Pyrimidine Metabolism

IV. A COMPARISON OF NORMAL AND REGENERATING RAT LIVER*

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(Received for publication, March 11, 1959)

It has been shown that uracil can be incorporated into ribonucleic acid of the normal rat tissues (2). However, this incorporation becomes significant only when the enzymes associated with the catabolism of uracil are saturated with their substrates. When this is achieved, the incorporation of uracil into RNA increases with substrate concentration. At high concentrations the extent of incorporation of uracil, uridine, uridylic acid, and of orotic acid into RNA becomes of the same order of magnitude.

These experiments showed further that an inverse relationship exists between the capacity of a tissue to synthesize RNA and its capacity to degrade uracil. A group of normal, nondividing tissues (rat and mouse liver) has been shown to have a high catabolic capacity for uracil and a low capacity to incorporate uracil into RNA. In contrast, a group of normal but rapidly dividing tissues (intestinal mucosa, regenerating rat liver) and also a group of abnormal tissues (hepatoma (2) as well as Ehrlich ascites cells (3)) have a low capacity for the degradation of uracil, whereas they actively incorporate this pyrimidine into RNA. An interplay between the degradative and the synthetic pathways for uracil could constitute an important means for the control of RNA synthesis. From a consideration of these facts the possible existence of a cellular homeostatic mechanism involved in the regulation of RNA synthesis was proposed (2).

In order to explore further the details of such a homeostatic mechanism we have studied the changes which occur in the anabolic and catabolic capacities of enzymes involved in nucleic acid metabolism. The aim has been to contrast these capacities in normal rat liver with those occurring in rat liver during the course of regeneration induced by partial hepatectomy. Variations in the catabolic and anabolic rates could then be considered to reflect changes in the state of growth in cell types of like origin.

The results of these experiments give additional support to the contention that a homeostatic mechanism exists for the control of RNA synthesis. Evidence will also be presented which indicates that similar mechanisms may control the metabolism of thymine as well as that of thymidine 5'-phosphate and consequently exert an effect on DNA synthesis. Based on the results of this and previous work we wish to suggest that a transition from a high catabolic-low anabolic capacity to the inverse situation, in the reactions studied, may represent a necessary part of the process related to growth.

EXPERIMENTAL

Male albino rats, Sprague-Dawley x Wistar hybrids,1 180 to 200 gms., were partially hepatectomized under ether anesthesia, according to the procedure of Higgins and Anderson (4). After the hepatectomy, which was always performed between 9:00 and 11:00 a.m., the animals were returned to a normally lighted, temperature-controlled room. Purina laboratory chow and water were provided at all times. During one session a sufficient number of animals was hepatectomized to permit groups of at least three rats to be killed at 12-hour intervals after hepatectomy during a 6-day period. This procedure was repeated at another time and the data were pooled, so that each value in Fig. 1 represents the average mitotic count of six animals.

After decapitation of each animal the liver was removed immediately. A small portion of each of the two lobes was fixed in Bouin's solution for subsequent histological examination, and the bulk of the tissue was used for the enzymatic assays. The tissues saved for histological examination were embedded in paraffin, sectioned at 5 μ, and stained with hematoxylin and eosin. Mitotic activity in these rat livers at 12-hour intervals after partial hepatectomy was determined by dividing the surface of each section into high power microscopic fields (×430) and counting the number of cells in any phase of mitosis contained therein. Two hundred fields were scored for each sample of liver, and, for convenience, an average count was recorded as the number of dividing cells per 100 high power fields. The mitotic counts of six different rat livers were used to establish the mean relative mitotic activity at each 12-hour interval after partial hepatectomy.

The remaining liver was sliced free-hand and 1.5 gm. of liver slices were incubated with 0.518 μmole of uracil-2-C14 for 90 minutes at 37°, with shaking, in an air atmosphere in the presence of 5 ml. of the medium previously described (2). The results presented for the extent of uracil degradation are the average values of six samples. In each experiment duplicate control studies were performed; the values obtained were relatively constant throughout the experiment. These experiments also were repeated at other times and similar results were obtained.

The degradation of thymine-2-C14 was measured under conditions similar to those described for uracil except that each value obtained represents the average of five to six samples.

* A preliminary report of this communication has been presented (1). This investigation has been supported in part by grants from the American Cancer Society and from the National Institutes of Health, United States Public Health Service.
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‡ Predoctoral Fellow in Pharmacology, training grant CRTY-5012, United States Public Health Service.
1 Obtained from the Charles River Breeding Laboratories, Inc., Boston, Massachusetts.
obtained from two rat livers in the presence of 0.10 μmole of thymine-2-C¹⁴, and 0.5 gm. of liver slices and with a 20-minute incubation period. Mitotic index was not measured in these experiments.

The C¹⁴-labeled deoxyribonucleotides were the same as those previously described (5). The methods of assay used in these experiments also have been described (2). We are indebted to Dr. W. Prusoff for the thymine-2-C¹⁴ used in these experiments.

RESULTS

Time Relationships of Mitotic Index and of Catabolic Capacities of Regenerating Rat Liver Slices for Uracil—In Fig. 1 are plotted the variations with time obtained for the mitotic index in regenerating rat liver. These variations follow the same pattern of diurnal variation previously described (6) and show that the high mitotic index is obtained in the morning with a low level attained in the evening. This relationship remains constant regardless of the time of hepatectomy. If the partial hepatectomy had been performed at night the time-sequence would have remained the same in that the maxima and minima would have occurred at the same time of day as described above. As Fig. 1 shows, under the conditions of these experiments the first increase in the mitotic index occurred 24 hours after partial hepatectomy and most of the mitotic activity ceased after 96 hours.

The capacity of regenerating rat liver slices to degrade uracil-2-C¹⁴ decreases within 12 hours after hepatectomy and remains at a relatively constant but low level for about 96 hours. After this time it begins to increase and returns to normal within 120 hours after hepatectomy. This constant but low level of catabolic activity contrasts with the variability observed in the mitotic index.

Variation with Time in Degradative Capacity of Slices of Regenerating Rat Liver for Thymine-2-C¹⁴—Table I shows that the capacity of regenerating rat liver slices to degrade thymine-2-C¹⁴ remains equal to that of control tissue for 12 hours after hepatectomy; it is then reduced to, and remains at, a low level up to 96 hours after regeneration. This experiment was not pursued beyond 96 hours, but the same essential features were found to hold true for the degradation of thymine that have been described for the degradation of uracil.

Phosphorylation of Deoxyribonucleotides by Extracts of Normal and Regenerating Rat Liver—Extracts of normal and regenerating rat liver are able to convert the 5'-monophosphates of adenosine, guanosine, and cytidine to the corresponding triphosphates, as shown in Table II. In addition, this table shows that whereas extracts of regenerating rat liver are able to convert thymidine 5'-phosphate into the triphosphate, similar extracts prepared from normal rat liver do not have this capacity to any appreciable extent. These results are representative of a variety of methods of extraction and of various preparations.

DISCUSSION

The mitotic index shows diurnal variations with large fluctuations between the morning and the evening values. Three fairly equivalent peaks of mitotic activity were observed in this strain of rats; these occurred at 24, 48, and 72 hours after partial hepatectomy. Although the peaks of mitotic activity always appear in the morning hours, the time of appearance of the major peaks, as well as the number of such peaks, seems to vary in different strains. In the Sprague-Dawley strain of rats, two sharp maxima in mitotic activity occur: one at 48 and the other at 72 hours after partial hepatectomy (6). In the Wistar strain two maxima also occur; in this case, however, the first is observed at 24 and the second at 48 hours after partial hepatectomy (7). Interestingly enough the Slonaker strain appears to show only one major peak of mitotic activity at 24 hours (8).

The rate of uracil breakdown in the liver decreases immediately after hepatectomy, remains at a low level, and finally returns to normal after about 100 hours following the operation when most of the process of regeneration has taken place. During this period of low catabolic activity an increased synthesis of RNA occurs, as has been shown by Iecht and Rotter (9) as well as by other investigators (10–14). In a similar series of experiments, it has been shown (2) that when liver slices are incubated in a medium containing uracil, uridine, or uridine 5'-phosphate in concentrations 10-fold higher than those used in these experiments, it was possible to increase the rate of their incorporation

![Fig. 1. Variations in mitotic activity and degradative capacity of rat liver after following partial hepatectomy. Mitoses measured as number of mitoses per 100 high power fields (HPF).](http://www.jbc.org/)

TABLE I

<table>
<thead>
<tr>
<th>Time after hepatectomy</th>
<th>Per cent of control</th>
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<tr>
<td>hrs.</td>
<td></td>
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<tr>
<td>12</td>
<td>90</td>
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<tr>
<td>24</td>
<td>56</td>
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<tr>
<td>48</td>
<td>47</td>
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<tr>
<td>72</td>
<td>50</td>
</tr>
<tr>
<td>96</td>
<td>48</td>
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</tbody>
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TABLE II

<table>
<thead>
<tr>
<th>C¹⁴-substrate</th>
<th>Normal rat liver</th>
<th>Regenerating rat liver</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Monophosphate</td>
<td>Diphosphate</td>
</tr>
<tr>
<td></td>
<td>Diphosphate</td>
<td>Triphosphate</td>
</tr>
<tr>
<td>Deoxyadenyllic acid</td>
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<td>80</td>
</tr>
<tr>
<td>Deoxyguanylic acid</td>
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<td>60</td>
</tr>
<tr>
<td>Deoxycytidylic acid</td>
<td>10</td>
<td>&lt;5</td>
</tr>
<tr>
<td>Thymidylic acid</td>
<td>95</td>
<td>&lt;5</td>
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into RNA. Such high levels of uracil probably do not obtain under the conditions ordinarily encountered in vivo. However, the decrease in the capacity for uracil catabolism noted after partial hepatectomy would tend to conserve uracil and its derivatives (either preformed or from orotic acid), and thus increase their concentration in the acid-soluble pool. A concomitant result of this conservation might be the observed increase in the rate of RNA synthesis. At the present time it is not possible to evaluate the effect of the tissue uracil pool size on the relative dilution of the added radioactive uracil, but experiments along these lines are in progress.

The experiments which relate to the degradation of thymine point out a basic similarity in the metabolism of thymine and uracil. In a system in which nucleic acids must be synthesized, a prior event is the decrease in the activity of those enzymes associated with the catabolism of precursors. The capacity of regenerating rat liver to degrade thymine falls to about 40 per cent of normal within 24 hours after hepatectomy and remains low for at least 96 hours. Since the results obtained for thymine and for uracil were derived from different experiments, it would be hazardous to emphasize the relative timing for the decreases in catabolic capacities observed for these two pyrimidines. Various investigators (9, 11, 13–15) have shown that the synthesis of deoxyribonucleic acid also increases during the process of regeneration. All these reactions return to normal rates upon completion of regeneration.

Whereas the deoxyribonucleotides of cytosine, adenine, and guanine can be phosphorylated by both normal and regenerating rat liver, only the regenerating liver is able appreciably to phosphorylate thymidine 5'-phosphate. Kornberg (16) and Bessman et al. (17) have demonstrated that in an enzyme system obtained from Escherichia coli, the synthesis of DNA is greatly enhanced in the presence of a full complement of the deoxyribonucleotide triphosphates which function as precursors; this is in contrast to the very low rate of polymerization observed when even one of these precursors is lacking. Bollum and Potter (18) as well as Mantzavinos and Canellakis (19) have demonstrated a similar phenomenon with enzymes extracted from regenerating rat liver which incorporate deoxyribonucleotides into DNA. Since the substrate concentration of all deoxyribonucleotides involved in the synthesis of DNA is critical, it is apparent that the concentration or activity of an enzyme system responsible for the synthesis of a precursor for the polymerization reaction would afford a regulatory mechanism for the synthesis of DNA. As a corollary of this hypothesis it might be possible that variations in the levels of these enzymes may result in correspondingly graded capacities of a tissue to synthesize DNA. This key system appears to involve an enzymatic mechanism concerned with the conversion of the monophosphate of thymidine to the triphosphate.

In the accompanying formulation (Scheme I) we have attempted a summary of our studies as they pertain to the existence of an homeostatic mechanism related to the regulation of nucleic acid synthesis. Nondiving tissues have active catabolic pathways which would make uracil, thymine, and their phosphorylated derivatives unavailable for synthetic reactions. This plus the block at site 2 results in both a direct inhibition of the synthesis of DNA and a further indirect inhibition of the formation of RNA. Regenerating rat liver and other actively dividing cells phosphorylate TMP and in addition are blocked at site 2. This impediment would tend to channel the anabolic products of these pyrimidines toward the synthesis of the nucleic acids. From the work of Reichard and Skold (3) it appears that in the Ehrlich ascites cells and in regenerating rat liver an increased synthesis of nucleotides from uracil can be observed. Therefore, this would further accelerate the reactions along the path of synthesis of RNA and indirectly along that of DNA synthesis.

Our principal concern in these and related studies has been to evaluate the enzymatic activities under experimental conditions most closely approximating those of the intact cell under normal conditions. For this reason, when possible, whole cell preparations rather than homogenates have been utilized. This technique gives a measure of the effective enzyme levels, which may be defined as the operating level of the enzyme in the cell under the conditions of assay. The effective enzyme level may well be different from the maximal enzyme capacity, since optimal conditions, i.e., pH, substrate concentration, and so on, may not exist within the cell. In addition, cellular localization of the enzyme or side reactions which may be competing for the substrate or product would affect enzyme rates. A measure of the total enzyme content extractable from the cells by homogenization or other procedures is, when the enzymes investigated are not unduly labile, an indication of the maximal enzymatic potential of the cell and need not reflect their effective level in the cell. The interpretation of data such as these presented is fraught with uncertainties, predominant among which is the relevancy of the correlated variables to the biological phenomenon under study. We believe that measuring the effective enzyme level rather than the enzyme potential of the cell at least increases the significance of the measurements.

The high catabolic and low anabolic capacities with respect to uracil and thymine, observed in normal, nondividing tissues, contrasts sharply with the low catabolic and high anabolic capacities noted in growing tissues. This could represent a crucial transition occurring in the process of growth and malignancy.

**SUMMARY**

1. Regenerating rat liver shows a 24-hour rhythmicity in mitotic activity with the higher activity occurring in the morning. In the Sprague-Dawley x Wistar hybrid rats three major peaks of mitotic activity are apparent which occur at 24, 48, and 72 hours after partial hepatectomy.

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*Dr. O. Skold and P. Reichard, personal communications.*
72 hours after hepatectomy. These results are compared with those obtained by other authors with other strains of rats.

2. The capacity of slices of regenerating rat liver to degrade uracil and thymine is lower than that of normal rat liver.

3. Extracts of regenerating rat liver have an appreciably greater ability to phosphorylate thymidine 5'-phosphate to thymidine triphosphate as compared to extracts of normal rat liver.

4. From a consideration of these observations it is proposed that a cellular homeostatic mechanism exists which is involved in the regulation of ribonucleic and deoxyribonucleic acid synthesis.

REFERENCES


Pyrimidine Metabolism: IV. A COMPARISON OF NORMAL AND REGENERATING RAT LIVER
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