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The Synthesis of Polynucleotides and Protein in Regenerating Rat Liver

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A study has been made of the changes in quantitative content of PNA and DNA phosphorus and protein nitrogen in regenerating liver 8 to 80 h after hepatectomy. This study has been correlated with a comparable one made previously on incorporation of ^{15}N-labeled glycine into these same substances, in an attempt to compare the time curves of turnover and synthesis.

During the first 80 h of regeneration the protein, lipid, and DNA content of liver remained relatively constant, *i.e.* these substances increased proportionately with liver weight. In contrast, the content of PNA per mg protein nitrogen increased from the original level to a maximum at 40 h.

The relative increases in these materials, over the normal values, were plotted and compared with that of the liver itself. From these, rates of synthesis could be estimated. PNA showed the first and most rapid increase and attained maximum rate of synthesis between 20 and 25 h. Protein synthesis reached a peak at about 40 h, and the maximum for DNA occurred between these two points. The maximum for protein synthesis corresponded with the peak of PNA content.

Correlation of the time curves for synthesis and turnover showed that the peaks of incorporation and synthesis agreed for protein, and, to a large extent, for DNA. In the case of PNA, the second peak in incorporation correlated with the maximum of synthesis, but the initial high peak apparently represented some turnover process distinct from net synthesis of new molecules.

Other work in this laboratory had undertaken a study of polynucleotide and protein turnover in regenerating liver, using ^{15}N-labeled glycine [1]. From this, time curves of turnover during regeneration were determined for these substances. In an attempt to define to what extent these turnover figures could be due to actual net synthesis of new molecules during this process, another study was made of the changes in the quantitative content of these materials in liver during identical periods of regeneration. This study was made as nearly comparable as possible to the earlier one on isotope incorporation [1].

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EXPERIMENTAL

The experiments employed adult albino rats weighing 180—200 g. Eight groups of eight to twelve rats were subjected to partial hepatectomy, according to a previously described method [2], and the livers allowed to regenerate for a different period of time in each case — 8, 12, 16, 20, 30, 40, 60 and 80 h, respectively, from operation to death.

The hepatectomized animals were fed by stomach tube every four hours during the period of regeneration in order to insure an equal and adequate consumption of food. The diet administered was the same as that used in the comparable isotope experiments [1], except for the two longest periods of regeneration. The animals in these two groups were fed by stomach tube for only the first 24 h after operation, being given a diet containing 18 % casein instead of synthetic amino acids [3]; after this time they were fed 10 g per day of the same diet in dry form.

The regenerated livers from all animals were removed and weighed, and the extent of regeneration calculated as in the previous work [1]. The livers for each group were pooled, homogenized in alcohol, disintegrated by vibration [4], and extracted with alcohol-ether to remove lipids, in a manner corresponding to that described for other work [1], except that recoveries of all fractions were made quantitatively. The liver removed from one of the groups at the time of hepatectomy was taken as a sample of normal, non-regenerating liver and was treated in the same way.

The lipid extracts were evaporated to dryness, and the dry weight of the extracted material determined.

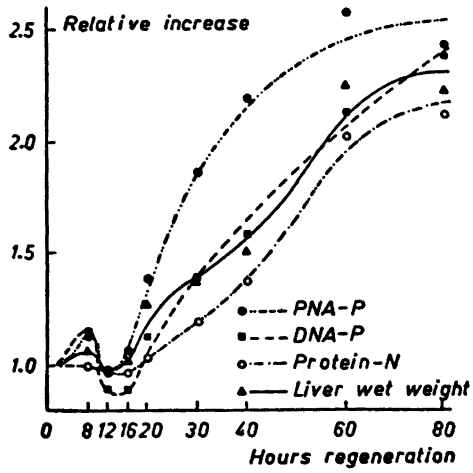
The tissue samples remaining after lipid extraction were treated according to Hammarsten [4] to extract mixed polynucleotides. The method was the same as that usually employed in this laboratory, involving extraction with saturated urea and saturated salt (sodium chloride and ammonium sulfate) solution, precipitation of the extracted polynucleotides with copper, decomposition of the copper salt with potassium acetate and solution of the residue with urea, and final precipitation of the free polynucleotides with alcohol. For this work, however, the exact procedure was somewhat modified in order to insure complete extraction. The extraction was performed on four to twelve weighed aliquots (around 100 mg each) of the original dry, lipid-free tissue, each aliquot being ground by hand during extraction in a small, conical centrifuge tube with a closely fitting ground glass piston. This centrifuge tube constituted a modified McShan-Erway grinder [5]; each tube and its piston were specially ground to fit very closely. The grinder was operated by hand to insure more complete disintegration, since the tissue became rather gummy during the first extraction.

The extracted mixed polynucleotides were hydrolyzed in alkali and separated into pentose nucleic acid (PNA) and desoxypentose nucleic acid (DNA) with lanthanum acetate at pH 2 [4,6]. Both the PNA supernatant and the lanthanum precipitate of DNA were combusted directly for quantitative phosphorus determinations, which were made on aliquots of each digest by the colorimetric method of Fiske and Subbarow [7].

Comparable weighed aliquots of the original dry, lipid-free tissue were also extracted twice with hot trichloroacetic acid (TCA), according to Schneider [8], to obtain a TCA-insoluble protein residue free of salt and other extraneous material. The dry weight of this protein residue was determined for each aliquot, and its nitrogen content estimated by a semi-micro Kjeldahl method.

RESULTS

The time curve of regeneration for these animals is given in Fig. 1, shown as the relative increase in the wet weight of the liver after hepatectomy. In absolute per cent regeneration (weight of regenerated liver as per cent of weight of original liver) the first seven of these groups corresponded very closely to the comparable groups from the previous isotope series [1]. Only the groups representing the two longest time periods showed regeneration much greater in extent. These two groups were fed the casein diet, which has been found in another series [3] to be associated with more extensive liver regeneration. The



amount of regeneration shown by the last two groups in the present series was even more than that previously observed on the casein diet, the figures here being greater than that found previously for 126 h of regeneration. These last two groups seem to have attained almost a plateau of growth, the extent of regeneration being no greater at 80 h than at 60 h.

The figures for the quantitative determinations on liver from these groups are shown in Table 1. This table shows for each group the extent of growth (per cent regeneration), the protein nitrogen content (nitrogen found in the TCA-insoluble protein residue) and the lipid content (dry weight of the residue from lipid extracts) of the dry whole liver tissue, and the content of PNA and DNA phosphorus, expressed per mg protein nitrogen. Because of irregular variations found, from preliminary estimations, in the glycogen content of the liver samples (reflected in the third column of Table 1, giving the protein nitrogen content), the protein nitrogen was considered to be the most constant as well as the most significant basis on which to express the quantitative figures for polynucleotide content.

Table 1.

Hours of regeneration	Per cent regeneration	mg protein N per 100 mg dry weight *	mg lipids per 100 mg dry weight *	μg PNA P per mg protein N	μg DNA P per mg protein N
0	34.6	7.52	19.8	24.9	7.21
8	36.8	7.25	25.3	28.2	8.33
12	34.0	8.31	24.7	25.2	6.65
16	35.3	7.81	23.7	27.6	6.67
20	44.1	6.29	26.3	33.5	7.85
30	47.6	6.64	26.0	38.9	8.40
40	52.4	7.19	27.8	39.8	8.31
60	78.1	6.90	31.7	31.8	8.23
80	77.4	7.39	27.0	28.6	8.09

* Dry weight of whole liver.

From Table 1 it can be seen that the protein content of the whole liver varied irregularly around a figure of approximately 7 mg protein nitrogen per 100 mg dry weight of liver. The lipid content, which was about 25 mg per 100 mg liver, was somewhat more constant, and there was no evidence of accumulation of lipid material during regeneration [9,10]. These substances, then, increased approximately proportionately with liver weight.

From the last column it can be seen that, except for slight irregularity in the early stages of regeneration, the content of DNA as expressed here also remained rather constant throughout the various time stages, *i.e.* the level increased proportionately with protein and with liver weight [cf.11]. The actual figure for DNA content was about 8 μg DNA phosphorus per mg protein nitrogen. Column five gives the values for PNA phosphorus and indicates that this constituent showed initial irregularities during the first 16 hours of regeneration and then increased steadily, compared with the level of protein, to a rather high maximum content at 40 h. If these nucleic acid contents are calculated on the basis of dry weight of liver, the initial irregularities in the curves are less, but the shape of both curves is essentially unchanged, with the maximum in PNA content falling at the same time point.

Figure 1 gives the time curves of regeneration for PNA phosphorus, DNA phosphorus, and protein nitrogen, as well as liver wet weight. These curves represent the change with time of the total amount of each of these constituents, expressed as relative increase over the zero point value. The zero point for each curve represents the content of that constituent in a unit of liver before regeneration has begun. For each time stage the total content of each constituent has then been calculated for the amount of liver expected at that stage of regeneration from this unit of liver. The curves serve to indicate when and with what speed each constituent was produced in the liver during the regeneration process. From them it can be seen that all these constituents increased rather steadily with time. The PNA began its increase somewhat earlier than the other substances and showed a somewhat steeper rise. It must be remembered that the increase in these constituents should actually be less than that seen here for the last two time points, especially the increase between 40 and 60 h, since these two groups had a high extent of regeneration which was out of proportion to that of the earlier groups. With this in mind, if the rates of formation of these materials are estimated from the curves, the maximum rate of PNA synthesis can be seen to have occurred between 20 and 25 h. Protein synthesis reached a maximum at about 40 h, and the peak for DNA occurred between these two points. If these results are compared with the figures in Table 1, it can be seen that the period of maximum rate of protein synthesis coincided with the peak of PNA content.

DISCUSSION

If the results of these quantitative determinations are correlated with those of the corresponding study on incorporation of ^{15}N-labeled glycine [1], some indications can be obtained as to the extent to which the time curves of isotope incorporation reflect net synthesis of protein and nucleic acid molecules. It must be remembered that the time periods recorded in the isotope series

were the duration of life after hepatectomy, and that isotope was administered 5 and 4 h prior to death, so, in making the correlation, these time points should be moved ahead to include this total period.

In the case of protein the highest peak of isotope incorporation was found at 39—44 h. This correlates with the time of most rapid synthesis of protein, as determined in the present series. The earlier, smaller peak of protein uptake of isotope, at 20—26 h, could also have included some lesser contribution from the synthesis of new molecules, since this synthesis had begun by that time. A correlation of the pattern for incorporation into protein with that for net synthesis helps to explain the relatively uncomplicated picture obtained for glycine uptake by protein during regeneration, and supports the claim [1] that this incorporation picture reflects changes in the actual protein rather than simply a biosynthetic pattern associated with this particular precursor.

It is of interest to note the correlation in time between the maximum content of PNA and the maximum rate of protein synthesis. A high PNA content during rapid growth and protein synthesis has been reported by many investigators for various systems of cell growth and function, and has been much discussed in relation to a postulated function of PNA in protein synthesis[2,9,12-27]. Without reviewing the evidence as to the pros and cons of this theory, it can be said that the results in the present study are in agreement with these previous studies in regard to high PNA content during the period of most rapid protein synthesis. However, it should be stressed that data such as these indicate only a time sequence and correlation and can throw no light upon the problem of mechanism for any relationship between these substances.

For DNA the maximum in rate of formation is broader than that for protein and thus harder to place exactly in time, but the curve would indicate that net synthesis of DNA could have materially contributed to the main peak of isotope incorporation around 30 h. This period of synthesis and incorporation also correlates rather well with the maximum of mitosis frequency *cf.*[28]. Actually certain cellular studies have indicated that DNA synthesis occurs during interphase, preceding mitosis [29-31], but the significance of this for a time study of total tissue changes is not clear. Price and Laird [32], using chemical methods, have found a doubling of DNA content just before the maximum of mitosis in regenerating rat liver. Such results cannot be seen from these figures. The picture here is more one of steady increase in the DNA content of the liver, paralleling the total regeneration, and it is difficult to perceive any period of much greater synthesis *cf.* [11,33]. Since the incorporation curve, furthermore, showed variations throughout the time range studied, specific time point correlations are of somewhat doubtful value in the case of DNA.

With respect to the rate of PNA synthesis, in relation to the time curve for PNA incorporation of glycine, the data here show that synthesis attained a maximum between 20 and 25 h, which could have contributed to the second peak in PNA incorporation of isotope. However, the initial high peak of isotope uptake, at 9—14 h, occurred ahead of any material increase in PNA content. It would seem that this peak, therefore, must represent some turnover process quite distinct from net increase in number of molecules. Such turnover might be more likely to depend upon and vary with the precursor used, and its inter-

pretation is more difficult. Thus, it is apparent that the double maximum observed in PNA incorporation [1] reflects the biological complexity of the metabolic processes involved in and contributing to the turnover of this material, but at present the data are still too incomplete to allow a more exact definition of the nature of this complexity.

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