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Radiosensitivity of Various Tissues of the Rat with Special Regard to Deoxycytidine-2-^{14}C Metabolism in Vitro

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Deoxycytidine-2-^{14}C의 代謝면으로 본 흰쥐의 여러 組織의 放射線感受性

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摘 要

흰쥐에 400R의 X線을 一時全身照射하여 肝, 脾臟 및 胸腺組織에 있어서의 DNA合成, DNA分解, CdR-aminohydrolase의 活性 및 酸素消費量에 미치는 放射線의 影響을 이들 組織의 放射線感受性과 關連시켜 比較檢討하였고 特히 放射線에 의한 CdR-2-^{14}C의 吸收率의 減少와 CdR-aminohydrolase 活性의 增加와의 關係에 대하여 考察하였다.

放射線에 의한 組織의 變化는 放射線의 作用期와 組織의 再生期에 따라서 判異하게 나타난다. 作用期에 있어서는 DNA 前驅物質의 吸收率, 組織內 DNA의 含量 및 酸素消費量은 顯著한 減少를 나타내나 CdR-aminohydrolase의 活性은 오히려 增加한다. 再生期에 있어서는 먼저 CdR-aminohydrolase의 活性의 回復이 앞서 일어나고, 뒤이어 CdR-2-^{14}C의 吸收率과 DNA含量에 回復이 일어나며 다시 그 뒤를 이어 酸素消費量에 回復이 일어난다.

이러한 여러가지 變化는 定性的인 面에서는 세組織에서 거의 같은 樣相을 나타내는 것으로 추정되나, 定量的인 面에서는 組織의 放射線感受性의 差異, 즉 細胞의 死滅, 生殘細胞內에 있어서의 DNA의 合成이 遲延되는 期間의 長短 및 再生率등에 따라서 다르다.

아울러 放射線照射에 의한 DNA의 合成 및 分解에 따르는 生化學的인 機作을 CdR-aminohydrolase의 活性增加의 觀點에서 考察하였다.

INTRODUCTION

Several types of deoxyribosyl compounds have been found in the acid soluble fraction of normal and malignant tissues (Potter et al. 1955, Schneider 1955, 1957, Schneider et al. 1957, Potter et al. 1957, LePage 1957, Sugino 1957). Studies on the isolation of the deoxyribosyl compounds of rat liver and blood indicated that deoxycytidine (CdR) accounted for almost the entire microbiological activity of the tissue extracts (Schneider 1955, 1957). Rotherham and Schneider(1958) investigated the concentration of deoxynucleoside, deoxynucleoside monophosphate,

and digestible deoxynucleotides in tissues of number of normal and malignant tissues of the rat and mouse, and found that the levels of these nucleotides and digestible nucleotides are higher in malignant than in normal tissue, relative to the concentration of nucleosides. Rotherham and Schneider (1958, 1960) have demonstrated that deoxyribosides are present in rat and mouse tissues and, indeed, that CdR is the major component found circulating in the blood with an apparent threshold.

Parizek et al. (1958) reported an increased excretion of Dische-positive material in rat urine the first day after irradiation, with a linear dose-response in the range of 10 to 600R. CdR was identified as the major component of this radiation-induced elevation of urinary deoxyribosyl compounds). Subsequent reports, although limited in most instances by the use of indirect methods for CdR, have demonstrated: (a) a confirmation of the increased urinary excretion of Dische-positive substances and/or CdR after ionizing radiation (Parizek et al. 1958, Berry et al. 1963); (b) that this phenomenon, in general, occurs within 24 to 36 hours after irradiation in many mammalian species; and (c) that there appears to be some indication of a dose response relationship at both sublethal and lethal doses (Parizek et al. 1958).

The significance of these observations, however, has not been fully explored or adequately explained. The occurrence of increased quantities of CdR in response to irradiation has implications both in relation to its use as a biochemical indicator of radiation exposure, and also in investigating the mechanisms involved in radiation injury. At present, the specific mechanism underlying the increased excretion of CdR after irradiation is not known and is beyond speculations that this represents an intermediary product of DNA catabolism and/or an interference in DNA synthesis.

The metabolism of CdR-2-^{14}C by hepatoma cells was studied by Schneider and Rotherham (1961), showing that hepatoma cells, in addition to incorporating the nucleoside into DNA, convert most of the added CdR to deoxyuridine (UdR). The activity of CdR-aminohydrolase, which deaminates CdR to UdR in the liver of various species including man was investigated by Zicha and Buric (1969). An enormously high activity was found in human liver and the lowest activity was found in rat and pig livers. This finding indicated that there is obviously a different transformation or different degree of this transformation between rats and other animals, and that the suitability of deoxycytidinuria as a biochemical indicator of postirradiation damage in animal of high CdR-aminohydrolase activity remains an open question.

In the present study, the rate of incorporation of CdR-2-^{14}C into DNA, levels of tissue DNA, specific activity of CdR-aminohydrolase, together with oxygen uptake in the liver, spleen and thymus at varying times postirradiation were investigated in an attempt to observe the possible origin of elevated levels of CdR in rat after sublethal total-body irradiation from the point of view of DNA metabolism, principally that of CdR-2-^{14}C, in connection with the radiosensitivity of various tissues.

MATERIALS AND METHODS

Male Sprague-Dawley rats, weighing from 96 to 146g and from 2 to 3 months old, were used in the present experiments. For each measurement 3∼7 rats were used and the data reported represent the mean ±S.D. The animals were exposed to a single whole-body irradiation of X-rays from a General Electric Maxitron 250 III therapy unit. The dose delivered was 400R and the radiation factors were: 230 Kvp, 10ma, Th II filter, approximately 17 R/min of dose rate at a distance of 50 cm.

The animals were sacrificed by exsanguination while anesthetized with ether at various time intervals following irradiation. The liver, spleen and thymus were excised, cleaned either in ice-cold Krebs-Ringer phosphate buffer pH 7.4 or in tris buffer pH 7.6 and homogenized in a glass tissue grinder or in a Teflon homogenizer.

In the incorporation experiment of CdR into DNA the tissue homogenates were incubated with 0.1 μCi CdR-2-^{14}C in 10 mM glucose-Krebs-Ringer phosphate buffer pH 7.4 in a Warburg flask at 37°C for 2 hours. To the incubation mixture was added 1.0 ml of ice-cold 0.5N perchloric acid to stop the reaction and allowed to extract at 4°C for 30 minutes, then centrifuged using International Portable Refrigerated Centrifuge Model PR-2 (at 2,000 rpm at 4°C for 15 minutes: subsequent centrifugation was done in a similar manner). The residue was recovered and washed with absolute ethanol containing 0.2N potassium acetate and then centrifuged. The residue thus obtained was again extracted with 1 ml of ethanol-ether (3:1) mixture at 50°C for 30 minutes and centrifuged. The lipid-free residue was resuspended in 1.0 ml of 0.1N potassium hydroxide and incubated at 37°C for 18 hours. To the solubilized cell material was added 0.034 ml of 6N hydrochloric acid and 1.0 ml of 0.5N perchloric acid, and allowed to precipitate to form at 4°C for 30 minutes and again centrifuged. Then the residue, which contained DNA and protein, was resuspended in 1.0 ml of 0.5N perchloric acid and heated at 90°C for 10 minutes, then cooled and centrifuged.

The DNA-containing supernatant was then subjected to radioactivity counting. For the counting of radioactivity of incorporated CdR-2-^{14}C into DNA, the supernatant was applied to a stainless steel planchet and allowed to dry under an infrared lamp. The counting was made in a TGC-14 gas-flow type carbon counter.

In the degradation experiment of DNA following irradiation, the same procedure as described for the incorporation experiment was employed, except that the tissue homogenate was incubated without CdR-2-^{14}C and the DNA quantitation was made spectrophotometrically for the DNA-containing supernatant. For the measurement of amount of DNA, diphenylamine reagent was prepared by adding 1.5g of twice recrystallized diphenylamine and 1.5 ml of concentrated sulfuric acid to 100 ml of redistilled glacial acetic acid. To 3 ml of diphenylamine reagent was added 1.5 ml of supernatant and the incubation was made at 37°C for 18 hours. At the end of incubation, absorbance at 600 nm was read in a MPS 50 L multipurpose recording spectrophotometer. Highly polymerized DNA obtained from Nutritional Biochemicals Corporations was used as the

standard.

In the CdR-aminohydrolase experiment, tissues were homogenized with about 8-fold greater volume of tris buffer pH 7.6. To 0.5 ml of homogenate, 0.1 ml of CdR solution (30 mM) was added at a final concentration of 5mM, and the system was enriched with 0.5 μCi of CdR-2-^{14}C (specific activity 42.3 mCi/mM; Département des Radioéléments, CEA, France). The system was incubated for 60 minutes at 37°C and inactivated in a boiling water bath for 2 minutes; 600 μg of UdR in 0.1 ml was added as carrier and the supernatant was separated chromatographically on Whatman No. 1 paper using a solvent system of butanol-acetic acid-water (120 : 30 : 50) for about 18 hours. The radioactive spot was cut out and transferred to a stoppered test tube, added 4 ml of distilled water and left the tube at room temperature for 3 hours. One ml each of eluate was pipetted off and counted in a liquid scintillation counter (ALOKA LSC 601) using PPO and POPOP dissolved in spectrograde benzene as liquid scintillator.

In the respiration experiment, oxygen uptake was measured for 1 ml each of homogenate incubated with 10 mM glucose by a conventional Warburg manometry in a flask having a total volume of 3.2 ml.

RESULTS

Incorporation of CdR-2-^{14}C into DNA after irradiation

Rate of incorporation of CdR-2-^{14}C into DNA of normal and X-irradiated rats at various time intervals after irradiation is shown in Table 1 and Fig. 1.

During the first 3 days after irradiation, a marked decrease in the rate of incorporation in the liver, spleen and thymus was noted as well as a sharp decrease in total weight of the spleen and thymus as revealed by the dry weight determination. Subsequent increase in the rate of incorporation occurred by day 5 in all tissues.

As is evident from Fig. 1, the rate of CdR-2-^{14}C incorporation into DNA was markedly inhibited in the thymus at days 1-3, compared to the liver and spleen, though the decreasing pattern was quite the same. The rate of increased incorpora-

Table 1. Rate of incorporation of CdR-2-^{14}C into DNA at varying times postirradiation. The change in dry weight of the spleen and thymus reveals a similar pattern, as does the rate of incorporation. The figures in the parenthesis indicate rate of incorporation as per cent of normal.

Group	Specific activity (cpm/100 mg tissue)		
	Liver	Spleen	Thymus
Control	826±129 (1.00)	9,902± 520 (1.00)	3,080±456 (1.00)
Irradiation			
1 hour	1,147±273 (1.40)	9,008± 415 (0.91)	2,615±230 (0.85)
1 day	336± 73 (0.40)	3,423± 675 (0.35)	173± 42 (0.06)
3 days	338± 79 (0.40)	4,753± 787 (0.48)	47± 12 (0.02)
5 days	1,649±327 (2.00)	14,485±1,248 (1.46)	2,010±160 (0.65)

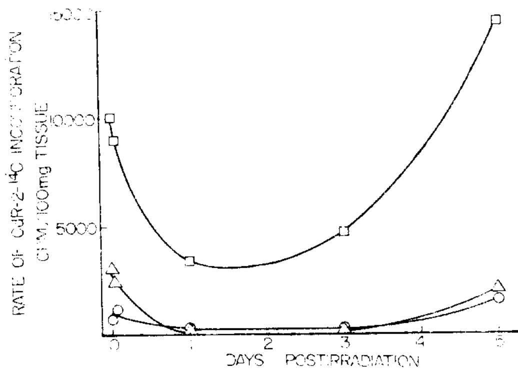

Fig. 1. Change in the rate of incorporation of CdR-2-^{14}C into DNA following irradiation. ○—○, liver; □—□, spleen; △—△, thymus.

tion at day 5 was about 1.5 times normal in case of the liver and spleen, whereas that in the thymus remained about 0.7 times normal indicating slower recovery.

Reduced precursor uptake and DNA content are thought of as functions of the dose received, the tissue studied, the time after irradiation at which the tissue is examined, and the type of the precursors used. Kelly et al. (1955) showed that in mice after 800 R, uptake into all the tissues examined was depressed 2 hours after irradiation, but by 24 hours the specific activity of intestinal DNA was back to the control level, whereas that in spleen and liver was still depressed. A comparison of the extent of the reduction at any one time in a number of different tissues is therefore misleading, and factors such as the normal mitotic rate and the proportion of highly radiosensitive cells have also to be taken into account in comparing the effects of irradiation on DNA synthesis in different tissues. As to the difference in the incorporation rate with the types of precursor, in the regenerating thymus during the first 2 days after 400 R of total-body X-irradiation, there was a sharp decrease in the rate of incorporation of the labeled TdR-^{3}H, CdR-^{3}H and ^{32}P into DNA. Thereafter, the rate of incorporation of TdR-^{3}H and ^{32}P showed a marked increase, reaching a maximum peak at day 3 1/2. At this time the increased incorporation rate of TdR-^{3}H ranged up to almost twenty times normal, whereas that of CdR-^{3}H rose to six times normal, reaching a peak at day 4 1/2 (Sugino et al. 1963).

Degradation of DNA after irradiation

Amounts of DNA in the liver, spleen, and thymus of normal and irradiated rats at various time intervals following irradiation are shown in Table 2 and Fig. 2. The DNA content is expressed in terms of mg DNA per 100 mg dry tissues and per cent of normal in each case. The DNA content in unit weight of tissues is variable from tissue to tissue, and the highest content was observed for the thymus. A marked decrease in the DNA content in radiation reaction period of first day after irradiation in the spleen and thymus was noted as well as a sharp decrease in total weight of the spleen

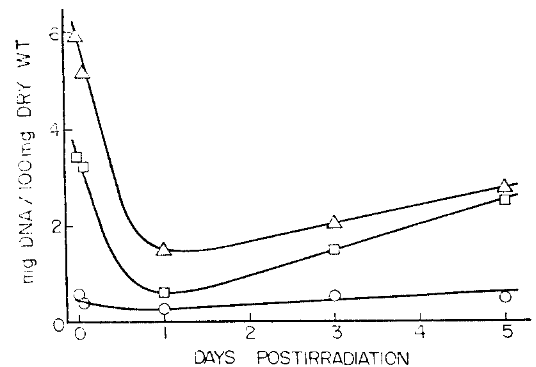

Fig. 2. Change in the amount of tissue DNA following irradiation. ○—○, liver; □—□, spleen; △—△, thymus.

Table 2. Amount of tissue DNA at varying times postirradiation. The change in dry weight of the spleen and thymus reveals a similar pattern, as does the amount of DNA if expressed in terms of concentration of DNA per unit of the gland. The figures in the parenthesis indicate DNA levels as per cent of normal.

Group	mg DNA/100 mg tissue		
	Liver	Spleen	Thymus
Control	0.55±0.06 (1.00)	3.40±0.09 (1.00)	5.90±0.08 (1.00)
Irradiation			
1 hour	0.40±0.07 (0.73)	3.25±0.08 (0.96)	5.10±0.07 (0.86)
1 day	0.30±0.07 (0.55)	0.65±0.08 (0.19)	1.50±0.08 (0.25)
3 days	0.55±0.08 (1.00)	1.50±0.07 (0.44)	2.10±0.07 (0.36)
5 days	0.55±0.06 (1.00)	2.60±0.08 (0.76)	2.85±0.09 (0.48)

and thymus as noted in the dry weight determination. The regeneration period was started from day 3 in all tissues and in case of the liver the recovery to normal levels was accomplished by day 5. This may be attributed, in part, to its marked radioresistance and to the exceedingly large regenerative capacity of the liver.

In case of the spleen and thymus the recovery was not completed by day 5, and rate of recovery of the thymus lagged somewhat behind that of the spleen. This result is in good agreement with those of Sugino et al. (1963), who showed a refinement of the methods in investigating DNA metabolism in regenerating thymus.

The evidence now available has established clearly that after irradiation there is usually a fall in the nucleic acid content of tissues, with DNA being especially affected. The present uncertainty is whether these changes are due to a primary action on the DNA-synthesizing mechanism or whether they result from cell death and/or changes in the population in the tissue studied.

Examination of Table 2 shows that decrease in DNA concentration has taken place as early as one hour after irradiation. The rate of decrease in these tissues one hour after irradiation was the most significant in the liver as low as 73 per cent of normal. At day 1, however, the rate of decrease in the spleen and thymus reached as low as about 20 per cent of normal, suggesting that any fall is likely to be due to a loss of sensitive cells from these tissues.

The activity of CdR-aminohydrolase after irradiation

The absorption spectra of CdR and UdR eluted from the radioactive spots following chromatography of reaction mixtures are shown in Fig. 3. The identity of these radioactive spots to the corresponding standards was proved by means of spectrophotometry and autoradiography.

The pattern of change in the specific activity of CdR-aminohydrolase, as expressed in terms of cpm of UdR/100mg tissues and per cent of normal, are shown in Table 3 and Fig. 4. Specific activity of CdR-aminohydrolase in normal tissues was highest in the spleen and lowest in the liver. During

Table 3. Specific activity of CdR-aminohydrolase expressed in terms of specific activity of labeled UdR converted from CdR-2-^{14}C at varying times postirradiation. The activity of UdR is obtained from the spots separated and eluted from chromatogram of 20 lambdas of supernatant of reaction mixtures. The figures in the parenthesis indicate specific activity as per cent of normal.

Group	Specific activity of UdR, cpm/100 mg tissue		
	Liver	Spleen	Thymus
Control	69± 8 (1.00)	188±20 (1.00)	100±15 (1.00)
Irradiation			
1 hour	56± 4 (0.81)	344±21 (1.83)	208±26 (2.08)
1 day	156±19 (2.26)	824±74 (4.38)	642±43 (6.24)
3 days	31± 6 (0.45)	496±24 (2.64)	398±31 (3.98)

days 0-2 postirradiation, a very sharp and marked increase in the levels of CdR-aminohydrolase was noted, reaching a maximum at the first day. The increase in the specific activity of this enzyme was 2, 4 and 6 times normal in the liver, spleen and thymus, respectively. At day 3 the levels began to return slowly towards normal levels.

A number of reports have appeared dealing with changes in enzyme activities in the liver, spleen and thymus of animals exposed to whole-body X-irradiation (Ashwell et al. 1952, Eichel 1955, Smith et al. 1957, Feinstein 1956, Weymouth 1958, van Lancker 1960, Sugino et al. 1963). Increases, decreases, or no change in these activities have been demonstrated, depending on the enzymes and the experimental conditions, but many of the reports have not evaluated the change in terms of the large decrease in the spleen and thymus size which follows irradiation (Lacassagne et al. 1958).

Smith and Low-Beer (1957), in the discussion in their paper involving the effect of

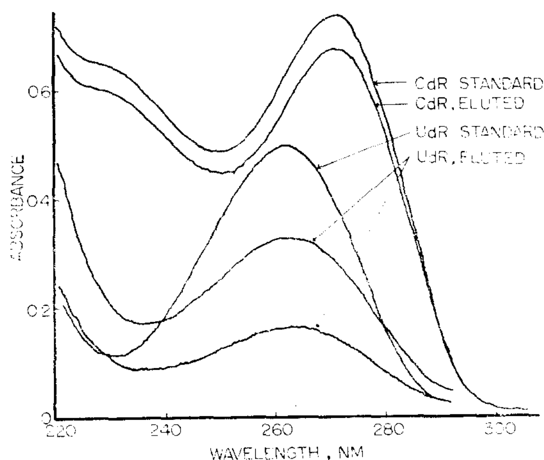

Fig. 3. Comparison of the spectra of CdR-2-^{14}C and labeled UdR, eluted from the chromatograms of reaction mixture, with those of the standard. The labeled UdR formed by the action of CdR-aminohydrolase is proved spectrophotometrically to be identical with UdR standard.

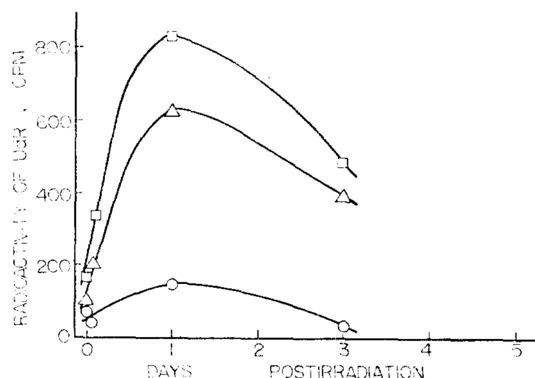

Fig. 4. Change in the activity of CdR-aminohydrolase following irradiation. ○—○, liver; □—□, spleen; △—△, thymus.

whole-body X-irradiation on some pyrimidine-metabolizing enzymes of rat tissues, grouped spleen enzymes studied by various investigators into three categories according to their response to X-irradiation. They indicated that one group of enzymes, whose total organ activity remains essentially constant regardless of splenic involution, but whose specific activity increases, could be thought of as residing in radiation-resistant cells.

One explanation (Eichel et al. 1960) of specific activity increases, in the face of a loss of tissue nitrogen, might be a selective retention of enzyme nitrogen. Alternatively the enzyme might be localized in cells which are resistant to radiation. Where 50 per cent nitrogen loss occurs, therefore, specific activity increases amounting to as much as 100 per cent cannot be interpreted as true activation. In this instance, only an increase in enzyme specific activity of greater than 100 per cent can be considered to be due, in part, to activation or synthesis. The results of the present investigation were thus taken as real increase of CdR-aminohydrolase one day after irradiation.

Since impaired energy production in the mitochondria is not likely of immediate and direct importance for the inhibition of DNA synthesis, it seems logical next to study the effect of radiation on some of the enzymes involved in the synthetic process. In the case of regenerating rat liver, usually following partial hepatectomy by surgery, it was found possible to inhibit or delay the synthesis of DNA, that normally occurs about 24 hours after the operation, providing that the animals were irradiated before maximum rate of synthesis has been attained (Cater et al. 1956, Kelly et al. 1957, Beltz et al. 1957). Analyses of the regenerating liver system have revealed that, prior to the inhibition of DNA synthesis, there is a great increase in the kinases responsible for the phosphorylation of thymidine to thymidine triphosphate (Bollum et al. 1959, Weissman et al. 1960), and also a considerable increase in the polymerizing enzyme (Bollum et al. 1959). The kinases apparently represent the rate limiting steps in the synthesis of DNA by regenerating liver. In other rapidly proliferating tissues such as spleen and thymus, however, the various kinases and polymerase occur in abundant amounts. That these enzymes are present throughout the cell cycle, and are not necessarily induced *de novo* with every round of DNA replication, has been indicated in several instances (Billen 1960). In this respect, then, the regenerating liver does not appear to be typical of rapidly dividing tissues, and the results obtained, however significant in their own right, ought not to be indiscriminately extrapolated to other cell systems.

The uptake of oxygen after irradiation

Uptake of oxygen at varying times post-irradiation is shown in Table 4 and Fig. 5. Oxygen consumed per unit weight of tissues was highest in the thymus and lowest in the liver. Although the difference between these two tissues exists, the rate of oxygen uptake takes a similar pattern of change, exhibiting the maximum decrease at day 3 postirradiation, and the degree of decrease was most remarkable in the case of the thymus.

The oxygen uptake, however, is a function

Table 4. Uptake of oxygen at varying times postirradiation. The change in dry weight of the spleen and thymus reveals a similar pattern, as does the change in oxygen consumed. The figures in the parenthesis indicate uptake of oxygen as per cent of normal.

Group	μl Oxygen consumed/mg tissue/hr		
	Liver	Spleen	Thymus
Control	1.03±0.07 (1.00)	1.13±0.03 (1.00)	1.65±0.08 (1.00)
Irradiation			
1 hour	0.84±0.06 (0.82)	1.01±0.05 (0.89)	0.85±0.04 (0.51)
1 day	0.62±0.02 (0.60)	0.43±0.04 (0.38)	0.12±0.03 (0.07)
3 days	0.58±0.03 (0.56)	0.32±0.03 (0.28)	0.12±0.03 (0.07)
5 days	0.83±0.05 (0.80)	0.56±0.05 (0.50)	0.37±0.04 (0.22)

of a large number of oxidations, any one of which may be damaged without alteration in the oxygen uptake unless this oxygen uptake is oriented toward the oxidative reaction catalyzed by the altered enzyme. It was early suggested that impairment of the energy-production in the cell may be responsible for the inhibition of both DNA synthesis and mitosis following irradiation. Irradiation of rats *in vivo* was found to reduce the capacity of mitochondria of the spleen cells to carry out oxidative phosphorylation, which is responsible for the bulk of the ATP production (Potter et al. 1952, Maxwell et al. 1952). However, these effects were not immediate but were delayed several hours, in contrast to the interruption of mitosis and, in many cases, also the decrease in the DNA synthesis. The effect of X-rays, given in a single dose to rats by whole-body irradiation at doses varying from 100 to 900 R, on the respiration, glycolysis, and rate of oxidation of a number of substrates was studied by Barron(1946). The measurements were performed on tissue slices of spleen, liver, kidney and others. The respiration of most of these tissues is diminished soon after irradiation. Inhibition of the oxygen uptake is increased when measurements are made in the presence of pyruvate, succinate, D-amino acid and so forth.

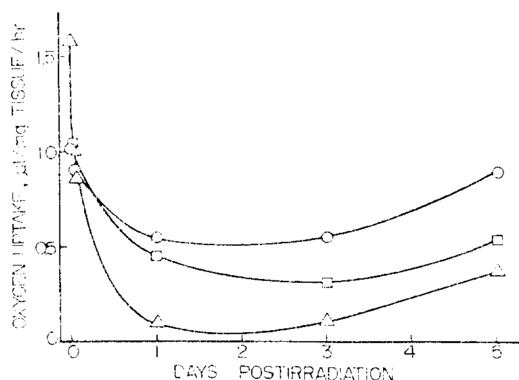

Fig. 5. Change in the uptake of oxygen following irradiation. ○—○, liver; □—□, spleen; △—△, thymus.

DISCUSSION

The origin of elevated levels of CdR in rat after sublethal whole-body X-irradiation was studied from the point of view of DNA metabolism (principally that of CdR metabolism) in connection with the radiosensitivity of the liver, spleen and thymus. As shown in Tables 1, 2, 3, and 4, these events can be divided into two periods, the radia-

tion reaction period and the regeneration period. The radiation reaction period is characterized by cellular death with the resultant decrease in the weight of the spleen and thymus and a decrease in the rate of incorporation of CdR into DNA and in the DNA content in three tissues (Figs. 1 and 2).

During this same period, the rate of oxygen uptake, expressed as per cent of normal, was greatly decreased approximating 60, 30, and 10 per cent of normal in the liver, spleen and thymus, respectively. The maximum decrease was observed at day 3, which is slower in appearance compared to those in the case of incorporation and degradation experiments. This probably indicates that impaired energy production is not likely of immediate and direct importance for the inhibition of DNA synthesis. In this connection CdR-aminohyrolase activity was measured because it seemed logical to study the effect of radiation on some of the enzymes involved in the synthetic process. As is evident from Table 3, a very sharp and marked increase in the levels of CdR-aminohydrolase, expressed in terms of amount of UdR converted from CdR was observed, reaching a maximum at the first day postirradiation. This finding is of particular interest in view of the fact that many enzymes involved in the DNA synthesis were found to be decreased by irradiation with resultant decreased DNA synthesis.

During the regeneration period, a similar pattern of recovery toward normal levels was noted, with decreasing order of the liver, spleen and thymus in all events tested.

Although several studies have led to the interpretation that the elevated levels of CdR in urine as well as in blood represent either a result of an interference in DNA synthesis and/or increased DNA degradation by irradiation, the mechanisms involved in these changes have not been completely understood. The results obtained by Guri et al. (1967, 1968) indicated that the spleen is an important site in the enhanced CdR excretion. The present results indicate the contribution of the thymus as well as of spleen to the elevated levels of deoxypyrimidine, evidenced by the decreased incorporation of CdR-2-^{14}C into DNA and by the decreased DNA content in the spleen and thymus. As shown previously, the maximum decrease in these events was observed at day 1, and consistent with this explanation was the observation that the elevated levels of CdR occurred within the first 24 hours after irradiation (Guri et al. 1967) and therefore parallels the time at which the maximum loss of DNA from spleen occurs (Cole et al. 1957, Nygaard et al. 1960). A correlation between DNA degradation and CdR excretion is also suggestive by the similar temporal sequence in the urinary CdR increase and the appearance of polydeoxynucleotide in the spleen (Cole et al. 1957). As recently demonstrated, the appearance of polydeoxynucleotide represents a catabolic process in the radiation-induced loss of cellular and tissue DNA and is thought to be mediated through the increased activity of specific DNases following irradiation (Swingle et al. 1967). In an analogous manner the elevated levels of pseudouridine after irradiation has also been attributed to the degradation of soluble RNA in the spleen.

Although these evidences imply that the degradation of DNA, as a result of enhanced specific DNase activity, is a prominent factor in the elevated levels of CdR, the role of an additional impairment in DNA synthesis has to be considered. Parizek et al. (1958) postulated that the specificity of CdR as an indicator of DNA degradation was in part related to the normal deficiency of enzymatic pathways for its utilization. However, CdR has been shown in the hepatoma cells to be not only actively deaminated, possibly at the nucleotide level with the subsequent accumulation of UdR, thymidine and uracil, but to be incorporated as well in DNA (Schneider et al. 1961, Sugino et al. 1963). Further, the incorporation of CdR-2-^{14}C into DNA has been observed to be depressed in irradiated thymus and regenerating liver (van Lancker 1960, Sugino et al. 1963) and is in good agreement with the results from the present experiments. As to the possible mechanisms involved, the extensive reports on the radiation-induced damage of DNA priming ability and/or its enzymatic activation indirectly imply some inherent alteration in CdR utilization (Lehnert et al. 1963). In addition to this, the fall in tissue levels of the crucial enzymes, deoxycytidylate deaminase and thymidylate synthetase, indicates that the block in DNA synthesis

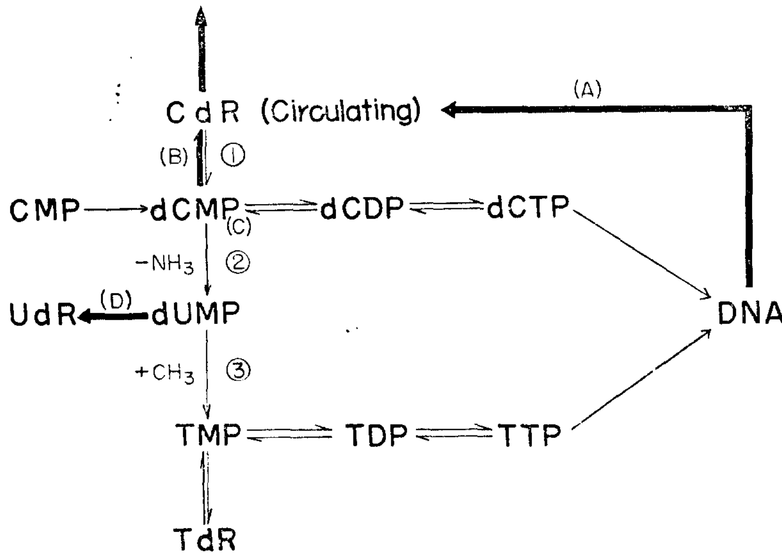

Fig. 6. Schematic diagram of some of the steps involved in the incorporation of pyrimidine derivatives into DNA and in the degradation of DNA following irradiation, in connection with the increased levels of CdR-aminohydrolase with resultant decreased CdR-2-^{14}C incorporation into DNA. Metabolic change after X-irradiation probably represents a function of increased DNA destruction (A), loss of intracellular CdR through cell death (B), and an interference in its utilization (C), which could be attributed to an inhibition of nuclear phosphorylation of nucleoside precursor (1), a decrease in activity of the enzymes dCMP deaminase (2) and thymidylate synthetase (3), and an increase in activity of CdR-aminohydrolase (D).

may reside at an early stage in the pathway of CdR incorporation into DNA (Sugino et al. 1963).

The elevated activity of CdR-aminohydrolase one day after irradiation is of special interest in the interpretation of reduced DNA synthesis by irradiation. The normal levels of CdR in the rat liver was found to be the lowest among several animals tested including man (Zicha et al. 1969). The activity of this enzyme was greatly enhanced, reaching as high as 6 and 4 times normal in the thymus and spleen, respectively.

A scheme summarizing the possible biochemical route of DNA synthesis and degradation after irradiation is suggestively given in Fig. 6. The physiological significance of enhanced activity of CdR-aminohydrolase in relation to the intermediary metabolism of the pyrimidine compounds is at present highly conjectural.

Experiments with homogenates of the hepatoma cells demonstrated that deoxycytidylate, but not CdR was rapidly deaminated (Schneider et al. 1961). The mechanism of formation of UdR was thought to be involved in initial phosphorylation followed by deamination and dephosphorylation. The increased activity of CdR-aminohydrolase results in the decreased dUMP or TMP, together with the decreased activity of dCMP deaminase and TMP synthetase, resulting decreased incorporation of TMP and/or dCMP as shown in the incorporation experiment, with resultant interference in the overall utilization of CdR in DNA synthesis. Further studies along these lines are therefore indicated.

SUMMARY

The effect of 400 R of whole-body X-irradiation on DNA synthesis, DNA degradation, CdR-aminohydrolase activity and oxygen uptake in the liver, spleen and thymus of the rat has been studied in connection with the radiosensitivity of these tissues. The rate of CdR-2-^{14}C incorporation has been followed during the postirradiation period and has been correlated with the increased levels of CdR-aminohydrolase activity during this period.

The postirradiation period comprises radiation reaction and tissue regeneration periods. During the period of radiation reaction, markedly decreased precursor incorporation, decreased tissue levels of DNA and decreased uptake of oxygen are noted as well as an increase in the CdR-aminohydrolase activity. The period of regeneration appears to consist in two discrete phases. The first phase reveals a return of CdR-aminohydrolase activity and the second phase is highlighted by a markedly increased rate of labeled CdR incorporation.

Various events occurring during the radiation reaction period and the regeneration period in the three tissues studied can be considered qualitatively the same, differing only in the degree of acute cell death, in the duration of the delay of DNA synthesis in the surviving cells, and in the rate of recovery resulting from accelerated cell replication during the period of regeneration.

A possible biochemical mechanism involved in the DNA synthesis and degradation, in connection with the increased levels of CdR-aminohydrolase after irradiation, has been briefly discussed.

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