hour regeneration stages. It may help to explain the altered serine to glycine ratios at 26 and 32 hours. In general it is interesting that the cell nuclei protein resembles the cytoplasm protein as closely as is indicated by the figures for glycine, serine and total hydrolysate. From these figures the total protein in both parts of the liver cell seem to have much the same turnover.

SUMMARY

Figures are reported for the uptake of N^{15} from injected glycine into various amino acids isolated from the protein of both cytoplasm and nuclei in normal and regenerating liver. The N^{15} has been found to be distributed to all of the compounds isolated from these proteins, the extent of transfer being for the most part in line with a general pattern of nitrogen distribution observed earlier for protein of normal rat liver after administration of various labeled amino acids. Contents of N^{15} higher than those in the general pattern support a direct conversion of glycine to serine and serine to cystine. These high levels are found in all these experiments with administered glycine. A high incorporation of isotope into glutamic acid can be seen in all cases, and the ratios of the levels in other amino acids to that in glutamic acid are about the same at all the different regeneration stages as in normal liver. However, the ratio of the level in glycine to that in glutamic acid is very high at early stages of regeneration and decreases steadily with increasing hours of regeneration to approach the ratio found in normal, non-regenerating liver. It is concluded that the utilization of the administered tracer is poorer at early stages of regeneration, with respect to N^{15} transfer to glutamic acid, but that the nitrogen distribution from glutamic acid to the other amino acids proceeds to about the same extent in all cases.

The actual levels of N^{15} in glycine are considered to be indicative of the extent of synthesis of new protein. For cytoplasm protein these show a maximum at 56 hours of regeneration and a lower peak at 26 hours. For cell nuclei protein the maximum is found at 26 hours and the second peak at 56 hours is slightly lower. These differences may well be due to contributions of different individual proteins which have maxima of synthesis or turnover at different periods of regeneration.

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