BIOCHEMISTRY AND BIOPHYSICS

INCORPORATION OF RADIOACTIVE S^{35}-METHIONINE AND C^{14}-GLYCINE INTO THE PROTEINS OF WHOLE LIVER TISSUE AND OF LIVER CELL NUCLEI, AT VARIOUS TIMES AFTER PARTIAL HEPATECTOMY

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Researches conducted in the Biochemical Laboratory of the P. A. Gertsen Oncological Institute [2, 4, 9] have shown that tumor cell nuclei differ from those of ordinary tissues in the relative proportions of their protein fractions. Malignant tumor cell nuclei show a pronounced rise (by a factor of 5-10) in the content of residual protein fractions, at the expense of nucleoprotein and acid protein fractions. In order to ascertain the specificity of these changes, a study was made of the composition of the cell nuclei of other rapidly growing tissues (embryonic and regenerating) [3]. During the early stages of compensatory growth of liver cells following partial hepatectomy the cell nuclei of regenerating tissue to a certain extent resemble, as regards their protein fractions, the nuclei of tumor cells. This allows us to draw a parallel between the dedifferentiation of tissues, taking place at this stage of regeneration, and the anaplasia of tumors [3].

Experiments on the incorporation of labelled amino acids into whole tissue proteins and into the nuclei of malignant tumor cells in vivo showed that the nuclear proteins of tumor cells took S^{35}-methionine and C^{14}-glycine 3-4 times more slowly than did the whole tissue proteins or the nuclear proteins of normal liver [5]. It remains to be shown to what extent this phenomenon is characteristic of tumor tissue, or whether it applies to all rapidly growing tissues, and whether it bears any relation to differentiation of tissues.

The present paper is devoted to a study of the rate of incorporation of S^{35}-methionine and C^{14}-glycine into the proteins of whole tissue and cell nuclei at various stages of compensatory growth following partial hepatectomy.

EXPERIMENTAL METHODS

The abdominal cavity of rats weighing 130-150 g was opened under ether anesthesia, and a part of the liver was ligated and excised. At various times after the operation (2, 5, 10, 15, and 20 days) injections of S^{35}-methionine were given subcutaneously, at a dosage of 3000 to 6000 impulses per minute per g body weight. The animals were killed by decapitation 30 minutes and 3 hours after the injection, and the liver was taken for examination, after trimming off the necrotic parts. The cell nuclei were isolated by Dounce's method, at pH 6.8 [12]. In some of the experiments the nuclei were isolated in a 1% citric acid medium [13]. Parallel with this we performed a series of experiments on the incorporation of labelled amino acids into the proteins of liver slices in vitro. The experiments were conducted according to the method described in an earlier paper [6].

The proteins were processed in the usual way. Radioactivity was measured by means of a Geiger counter, on a 10 mg sample of protein spread evenly over the surface of aluminium discs, diameter 18 mm. The results were calculated as the ratio of impulses per minute per g of protein and per g body weight.

TABLE 1

Incorporation of S^{35}-Methionine into the Proteins of Whole Tissue, Nuclei, and Nuclear Fractions of Regenerating Rat Liver at Various Times after Partial Hepatectomy. Three Hours After Injection of Radiomethionine. The Nuclei were Isolated in Neutral Solution. Impulses per min. per g of Protein; Impulses per min. per g Body Weight

Experiment	№ 9			№ 11				№ 14						№ 16					
	days after partial hepatectomy																		
	5	10	15	20	2	4	11	2	4	11	14	2	6	10	15	18
Whole tissue proteins	3.4	3.4	5.6	6.2	8.0	6.2	9.3	5.1	5.1	3.3	3.9	9.5	7.2	4.8	5.4	5.3
Nuclear proteins	1.0	3.3	7.1	7.2	5.7	3.6	8.1	4.9	3.5	—	3.8	8.0	5.5	6.5	4.7	5.3

EXPERIMENTAL RESULTS

Our experiments on the incorporation of S^{35}-methionine into the proteins of whole liver tissue and into the cell nuclei of regenerating liver of rats showed (Table 1) that the highest radioactivities were found in the proteins of whole liver tissue during the first few days after partial hepatectomy (for example, on the second day), after which the activity fell, and again rose in the terminal stages of regeneration. On the 2nd day after partial hepatectomy the nuclear proteins incorporate S^{35}- methionine at a somewhat slower rate than do those of whole tissue. By the 4 – 6th day the radioactivity of the nuclear proteins had fallen by $1\frac{1}{2}$ – 2 times, and it regained its earlier level only on the 15 – 20th day after the operation.

Fractionation of the nuclear proteins [4] was performed in some of the experiments only, owing to the insufficient amounts of available material.

The results of these experiments showed that the highest radioactivity was found in the acid protein fraction, and the lowest in the residual proteins.

The data for incorporation of C^{14}-glycine into the proteins of regenerating liver tissue are presented in Table 2. In these experiments the cell nuclei were isolated in acid medium; inclusion of radioactivity into the nuclear proteins at different stages of regeneration did not differ appreciably from that for whole tissue proteins.

Our results for rate of incorporation into tissue slices in vitro confirmed the findings reported in the literature, that regenerating liver tissue takes up labelled amino acids faster than does normal liver tissue (Table 3).

Since we found, in our earlier experiments [6], that products of hydrolysis of ribonucleic acid had an activating effect on incorporation of labelled amino acids into the proteins of normal liver slices, and that this effect did not apply to tumor tissue, we thought it would be of interest to repeat this experiment with slices of regenerating liver at various times after partial hepatectomy.

Slices of regenerating liver, weighing 150-200 mg, were immersed in a medium containing C^{14}-glycine and 0.05% of ribonucleic acid hydrolysis products.

The results of this experiment (Table 3) showed that the rate of incorporation of C^{14}-glycine into proteins of tissue slices taken on the second day after partial hepatectomy was unaffected by ribonucleic acid hydrolysis products.

TABLE 2

Incorporation of C^{14}-glycine into the Proteins of Whole Tissue and Cell Nuclei of Rat Livers, Isolated in Acid Medium, at Various Times after Partial Hepatectomy, 3 Hours after Introduction of C^{14}-Glycine expressed as impulses per min. per g of protein : impulses per min. per g weight

Experiment	№ 20				№ 21				
Day after hepatectomy	2nd	6-th	9-th	20	3-rd	6-th	10-th	14-th	20-th
Whole tissue	5.5	3.7	3.6	2.7	4.7	4.1	3.7	2.9	2.9
Nuclei	6.2	3.7	3.6	3.9	4.6	4.2	2.7	2.7	2.7

TABLE 3

Incorporation of C^{14}-Glycine into the Proteins of Regenerating Rat Liver Slices, at Various Times after Partial Hepatectomy, and the Effect on this Process of Hydrolysis Products of Ribonucleic Acid

Days after hepatectomy / Incubation conditions	2-d	6-d	14-d
Without addition of ribonucleic acid hydrolyzate	1.17	1.07 1.07	1.1 1.1
In presence of ribonucleic acid hydrolyzate	0.89 1.14	1.33 1.67	2.7 2.9 2.5

* The slices were incubated in Robinson's medium [14] for 50 minutes at 37.8°, in an atmosphere of O_2.

A slight activating effect became apparent on the 6th day, and on the 14th day addition of RNA hydrolyzate raised the rate of incorporation of labelled amino acid by 2 – 2½ times.

It appears from our findings that the synthesizing activity of the nucleus varies at different stages of the regeneration process. Thus the rate of incorporation of labelled amino acids into nuclear proteins is lower on the 4 – 6th day after partial hepatectomy than into whole tissue proteins. It should be noted that at this stage of regeneration, characterized by undifferentiated growth, the cell nuclei to some extent resemble those of tumor cells, the proteins of which incorporate labelled amino acids 3 – 4 times more slowly than do those of whole tissue [5]. At a late stage of regeneration (15 – 20th day), the relative rate of incorporation of amino acids into cell nuclei rises. At this stage, nuclear proteins incorporate S^{35}-methionine at a rate equal to that for whole tissue proteins, and in this respect they may be said to resemble the proteins of normal liver cell nuclei.

Somewhat different results were found for incorporation of C^{14}-glycine into liver regenerate proteins, and in particular into the proteins of the cell nuclei, when these were isolated in an acid medium. In this case we

could find no difference between the rates of incorporation into whole tissue and nuclear proteins.

As was shown by our previous researches [7], when nuclei are isolated in acid media a certain amount of basic proteins, the radioactivity of which is low, is extracted into the solution, as a result of which the radioactivity of the remaining proteins rises, and approaches that of whole tissue proteins.

Our in vitro experiments confirm published reports that the rate of incorporation of labelled amino acids into the proteins of regenerating liver slices is higher than for normal liver, but lower than for tumor tissues.

According to our data, the radioactivity of the proteins of regenerating liver may be expressed in relative units as 1.1, of normal liver as 0.66, of M-1 rat sarcoma as 2.5, and of Ehrlich mouse ascites tumor as 7.8 [6].

During the first few days after partial hepatectomy addition of ribonucleic acid hydrolyzate to regenerating liver slices has no effect on the rate of incorporation of amino acids into their proteins. Much the same was found to apply to M-1 rat sarcoma slices [6]. At later stages of regeneration, addition of ribonucleic acid hydrolyzate raises the rate of incorporation of amino acids by a factor of $2 - 2\frac{1}{2}$. This finding is in accordance with our earlier data for normal liver slices [6].

Thus the results of our research are consistent with published findings [1, 3, 8, 10, 11], and point to biochemical peculiarities of two stages of regeneration. Although the differences between the rates of incorporation of labelled amino acids into nuclear and whole tissue proteins of liver regenerates of the first stage of regeneration are not great (a factor of $1\frac{1}{2} - 2$, as compared with $3 - 4$ for tumor tissue), we may venture to suggest that there is some resemblance, in this respect, between the early stages of regeneration, distinguished by undifferentiated growth, and the anaplasia of tumor tissue.

Our data also indicate that at a late stage, that of differentiation, the proteins of regenerating liver resemble those of normal liver, as regards the rate of incorporation of S^{35}-methionine.

We regard the results of the present research as affording confirmation of the assumption that the nucleus is responsible for the synthesis of the more specific proteins, which determine the normal development and differentiation of the liver [5]. It may be concluded from our experiments that on the $4 - 6$th day after partial hepatectomy the liver cell nuclei incorporate S^{35}-methionine at a rate $1.3 - 2$ times slower than do the whole tissue proteins of the regenerate. On the $15 - 20$th day after partial hepatectomy the rate of incorporation of S^{35}-methionine is the same for nuclear and whole tissue proteins. Ribonucleic acid hydrolyzates do not affect the rate of incorporation of C^{14}-glycine into the proteins of liver regenerate slices taken on the 2nd day after partial hepatectomy, but they raise the rate of inclusion into tissues taken on the 6th day, to a small extent. By the 14th day the hydrolyzates raise the rate of inclusion of C^{14}-glycine by a factor of $2 - 2\frac{1}{2}$.

SUMMARY

The rate of inclusion of S^{35}-methionine and C^{14}-glycine into the proteins of whole tissue and cellular nuclei of rats liver at various periods following partial hepatectomy was investigated. It was established that on the 4th to 6th day following partial hepatectomy the process of inclusion of S^{35} into the cellular nuclei of the liver was 1.3 to 2 times slower than in the proteins of the tissue regenerate as a whole. The rate of inclusion of S^{35}-methionine into the proteins of the nuclei and the whole tissue of regenerating liver becomes equal on the 15th-20th day. On the second day after partial hepatectomy the products of hydrolysis of ribonucleic acid have no effect on the rate of inclusion of C^{14}-glycine into the proteins of sections of liver regenerate in vitro. A certain increase of inclusion is noted on the 6th day. Hydrolyzate of ribonucleic acid enhances inclusion of C^{14} from 2 to $2\frac{1}{2}$ times on the 14th day after the operation.

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* Original Russian pagination. See C. B. Translation.

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