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The Turnover of Polynucleotides and Proteins in Regenerating Rat Liver, Studied with Glycine-^{15}N *

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An investigation of polynucleotide and protein turnover in regenerating rat liver has been made using ^{15}N-labeled glycine. Ten time stages, between 10 and 80 h after operation, have been studied, and particular attention has been paid to the incorporation pattern during the early period after hepatectomy.

1. The maximum of total cell PNA purine incorporation occurred at 14 h, considerably ahead of any other maximum. There was a smaller, broader peak of uptake into PNA around 26 to 56 h.

2. Incorporation into DNA purines was less than into those of PNA but of the same order of magnitude; the exact ratio between the two nucleic acids varied at different times. The peaks of incorporation into the DNA purines occurred at 26—32 and at 56 h.

3. In both PNA and DNA the two purine bases were not identical either in absolute uptake of ^{15}N or in time changes of incorporation. Guanine consistently showed a greater uptake of isotope than did adenine, but the ratio between the bases varied with time.

4. Total protein and its glycine constituent both showed two peaks of incorporation, at 20—26 and at 44 h, respectively, considerably later than the initial PNA maximum.

5. Cytological studies located the peak of mitosis frequency around 32 h; this coincided rather well with the major peak of isotope incorporation into DNA. Estimations of the relative size of the various cell parts revealed that the nucleolus showed maximum size earlier than did the nucleus and cytoplasm.

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Previous work from this laboratory [1] undertook an investigation of polynucleotide and protein turnover in regenerating rat liver, as revealed by the incorporation of ^{15}N-glycine. Protein turnover was judged by the uptake of isotope into the glycine constituent of the total protein, and nucleic acid turnover by the incorporation of tracer into the purine bases, which have been shown to be specifically labeled by this precursor [2-7]. This work showed that the maxima of turnover of these substances, particularly the polynucleotides, occurred soon after hepatectomy, rather than in the later stages of regeneration which have been studied in certain other investigations [8-10]. Further work was therefore undertaken to examine the time curve more closely during this early period, under more carefully controlled experimental conditions but similar conditions of isotope administration.

EXPERIMENTAL

The animals used were adult albino rats weighing 180—200 g. The investigation included eleven groups of animals, each comprising five to ten rats; one group of non-hepatectomized rats represented the normal base line of no regeneration, while the other groups represented various time points of regeneration — 10, 12, 14, 17, 20, 26, 32, 44, 56 and 80 h, respectively (time from hepatectomy to death).

Partial hepatectomy was performed as described in previous work [3]. The wet weight of the extirpated portion of liver was recorded in each case and the weight of the remaining portion calculated, as in earlier work [1]. The weight of the total original liver was thus recorded.

Since the voluntary consumption of food by the hepatectomized animals is considerably below normal, especially at early stages after operation, all rats were fed a definite amount of food by means of stomach tube. The diet [11] * was made up as a water suspension containing 10 g per 24 ml, and each rat was given 4 ml of this suspension per 200 g (weight at the beginning of the experiment) every 4 h. Feedings of the hepatectomized rats were begun 4 h after operation. The non-hepatectomized animals were fed in this manner for 32 h, with the last feeding being given 4 h before death.

Labeled glycine (32.9 atom per cent excess ^{15}N) was injected subcutaneously in aqueous solution. The total dosage was 200 mg per 200 g (weight at the time of first injection) divided in half and given in two injections, 5 and 4 h, respectively, before death.

The livers from all animals were removed and weighed. From these figures and the calculated weights of the total original liver, the extent of regeneration was determined (weight of regenerated liver as per cent of weight of total original liver).

Samples of each liver were taken for determination of water content and for cytological examination. In the cytological studies the relative size of the nucleus, nucleolus, and cytoplasm areas (optical cross-section areas), were determined as described previously[1]; the mitosis frequency was also measured [1].

The livers from each group of animals were pooled in alcohol, homogenized, and dried. The material was then disintegrated by vibration with glass beads [12] and extracted with boiling alcohol-ether to remove lipids. Mixed polynucleotides were extracted by the method of Hammarsten [12], hydrolyzed in alkali, and separated into pentose nucleic acid (PNA) and desoxypentose nucleic acid (DNA) with lanthanum nitrate.

The PNA mononucleotides were purified by precipitation with mercuric nitrate and decomposition with hydrogen sulfide, and the purine bases were split off by hydrolysis in

* This synthetic amino acid diet was originally set up as a basis for further investigation on the effect of single amino acid deficiencies on liver regeneration. However, preliminary experiments indicated that a deficiency of one of the essential amino acids produced no decrease in this liver growth or in the incorporation of glycine, and in later work this diet was replaced by a less elaborate one containing 18 % casein instead of the synthetic amino acids. The authors are grateful to Dr. Arvid Wretlind for advice on the composition of the diet and for assistance with the animal experiments.

acid and precipitated with silver, according to established methods [13,14]. The silver purines were decomposed with hydrochloric acid [13], excess hydrochloric acid removed *in vacuo*, and the free bases isolated and purified by starch chromatography, using a solvent system of butanol-water [15]. The pyrimidine bases were not analyzed.

The lanthanum precipitate of DNA was decomposed with potassium carbonate and the DNA precipitated with acid alcohol and dried [16]. The DNA was then hydrolyzed with dry hydrochloric acid in methanol [17]. The purine hydrochlorides were dissolved in water, the excess hydrochloric acid removed, and the pure bases again separated on starch.

All purine bases from both PNA and DNA were analyzed for ^{15}N content in the mass spectrometer, nitrogen being determined by a semi-micro Kjeldahl procedure. Purity was checked in each case by determination of the ultraviolet light absorption and the ratio $E_{max}/\mu g$ N per ml.

The protein residue from the polynucleotide extraction was treated twice with hot 5 % trichloroacetic acid, according to Schneider [18], to remove any remaining traces of polynucleotides. It was then dried, hydrolyzed with 6 N hydrochloric acid, and separated into the constituent amino acids by starch chromatography, according to Åqvist [19]. Both the total unhydrolyzed protein and the isolated glycine constituent were analyzed for excess ^{15}N.

RESULTS

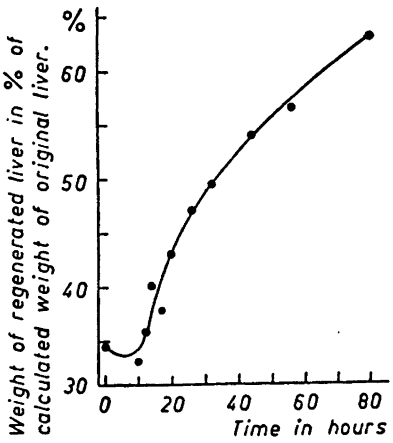
The average extents of regeneration found for these various time stages are shown in Fig. 1. It can be seen that after a slight initial drop the liver weight increased to 63 % of the original weight by 80 h. The differences in extent of regeneration at the earlier time stages are within the limits of biological variation and probably do not indicate significant deviation from the curve of steady increase.

Table 1 shows the figures for the atom per cent excess ^{15}N found in the PNA and DNA purines as well as in the total protein and its glycine constituent. The figures are calculated on the basis of 100 % ^{15}N in the administered glycine.

From these figures it can be seen that when the turnover picture is examined with time intervals so close together in the period soon after hepatectomy, the trends appear to be more complicated than could be seen from previous work with regeneration stages farther apart. The highest peak of PNA incorporation occurred as early as 14 h after operation, considerably ahead of any other maximum. In agreement with earlier work in this laboratory on glycine-^{15}N incorporation *in vivo* [1,3,20], the uptake into guanine was considerably greater than that into adenine, but the ratio between the two bases varied at different regeneration times. The time changes for the PNA purines showed that after 14 h guanine incorporation fell off rapidly to increase again around 26 h, while adenine incorporation decreased somewhat more slowly and then rose to a broader maximum between 26 and 56 h.

The incorporation into DNA was lower than that into PNA, although the figures for both nucleic acids had the same order of magnitude. The exact ratio between PNA and DNA varied with time, as did that between the two purine bases. As in the case of PNA, the DNA guanine showed a greater uptake of tracer than did adenine, but again the two purines did not show the same changes with time, both exhibiting considerable variation with both increases and decreases during the total time range. The highest incorporation was found at 26—32 and at 56 h.

From Table 1 it can also be seen that the total protein and the protein glycine both showed two peaks of isotope incorporation, also in the later stages,



at 20—26 and 44 h, respectively. This is in agreement with previous work [1]. It should be emphasized that this maximum is considerably later than the initial high peak of PNA incorporation.

The results of the cytological studies also showed considerably more variation than previously observed for regeneration stages farther apart in time [1,21]. The nucleolus was the first cell part to increase in size (optical cross-section area), reaching a maximum as early as 10—20 h. Both nucleus and cytoplasm approached maximum size around 26 h. The peak of mitotic activity came rather late in this series, based on the average of results for all livers within each group; the mitosis frequency remained at the normal low level through 20 h of regeneration and then increased to a maximum of about 10 % (per cent of cells counted which were undergoing mitosis) by 32 h. With such close time

Table 1.

Hours of regeneration	^{15}N excess * PNA ** Adenine	PNA ** Guanine	DNA Adenine	DNA Guanine	Protein ** Total	Protein ** Glycine
0	0.122	0.191	0.067	0.134	0.503	3.88
10	0.661	0.762	0.092	0.184	0.731	3.99
12	0.572	0.796	0.116	0.375	0.721	4.06
14	0.754	1.54	0.102	0.415	0.791	4.53
17	0.410	1.10	—	—	0.796	—
20	0.298	1.28	0.113	0.222	0.960	5.43
26	0.517	1.35	0.182	0.771	0.943	6.11
32	0.441	1.15	0.276	0.569	0.926	5.63
44	0.498	0.858	0.110	0.264	1.03	6.14
56	0.491	0.517	0.309	0.519	0.870	5.52
80	0.173	0.434	0.060	0.093	0.819	4.93

* The values are calculated on the basis of 100 % ^{15}N in the administered glycine.

** The curves for the time changes of ^{15}N content in the PNA purines and in the protein have been previously published [23].

intervals it was observed that mitotic activity increased very sharply at a particular time point for each liver, but examination of the individual livers indicated considerable variation among animals in the time point at which this began. Thus, around 26 to 44 h, each liver showed either great mitotic activity or very little mitosis, and the composite figure for each time interval could only be obtained by averaging the extremes for each group.

If the cytological findings are compared with the findings on isotope incorporation, it can be seen that the maximum of mitosis frequency correlated rather well with the major peak of isotope incorporation into DNA. Such correlation has also been emphasized in earlier work [1,22]. By and large, however, the appearance of the cell parts, as expressed in the other cytological magnitudes, does not seem to have a direct relationship to the chemical events reflected in the pattern of isotope incorporation.

DISCUSSION

As pointed out in previous work [1,23], an ideal precursor for isotope experiments would be incorporated directly, or by identical pathways, into all of the materials being analyzed. This situation can almost never be realized for a variety of substances, but a direct incorporation is approached in the uptake of glycine by protein[23]. In this case, therefore, the turnover indicated by glycine incorporation might be assumed to approximate that of the protein itself, with time changes being indicative of actual changes in the protein turnover rather than simply variations in biosynthetic patterns associated with this particular precursor. Explanation of the double maximum in the protein uptake of isotope could, then, be sought in the protein itself. Such a double maximum has been observed previously in this laboratory in a study similar to this [1] as well as in an investigation of the incorporation of ^{15}N-glycine into all the constituents of protein [24]. The double peak has been discussed in this earlier work in connection with heterogeneity of the protein fraction; since the fraction is a composite of cell proteins, the figures for isotope incorporation would be affected by variations in the composition of the total fraction.

Although the incorporation of glycine into polynucleotide purines has also been shown to be specific [2,4–7], the pathway is a complex one [25-29] and the uptake of this amino acid into the purine molecule might be expected to be somewhat more complicated than that into protein; the results in the present study would support this idea. The differences between the two purine bases, not only in extent of incorporation but also in pattern of time changes, indicate non-identical patterns of incorporation in the two cases *cf.* [30-33]. Studies on differences in the pattern of nucleic acid turnover revealed by the incorporation of various precursors [34-39] have served to emphasize the complexity of this problem. Thus, it is known that parts of the pattern found in these results, such as the high guanine to adenine ratios and the comparatively low PNA to DNA ratios, are typical of glycine incorporation and are not as evident with certain other nucleic acid precursors [3,4,6,16,20,23,34,36-44]. It is also apparent that these ratios reflect incorporation by purine bases themselves and are not due only to differences in uptake by the pyrimidine or carbohydrate portions

of the nucleic acid molecule [cf. 36]. Possible factors contributing to differences between dissimilar precursors include the availability of any particular one for biosynthesis in a specific tissue, dilution by unlabeled pools of intermediates, and the metabolic effects of enzyme distribution and activity. Moreover, the incorporation pattern for any one precursor could be affected by independent turnover of specific parts of the molecule being synthesized, so that the problem is the extent to which the incorporation is representative of overall nucleic acid turnover. In the light of the known differences in turnover results, it would seem highly probable that there are different paths of biosynthesis of nucleic acid components and that these paths may coexist in one tissue. Since we know very little about how such paths may vary in importance during function, data with various precursors must be correlated before the incorporation of any particular one can be fully evaluated as an accurate reflection of total nucleic acid turnover during liver regeneration. In addition to reflecting any such effects of a particular precursor, the nucleic acid turnover pattern, like that of the protein, could be affected by any heterogeneity of the material.

All such variations could affect the time changes for both PNA and DNA. The DNA figures here showed particularly irregular changes, but since glycine incorporation into DNA is high even in non-dividing tissue [cf. 41,45], the isotope uptake here might be expected to show variations due to DNA synthesis not associated with cell division, or even to some dynamic turnover having no relation to actual synthesis. The factors mentioned above could enter into such turnover; heterogeneity of DNA has been specifically pointed out [46-48]. DNA is considered to be metabolically a rather stable cell component. However, it may also be that at least the smaller variations in DNA incorporation here represent merely biological variation during regeneration (*cf.* the results on mitosis frequency). This question of the reproducibility of the biological phenomenon being studied must be kept in mind in evaluating all of these time curves, since it is virtually impossible to know to what extent the groups representing the various time points actually correspond to each other in regeneration.

The time changes in PNA incorporation of isotope seemed considerably more distinct, and the double maximum in this incorporation, revealed by this examination of such close time intervals, was of considerable interest. It appeared to be of value to try to investigate further the significance of this curve for PNA in terms of some of the variables discussed above. One possible explanation of the double peak in incorporation was cellular heterogeneity of the PNA; the PNAs from nucleus and cytoplasm are known to show great differences in the absolute uptake of various precursors [1,3,36-38, 49-53] and might well vary also in their changes of incorporation with time. Another possibility was a difference among the various nitrogen atoms of the purines in rate of incorporation. Further work was therefore undertaken to explore these particular possibilities.

The distinction between turnover and synthesis is a difficulty always encountered in the evaluation of isotope incorporation pattern. It is evident that the pattern will be influenced by changes in the equilibrium between synthesis and degradation. In the case of both protein and nucleic acid it appeared desirable, therefore, to determine to what extent the turnover figures

here represented actual net synthesis of new molecules. Towards this end a comparable study was undertaken to determine the quantitative changes in the content of these materials in liver during regeneration.

REFERENCES

1. Eliasson, N. A., Hammarsten, E., Reichard, P., Åqvist, S., Thorell, B. and Ehrensvärd, G. *Acta Chem. Scand.* **5** (1951) 431.
2. Abrams, R., Hammarsten, E. and Shemin, D. *J. Biol. Chem.* **173** (1948) 429.
3. Bergstrand, A., Eliasson, N. A., Hammarsten, E., Norberg, B., Reichard, P. and Ubisch, v. H. *Cold Spring Harbor Symposia Quant. Biol.* **13** (1948) 22.
4. Heinrich, M. R. and Wilson, D. W. *J. Biol. Chem.* **186** (1950) 447.
5. Sutton, W. B., Schlenk, F. and Werkman, C. H. *Arch. Biochem. and Biophys.* **32** (1951) 85.
6. Heidelberger, C. and LePage, G. A. *Proc. Soc. Exptl. Biol. Med.* **76** (1951) 464.
7. Edmonds, M., Delluva, A. M. and Wilson, D. W. *J. Biol. Chem.* **197** (1952) 251.
8. Drabkin, D. L. *J. Biol. Chem.* **171** (1947) 395.
9. Novikoff, A. B. and Potter, V. R. *J. Biol. Chem.* **173** (1948) 223.
10. Lombardo, M. E., Cerecedo, L. R. and Reddy, D. V. N. *J. Biol. Chem.* **202** (1953) 97.
11. Wretlind, K. A. J. and Rose, W. C. *J. Biol. Chem.* **187** (1950) 697.
12. Hammarsten, E. *Acta Med. Scand. Suppl.* **196** (1947) 634.
13. Kerr, S. E. and Seraidarian, K. *J. Biol. Chem.* **159** (1945) 211.
14. Reichard, P. *J. Biol. Chem.* **179** (1949) 763.
15. Edman, P., Hammarsten, E., Löw, B. and Reichard, P. *J. Biol. Chem.* **178** (1949) 395.
16. Reichard, P. *Acta Chem. Scand.* **3** (1949) 422.
17. Vischer, E. and Chargaff, E. *J. Biol. Chem.* **176** (1948) 715.
18. Schneider, W. C. *J. Biol. Chem.* **161** (1945) 293.
19. Åqvist, S. E. G. *Acta Chem. Scand.* **5** (1951) 1031.
20. Reichard, P. *J. Biol. Chem.* **179** (1949) 773.
21. Stowell, R. E. *Arch. Pathol.* **46** (1948) 164.
22. Hammarsten, E., Thorell, B., Åqvist, S., Eliasson, N. A. and Åkerman, L. *Exptl. Cell Research* **5** (1953) 404.
23. Hammarsten, E. *CIBA Foundation Conference, Isotopes in Biochemistrty*, London 1951, p. 203.
24. Åqvist, S. E. G. *Acta Chem. Scand.* **5** (1951) 1065.
25. Greenberg, G. R. *Federation Proc.* **12** (1953) 651.
26. Goldthwait, D. A., Peabody, R. A. and Greenberg, G. R. *J. Am. Chem. Soc.* **76** (1954) 5258.
27. Hartman, S. C., Levenberg, B. and Buchanan, J. M. *J. Am. Chem. Soc.* **77** (1955) 501.
28. Goldthwait, D. A., Greenberg, G. R., and Peabody, R. A. *Biochim. et Biophys. Acta* **18** (1955) 148.
29. Levenberg, B. and Buchanan, J. M. *J. Am. Chem. Soc.* **78** (1956) 504.
30. Carter, C. E. and Cohen, L. H. *J. Am. Chem. Soc.* **77** (1955) 499.
31. Abrams, R. and Bentley, M. *J. Am. Chem. Soc.* **77** (1955) 4179.
32. Lagerkvist, U. *Acta Chem. Scand.* **9** (1955) 1028.
33. Lieberman, I. *J. Am. Chem. Soc.* **78** (1956) 251.
34. Furst, S. S. and Brown, G. B. *J. Biol. Chem.* **191** (1951) 239.
35. Goldthwait, D. A. and Bendich, A. *J. Biol. Chem.* **196** (1952) 841.
36. Payne, A. H., Kelly, L. S. and Jones, H. B. *Cancer Research* **12** (1952) 666.
37. Anderson, E. P. and Åqvist, S. E. G. *J. Biol. Chem.* **202** (1953) 513.
38. Tyner, E. P., Heidelberger, C. and LePage, G. A. *Cancer Research* **13** (1953) 186.
39. Moldave, K. and Heidelberger, C. *J. Am. Chem. Soc.* **76** (1954) 679.
40. Brown, G. B., Roll, P. M., Plentl, A. A. and Cavalieri, L. F. *J. Biol. Chem.* **172** (1948) 469.
41. Brown, G. B. *CIBA Foundation Conference, Isotopes in Biochemistry*, London 1951, pp. 149—150, 164.
42. LePage, G. A. and Heidelberger, C. *J. Biol. Chem.* **188** (1951) 593.

43. Abrams, R. *Arch. Biochem. and Biophys.* **33** (1951) 436.
44. LePage, G. A. *Cancer Research* **12** (1952) 277.
45. Malkin, H. M. *Biochim. et Biophys. Acta* **12** (1953) 585.
46. Chargaff, E., Crampton, C. F. and Lipschitz, R. *Nature* **172** (1953) 289.
47. Brown, G. L. and Watson, M. *Nature* **172** (1953) 339.
48 Bendich, A., Russell, P. J., Jr. and Brown, G. B. *J. Biol. Chem.* **203** (1953) 305
49. Jeener, R. and Szafarz, D. *Arch. Biochem.* **26** (1950) 54.
50. Barnum, C. P. and Huseby, R. A. *Arch. Biochem.* **29** (1950) 7.
51. Smellie, R. M. S., McIndoe, W. M., Logan, R., Davidson, J. N. and Dawson, I. M. *Biochem. J. London* **54** (1953) 280.
52. Smellie, R. M. S., McIndoe, W. M. and Davidson, J. N. *Biochim. et Biophys. Acta* **11** (1953) 559.
53. Smellie, R. M. S. in Chargaff, E. and Davidson, J. N., Eds., *The Nucleic Acids II*, New York 1955, p. 408.

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