DNA Synthesis in Morris Hepatoma 9618A and in Host Liver following Partial Hepatectomy in Rats Adapted to Controlled Feeding Schedules

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INTRODUCTION

Among the many types of transplantable Morris hepatomas, hepatoma 9618A has been reported as "karyotypically least deviated" (12, 16) because of its chromosome number and morphology, which have been found to be normal (10). Nevertheless, a number of metabolic alterations are present, and several reports cover a large range of information (4, 12, 15, 16, 19). This hepatoma is responsive to many dietary manipulations and environmental changes (19) with some enzymes and not with others, while maintaining a pattern that differs from that of host liver in many parameters. Earlier studies (14) on Morris hepatoma 7793 clearly showed diurnal variation in the rate of incorporation of thymidine into DNA and a marked change in the usual diurnal variations in DNA synthesis following the stimulus (i) of the controlled feeding schedules.

SUMMARY

DNA synthesis in Morris hepatoma 9618A, as reflected by thymidine incorporation, shows diurnal variations following the stimulus (i) of the controlled feeding schedules.

Partial hepatectomy performed on the tumor-bearing rats is able to abolish, almost completely, the incorporation of thymidine into the tumor DNA during the first 27 hr after the operation. After this transient depression, DNA synthesis is much higher than in the unoperated rats at the same time of day.

The host regenerating liver shows an alteration of the normal regulatory mechanisms for DNA synthesis, insofar as there is an early rise of the rate of incorporation of thymidine into DNA and a marked change in the usual diurnal variations in the rate of DNA synthesis following the stimulus (i) of the controlled feeding schedules.

MATERIALS AND METHODS

Animals. The experiments were carried out on Morris hepatoma 9618A (generation 6) transplanted into male Buffalo rats at Howard University, Washington, D. C. The tumor-bearing rats were shipped to the McArdle Laboratory soon after they were inoculated. The rats were housed, on arrival, in an air-conditioned, windowless room with an inverted and displaced lighting schedule, in which lights were on from 8:30 p.m. to 8:30 a.m. in a 24-hr cycle. The diet contained 30% protein (18), and food was supplied just before the lights were switched off and was removed 8 hr later, according to the "8 + 16" feeding schedule developed by Potter et al. (13, 18). Water was supplied ad libitum. The rats were killed 180 to 200 days after the transplantation.

Partial Hepatectomies. Partial hepatectomies were performed under ether anesthesia, with removal of the main lobes (66 to 72% of the liver was excised) as described by Higgins and Anderson (6). The operations were performed at 8:30 p.m. ± 30 min, as indicated in Charts 1 and 2.

Estimation of the Rate of DNA Synthesis. We estimated the rate of DNA synthesis by measuring the incorporation of TdR into DNA over a 1-hr period, recognizing that this parameter may not always strictly parallel DNA synthesis. TdR-methyl-H (specific activity, 6.45 Ci/m mole), purchased from New England Nuclear, Boston, Mass., was used to evaluate the rate of DNA synthesis. Radioactive TdR was diluted with sterile 0.9% NaCl solution to an isotopic concentration of 20 μCi/ml. Each rat received an injection i.p. of 50 μCi/100 g body weight and was killed by cervical dislocation 1 hr later, at the times of day indicated in the charts. The tumors and livers were quickly removed and dropped into cold NaCl solution. Connective tissue and necrotic tumor were carefully dissected away from the viable neoplastic tissue. Samples of the liver and of the tumor were homogenized in distilled water (9:1) with a Polytron homogenizer (Kinematica GmbH, Luzern, Switzerland; distributed through Brinkmann Instruments, Westbury, N. Y.).

Perchloric acid was added to an aliquot of the homogenate to a final molarity of 0.5. After centrifugation, the supernatant was saved and the residue was washed once with...
cold 0.5 M perchloric acid. The 2nd supernatant was added to the 1st one and was labeled "acid-soluble fraction." DNA was separated from RNA, as described by Munro and Fleck (9), and was assayed by the procedure of Ceriotti (3), slightly modified (2). Radioactivity was measured on a Packard Tri-Carb liquid scintillation spectrometer.

RESULTS

The lower part of Chart 1 reports the pattern of the incorporation rate of labeled TdR into DNA of 9618A tumor following partial hepatectomy. The same chart reports also the levels of the incorporation rate of TdR into DNA of the hepatomas from 4 intact animals per group measured at 2 different times of the day, based on earlier experience with other hepatomas (5, 14). The data are expressed as percentage of total uptake (defined as the sum of the radioactivities in DNA and in acid-soluble fraction 1 hr after the injection of the labeled precursor). The data have been expressed in this way in order to minimize the range of the individual variations due to differences in TdR transport or injection techniques. From the data (Chart 1, lower part) it appears that the rate of DNA synthesis in the tumor of intact animals (closed squares) exhibited a diurnal variation, showing a low value at 9:30 a.m. and a much higher value (3 times as much) at 9:30 p.m. These data are very similar to previous findings by Potter et al. (14) with Morris hepatoma 7793. A very different pattern was observed when the rate of TdR incorporation into DNA was measured after partial hepatectomy. There was a depression of the rate of the hepatoma DNA synthesis in the 1st 27 hr after the operation, which was constantly (from 16 to 27 hr) around a very low level of about 10 to 15% of total uptake. Thereafter, sometime between 27 and 37 hr after the operation, the incorporation rate of TdR into DNA began to increase, reaching maximum values between 41 and 45 hr after the operation (46% of total uptake). At 48 hr, the rate was down again to a value of about 30%, which corresponds to the level found at the same time of day in the intact rats.

The upper part of Chart 1 reports the pattern of the uptake of TdR into the hepatomas from 4 intact animals per group measured at 2 different times of the day, based on earlier experience with other hepatomas (5, 14). The data are expressed as percentage of total uptake (defined as the sum of the radioactivities in DNA and in acid-soluble fraction 1 hr after the injection of the labeled precursor). The data have been expressed in this way in order to minimize the range of the individual variations due to differences in TdR transport or injection techniques. From the data (Chart 1, lower part) it appears that the rate of DNA synthesis in the tumor of intact animals (closed squares) exhibited a diurnal variation, showing a low value at 9:30 a.m. and a much higher value (3 times as much) at 9:30 p.m. These data are very similar to previous findings by Potter et al. (14) with Morris hepatoma 7793. A very different pattern was observed when the rate of TdR incorporation into DNA was measured after partial hepatectomy. There was a depression of the rate of the hepatoma DNA synthesis in the 1st 27 hr after the operation, which was constantly (from 16 to 27 hr) around a very low level of about 10 to 15% of total uptake. Thereafter, sometime between 27 and 37 hr after the operation, the incorporation rate of TdR into DNA began to increase, reaching maximum values between 41 and 45 hr after the operation (46% of total uptake). At 48 hr, the rate was down again to a value of about 30%, which corresponds to the level found at the same time of day in the intact rats.

Chart 2. Incorporation of labeled TdR into the host liver DNA as a function of time of day in intact tumor-bearing rats and after partial hepatectomy. A, total uptake expressed as dpm/g of liver x 10^-3. o, values for unoperated rats at 2 different times of the day, repeated in the 2nd day; •, partially hepatectomized, tumor-bearing rats; ..., the pattern obtained after partial hepatectomy from normal rats and reported in detail elsewhere (1); vertical bars, S.E. of the mean. B, percentage of label in DNA. o, unoperated tumor-bearing rats, repeated as in A; o, partially hepatectomized tumor-bearing rats; ...••••, partially hepatectomized normal rats (1). When S.E. of the mean is not given, each point represents 1 rat. 8:30, 8:30 a.m.; 20:30, 8:30 p.m.
strains. DNA radioactivity (Chart 2B) is expressed as percentage of total uptake, as in Chart 1.

The unoperated tumor-bearing rats showed a very low incorporation rate of TdR into liver DNA, similar to that of normal adult rats in which the DNA synthesis is almost completely suppressed. In the partially hepatectomized rats, the 1st measurement of the rate of TdR incorporation into DNA was performed 16 hr after the operations, and a fairly high rate was found at this early time. Thereafter, the pattern of DNA synthesis showed a quick rise, reaching very high values between 25 and 37 hr after the operation. At 48 hr, a decrease was observed, and minimum values, still very high, were reached at 51 hr after partial hepatectomy. The total uptake of labeled TdR into the host liver (Chart 14) showed a very similar pattern, with high values between 25 and 37 hr after the operation.

DISCUSSION

The results from the present experiment showed significant interaction between the tumor and the host liver and vice versa. These interactions were demonstrated as (a) a transient inhibition of the rate of TdR incorporation into the tumor DNA, followed by a rather remarkable stimulation, and (b) an alteration of TdR incorporation into DNA in the host liver.

As far as the tumor is concerned, it is very difficult, with the present data, to understand fully the mechanisms involved that are responsible for the depression of the rate of DNA synthesis observed in the 1st 27 hr after the partial hepatectomy, or to understand the following stimulation. It seems reasonable to believe that the 1st lot of dividing liver cells, being highly important, is able to obtain from the blood stream the factor(s) required for DNA synthesis and cell replication. Indications of a similar transient inhibition of nucleic acid synthesis followed by a stimulation have been already reported by Wheeler et al. (20), who studied the 5123C Morris hepatoma.

More understandable results have been obtained from the host liver. In the intact tumor-bearing rats, the rate of DNA synthesis in liver was at a very low level. Therefore, it is not possible to find any diurnal variation by measuring the incorporation rate of a labeled precursor into DNA. Nevertheless, we can point out that, in the intact rats, the presence of the tumor apparently did not affect the normal control of the liver DNA synthesis, which is almost completely suppressed, as in the normal adult animals.

On the contrary, a very different picture was obtained when the stress and the stimulus(s) of the partial hepatectomy were introduced in the tumor-bearing rats. These large qualitative and quantitative differences become more evident when directly compared with the pattern of the rate of DNA synthesis in normal rat regenerating liver (Chart 2, dotted line). It may be significant that 16 hr after the operation the incorporation rate of TdR into DNA was already at a rather high level, while in the operated nontumor-bearing rats it was still at a background level.

Very recently, it has been reported (8) that rats pretreated with growth hormone or subjected to surgical stress respond to partial hepatectomy with an accelerated DNA synthesis, while Yesh and Oliver (21) reported that glucagon is able to stimulate the DNA synthesis in liver slices. The blood levels of growth hormone and glucagon in hepatoma 9618A tumor-bearing rats is not known, but it is possible that some of the factor(s) are present in a higher concentration, and (or) the presence of the tumor can be regarded as a continuous stress.

After an early rise, a very high level of the incorporation rate of TdR into DNA is reached (about 80% of total uptake is found in DNA) and is still very high at 37 hr. On the contrary, in the normal rats, there was a 1st sharp peak of the rate of DNA synthesis 23 hr after the operation, and a 2nd high level is reached only in the next 24 hr, with very low values between the 2 peaks.

An interesting point appears by taking into consideration the percentage of DNA radioactivity with respect to total uptake. In other words, from a calculation of the total amount of labeled precursor incorporated into DNA in the host regenerating liver, it appears that much more precursor is incorporated per g of liver and that many more cells are synthesizing DNA in the tumor-bearing regenerating rat liver than in the control regenerating liver during the 1st 48 hr after the operation.

A comparison of the patterns of total uptake of TdR into the tumor (Chart 1, upper graph) and into the host liver (Chart 24) indicates that no significant variations are found in the total uptake of the labeled precursor into the tumor, while large variations (following the pattern of DNA synthesis) are found in the host liver. This finding suggests that, in the host regenerating liver as in normal rats (1), a large requirement for TdR due to a large increase of DNA synthesis is able to increase the transport of the precursor molecule from the blood stream into the liver cells. On the contrary, it appears that in the tumor this is not the case or else that the tumor has a thymidylate pool large enough to satisfy the requirement of the increased rate of DNA synthesis found in our experimental conditions.

Our findings present evidence for reciprocal interactions between the tumor and the host liver which become evident (in terms of rate of DNA synthesis) only when partial hepatectomy is performed. Whether these interactions are simply a matter of competition for very low levels of circulating thymidine or whether more subtle interactions occur remains to be determined by future experiments with the use of varied routes of administration, levels of injected thymidine, and possibly injections of various hormones.

Since these experiments were completed, there have been important new findings that will affect further work on the interaction between hepatomas and host livers. Ferdinandus et al. (4) have shown an inverse relationship between TdR degradation and TdR incorporation into DNA in both regenerating liver and in hepatomas and newborn rat livers, using low concentrations of labeled TdR. Meanwhile, Rizzo et al. (17), using both labeled TdR and labeled orotic acid, have shown a 12-hr delay in the incorporation of precursors into DNA in regenerating liver in rats that were given a single injection of hydrocortisone at 19 hr after the operation, which is normally the end of the lag phase. Further studies based on these findings, coupled with controlled feeding and lighting and an examination of the acid-soluble pool, may shed further light on the regulatory mechanisms that interact when slow- or
fast-growing hepatomas are studied in rats bearing regenerating livers. In a previous report, Ono (11) commented that Morris hepatoma 7316A delayed DNA synthesis in regenerating host liver by about 24 hr and also lowered the maximum rate, while events in the hepatoma were unaffected. However, when Morris hepatoma 5123tc was used, the maximum rate in regenerating host liver was suppressed, but no delay was observed, and DNA synthesis in the hepatoma was enhanced. Wheeler et al. (20) studied formate-$^{14}$C incorporation into acid-soluble compounds and RNA and DNA purines in host liver and in Morris hepatoma 5123C (a different line) in rats that were partially hepatectomized, compared with sham-operated animals. They found that sham operation had very little effect on the hepatomas, but partial hepatectomy inhibited incorporation of formate into hepatoma DNA adenine by 89% at 16 hr and by 77% at 24 hr, while causing a 74% stimulation at 48 hr. These results are surprisingly similar to the findings in Chart 1, considering the differences in protocol. It appears that results will depend on the individual hepatoma line but that, before different lines are surveyed, more attention will have to be given to the development of a satisfactory protocol, in which controlled feeding schedules and measured food intake may need to be included.

REFERENCES

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