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Correlation between Deoxycytidineuria and CdR-aminohydrolase Activity following X-Irradiation*

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X線照射에 따르는 Deoxycytidineuria와 CdR-aminohydrolase의 活性變化와의 連關性

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摘 要

생쥐에 400 및 800 R의 X線을 一時全身照射한 뒤, 몇가지 臟器에서 Dische 陽性物質의 放出量과 CdR-aminohydrolase의 活性變化 및 尿중의 CdR量을 照射후의 時間經過에 따라 측정하여 放射線照射후 deoxycytidineuria의 增加機構의 一面을 究明하려고 시도했다.

CdR-aminohydrolase의 活性度는 照射후 1時間째에 小腸과 肝에서 顯著한 감소를 보였으며, 5~12시간 사이에 정상水準으로 복귀되었다가 3日째에 最大의 증가를 나타냈다. 한편, 脾臟과 血液의 경우는 對照群과 照射群에서 모두 극미한 活性만이 관찰되었다.

Dische 陽性物質의 放出量은 小腸의 경우 照射후 3~12시간 사이에 증가를 보였으며 6~9시간에 最大値를 나타냈다. 이에 반해서 肝, 脾臟 및 腎臟의 경우는 小腸에 비해 1/20 이하에 불과하였으며, 이들 조직은 照射후 증가하는 deoxycytidineuria에 직접 寄與하지는 않는 것으로 생각된다.

Deoxycytidineuria는 照射후 9~12시간에 最大値를 보였는데, 이의 相當量이 小腸에 연유하는 것으로 믿어지고, 특히 小腸의 CdR-aminohydrolase의 活性度의 변화와 깊은 關連이 있는 것으로 생각된다. 즉 放射線照射후 DNA分解産物중 CdR이 CdR-aminohydrolase의 活性이 격감하는 시기에 조직으로부터 血液으로 放出되고, 血液내에서는 별다른 量的변화를 거치지 않고 尿에 섞여 排泄되는 것으로 思料된다.

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INTRODUCTION

During the last decade, many authors observed that a total content of DNA is markedly decreased by sublethal or lethal doses of ionizing radiation in the thymus of the rat (Sugino, Frenkel and Potter, 1963), in *E. coli* (Pollard and Achey, 1966 ; Miletic, Kucan and Novak, 1964) and in *Haemophilus influenzae* (Stuy, 1961). Not only degradation of DNA after irradiation, but inhibition of DNA synthesis at first several days after irradiation was observed in various tissues of rats (Baltz, Lancker and Potter, 1957 ; Nygaard and Potter, 1960; Sugino, Frenkel and Potter, 1963). Above observations made it clear that products induced by irradiation and endogeneous deoxyribose derivatives would accumulate abnormally in the tissues of irradiated animals. If these derivatives do not meet further degradation at the tissue level, these are excreted finally in urine as forms of free nucleosides and of others.

It was also found that deoxycytidine (CdR) is the main deoxyribose derivatives present in the rat urine (Rotherham and Schneider, 1960 ; Solle and Shejbal, 1968). Several reports have indicated that the excretion of CdR in the urine of rats is elevated by whole-body X-irradiation and is proportional to the increasing doses of ionizing radiation (Shejbal, 1970 ; Parizek, Arient, Dienstbier and Skoda, 1958; Guri, Swingle and Cole, 1967). At present mechanisms underlying the increased excretion of CdR after irradiation is not known.

Specific activity of CdR-aminohydrolase which deaminates CdR to deoxyuridine (UdR) was investigated in the livers of various species of mammals including human (Zicha and Buric, 1969). These authors strongly suggested that there exists some relationship between the level of deoxycytidineuria and the activity of CdR-aminohydrolase; exceedingly low excretion of CdR in human urine is caused by an extremely high activity of the enzyme. As to the effect of whole-body X-irradiation on the enzyme, Kang (1972) observed that specific activity of this enzyme is elevated at first day after irradiation in several tissues of the rat.

In this report time course changes of CdR-aminohydrolase activity and of Dische-positive substances liberated from the small intestine of mouse along with the CdR excretion in the urine were observed after whole-body X-irradiation for further understanding of mechanisms underlying increased deoxycytidineuria.

MATERIALS AND METHODS

Male C3Hf mice supplied from the Radiation Medical Research Institute, from 2 to 3 months old, were used in these experiments. Pyrimidine derivatives and labeled compounds were purchased from the Sigma Chemical Co. and the Département des Radioéléments, respectively.

The mice were subjected to a single whole-body exposure of X-rays from a General Electric Maxima 250 III therapy unit. The doses delivered were 400 and 800 R. Radiation factors were: 230 Kvp, 10 ma, Th II filter, approximately 13 R/min at a distance of 50 cm.

Immediately after irradiation, the whole liver, spleen, small intestine and kidneys were excised out and sectioned into about 2 cubic mm slices. The slices were washed twice with tris-HCl buffer (isotonic solution for mouse plasma, 0.335M, pH 7.6) and to this slices was added 1 ml each of buffer and incubation was made at 37°C for 3 hours interval.

At the end of incubation, the incubation mixtures were centrifuged at 3,000G for 5 minutes and the supernatant was taken in a test tube for Dische-Stumpf reaction. The remaining residue was again incubated for 3 hours after addition of fresh buffer. In the course of repeated incubation period, recovered supernatant obtained at every 3 hours interval were inactivated at 100°C for 10 minutes and were employed for determination of the amount of Dische-positive substance.

Urine collections were made from 5 mice at every 3 hours period following irradiation. Collected urines were stored at −10°C until used. Control urines were similarly obtained. Urine samples were subjected to a Dische-Stumpf reaction after adjusting to equal volume of 3 ml with distilled water. In the present experiment, a modified Dische-Stumpf method was employed; To 0.35 ml of sample was added 20μl of 0.3% cysteine solution. After thorough mixing, 5 ml of concentrated sulfuric acid was added. When the reaction was completed for 22-24 hours at room temperature, the optical density was read with a Multipurpose Recording Spectrophotometer. Optical density of CdR was determined by substracting Anonspecific from Aspecific, in order to exclude optical density of interfering substances, as described by Solle and Shejbal (1958).

Irradiated animals were sacrificed by exsanguination while anesthetized with ether at different time intervals after irradiation. The entire small intestines were immediately removed, opened longitudinally, washed three times with cold tris-HCl buffer (0.05M, pH 8.0) and homogenized in a Teflon glass homogenizer with three-fold greater volume of buffer for 2 minutes. The homogenetes were then centrifuged in a International Portable Refrigerated Centrifuge at 8,000G for 20 minutes. The supernatants were properly diluted to contain 5.0 mg protein with buffer and were adopted for an enzyme source. All the procedures otherwise noted were carried out at 4°C. The amount of protein in the supernatant was determined by spectrophotometry at 540 nm after the reaction with modified Weichselbaum's reagent using bovine serum albumin as a reference. The activity of CdR-aminohydrolase was determined by a modified method of Zicha and Buric (1959) as described in the previous paper (Kang, Rhee and Cho, 1974).

RESULTS

Fig. 1 shows the amount of Dische-positive substance liberated over the control from the small intestine at every 3 hours interval following irradiation in terms of optical density. As is evident from the figure, increased liberation was observed from 3-12 hours, reaching a maximum 6-9 hours after irradiation. However, the liberated Dische-positive substances from the liver, spleen and kidney after irradiation were found to be less than one twentieth of that from the small intestine. In view of these facts the increased CdR in the urine following irradiation might at least partly be associated with the increased liberation of Dische-positive substance from the small intestine.

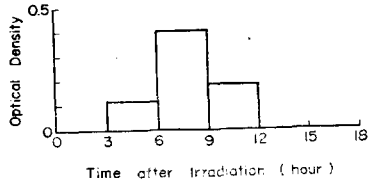

Fig. 1. Relative amount of Dische-positive substance liberated from the small intestine for every 3-hour period following X-irradiation.

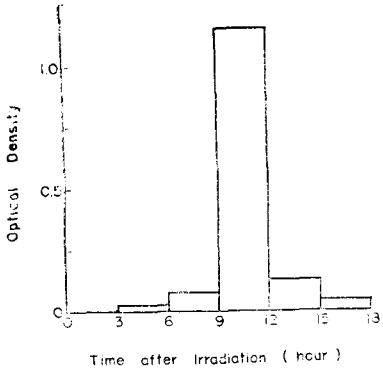

Fig. 2. Relative amount of CdR excreted in the urine for every 3-hour period following X-irradiation.

Similarly, Fig. 2 indicates the amount of Dische-positive substance over the control in the urine collected at every 3 hours interval following irradiation. It has been ascertained by Solle and Shejbal (1968) that the Dische-positive substance found in rat urine was CdR. As shown in figure 2, the amount of CdR in the urine begins to increase as early as 3 hours postirradiation, reaching a maximum from 9-12 hours, followed by a recovery to normal level henceforth.

In Fig. 3 are shown the time course changes of specific activity of CdR-aminohydrolase in the small intestine and in the liver after irradiation as expressed in terms of mμ moles of deamination products per mg protein for 30 minutes. The enzyme activity in the small intestine revealed an abrupt decline one hour after irradiation with 800 R and a return to normal level at 6 hours, followed by an elevation from 12 hours through 7 days. Especially interesting and significant finding lies in the fact that from 3-7 days postirradiation the increased enzyme activity was so great that all of the added substrate, 20 mμ moles per mg protein, was consumed off in 30 minutes incubation. In the case of liver, however, which exhibits an enzyme activity of about one fourth of the small intestine, revealed a sluggish change in enzyme activity compared to the small intestine to irradiation. A maximum enzyme activity was found to appear 3 days after irradiation, reaching as high as one and half times the normal.

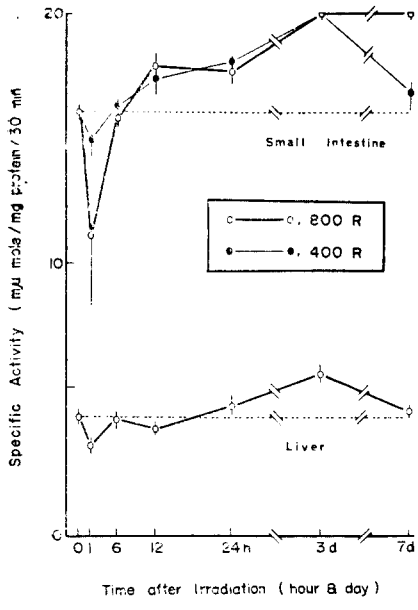

Fig. 3. Time course change of CdR-aminohydrolase activity in the small intestine and liver following X-irradiation, as expressed in terms of specific activity.

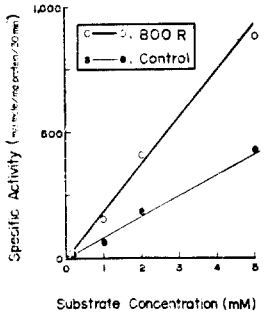

Fig. 4. The ffeect of substrate concentration on the specific activity of CdRaminohydrolase in the small intestine for the control and irradiated animals.

A similar pattern of change in the enzyme activity was observed in both animals irradiated with 400 and 800 R, except a recovery to normal level at 7 days after 400 R than after 800 R. Because the small intestine irradiated with 400 R revealed a maximum increase 3 days after irradiation, the maximum increase in enzyme activity of the small intestine, in general, might be concluded to appear 3 days after irradiation.

The CdR-aminohydrolase activities in the spleen and the whole blood of both the control and irradiated animals were not detectable with the method employed in the present experiments. This finding, however, is in good agreement with the observation of Creasey (1963) and Rothman *et al.* (1970) that there is only a trace of enzyme activity in the spleen and whole blood of mice.

In Fig. 4 a comparison is made of specific activity of CdR-aminohydrolase in the small intestines of the control and irradiated animals measured 7 days after irradiation, at various substrate concentrations ranging from 0.17-5 mM. As is evident from the figure, the specific activity of the enzyme in irradiated animal kept a linear increase of twice the control in whole ranges of substrate concentration, and a saturation was not attained even at 5 mM.

In Fig. 5 is shown the heat resistivity of CdR-aminohydrolase in the small intestines of both the control and irradiated animals following various temperature treatments for 5 minutes before incubation. The activity of the enzyme was expressed as percent of the control incubated at 37°C for 30 minutes. The inactivation temperature for both groups was found to be likely 80°C. Enzyme activity of both groups kept a fairly moderate level up to 70°C, followed by an abrupt inactivation at 80°C. This finding is suggestive of the fact that there would not occur confor-

mational change in the enzyme structure by irradiation.

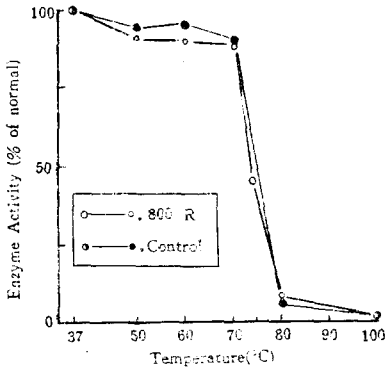

Fig. 5. The effect of temperature on the CdR-aminohydrolase activity, as expressed in terms of percent of normal which was pretreated to 30°C.

DISCUSSION

The time course changes of CdR-aminohydrolase activity and of Dische-positive substance after whole-body X-irradiation with 400 and 800 R were followed in several organs of the mice. It was reported that the marked decreases in total content of DNA and in the rate of incorporation of labeled pyrimidine nucleosides in several organs of experimental animals were detected at first 2 days after irradiation (Nygaard and Potter, 1960 ; Sugino, Frenkel and Potter, 1963 ; Kang, 1972 ; Tsubouchi and Matsuzawa, 1973). Above reports suggest that the decomposition products derived from the accelerated DNA degradation and inhibition of DNA resynthesis would undergo degradation pathways in the tissues. In connection with this line of researh, Pollard and Achey (1966) observed that *E. coli* cells heated to 70°C for 10 minutes showed no DNA degradation after irradiation. This finding strongly suggests the possibility that the DNA decomposition is not induced by a direct effect of radiation but by a participation of enzymes concerned with the degradation processes.

Cytosine derivatives, the degradation products of DNA in the small intestine of irradiated mice would further converted to other product by either deoxycytidine monophosphate (dCMP) aminohydrolase or CdR-aminohydrolase irreversibly. It was reported by Maley and Maley (1959) that the activity of dCMP-aminohydrolase in the small intestine is not so high, and that the activity in the rat thymus decreased sharply 0-2 days after irradiation with 400 R, reaching a maximum decline at second days (Sugino, Frenkel and Potter, 1963). On this account, the radiation-induced dCMP seems not to be affected by dCMP-aminohydrolase at least for two days following irradiation, resulting in degradation of dCMP to CdR which is correlated with CdR-aminohydrolase. It was previously shown that the metabolic pathway for conversion of CdR to either cytidine or cytosine is not available in the mouse small intestine (Kang, Rhee and Cho, 1974).

As shown in Fig. 3, the activity of CdR-aminohydrolase in the small intestine revealed a sharp decrease at 1 hour, followed by a moderate recovery to normal

level at 6 hours postirradiation. Thus, a decline in the enzyme activity 0-6 hours after irradiation is suggestive of the possibility that some of CdR derived from irradiation would accumulate and then released from the tissue without further degradation to other substances. Release of radiation-induced Dische-positive substances into blood stream from the small intestine occurred from 3-12 hours after irradiation, exhibiting a maximum at 6-9 hours as shown in Fig. 1.

The activity of CdR-aminohydrolase in the whole blood was not detectable in the control as well as in the irradiated animals. This fact is a strong support for the prediction that the released CdR into the blood stream would be excreted in the urine without considerable change in the amount of CdR. Guri *et al.* (1968) reported that an elevated plasma level of CdR appeared 4 hours after irradiation in the rat and also deduced that maximum deoxycytidineuria 4-8 hours after irradiation (Guri *et al.*, 1967) would be caused by a rapid filtration in the kidney. As shown in Fig. 2, the present finding that a maximum deoxycytidineuria appear from 9-12 hours postirradiation is in good agreement with the above reports. Kang (1972) observed an increased activity of CdR-aminohydrolase in several tissues of rat one day after irradiation. Quite a similar result was obtained in the present experiment; an elevated activity of the enzyme in the small intestine and the liver of mice was marked one day after irradiation. These findings are in support of the observation made by Chen *et al.* (1968) that the amount of deoxycytidineuria significantly decreased one day after irradiation.

Tsubouchi and Matsuzawa (1973) observed that the wet weight of mouse small intestine did not change appreciably after irradiation and that total number of cells decreased while the content of protein per total epithelial cells increased three folds during 2-3 days after irradiation. Therefore, the increased specific activity of CdR-aminohydrolase of the small intestine 3 days after irradiation seems to be a real increase, and it is proposed that there might be a marked increase in the enzyme activity per cell, provided that the enzyme concerned is water soluble in the cell. A presumption could be made with the results shown in Fig. 5 that the conformational change in CdR-aminohydrolase would not occur by irradiation. Lipkin *et al.* (1963) reported a decrease of the total protein synthesis in the small intestine homogenate 2-3 days after irradiation. Accordingly, the increased enzyme activity could not be explained with either the conformational change of the enzyme or the increase in the number of the enzyme.

Many studies on dCMP-aminohydrolase have revealed that this enzyme is primarily associated with embryonic tissues (Scarano, Bonaduce and Detrocellis, 1962), neoplastic tissues and regenerating rat livers (Maley and Maley, 1959). It has also been extensively studied that the enzyme performs a biosynthetic role as a major supplier of DNA thymidylate in rapidly growing tissues and in

proliferating cells (Silber, 1967 ; Scarano, Bonaduce, and Detrocellis, 1962 ; Maley and Maley, 1959, 1960, 1964). On the contrary, CdR-aminohydrolase has been proposed to be an enzyme correlated with a catabolism in differentiated tissues (Creasey, 1963 ; Kang, Rhee and Cho 1974) and in cells ceased mitotic activity (Rothman, Zanjani, Gordon and Silber, 1970 ; Silber, 1967). Camiemer and Smith (1965) demonstrated that CdR-aminohydrolase activity which is very high in adult human liver is not detectable in prenatal liver. Judging from the above facts, it could be deduced that these two enzymes play an opposite role and occur reciprocally from each other.

Maley and Maley (1960) reported that no deoxyuridine was detected when CdR-2-^{14}C was substituted for dCMP-2-^{14}C in the aminohydrolase assay for hepatoma cells of rats which showed an elavated enzyme activity for the dCMP deamination. A similar result was also obtained in the regenerating livers. It could, therefore, be proposed that a means might be developed to control activities of these two enzymes by applying proper methods. Additional evidences in support of the proposition have been reported in experiments with rat thymus following whole-body X-irradiation. During the first 2 days postirradiation, a very sharp and marked decline in the levels of dCMP-aminohydrolase was seen by Sugino *et al.*(1963), and a significant increase in the levels of CdR-aminohydrolase was observed at the same period by Kang (1972). Although sufficient information is not yet available, a control means could be developed for the inhibition of excess DNA synthesis which occurs in rapidly proliferating cells such as tumor or cancer cells.

SUMMARY

This work was undertaken to elucidate some aspects of mechanisms underlying the increased deoxycytidineuria following irradiation in the mice, by observing Dische-positive substances liberated from tissues, the activity of CdR-aminohydrolase of tissues and the CdR excreted in the urine at various times after single whole-body exposure to 400 and 800 R of X-rays.

The activity of CdR-aminohydrolase declined markedly at 1 hour in the small intestine and liver, followed by a gradual rise reaching a maximum at 3 days after irradiation. In the case of the spleen and blood, however, only a trace of activity was observed in the control and irradiated animals.

The amount of Dische-positive substance liberated from the small intestine postirradiation was elevated from 3 to 12 hours, showing a maximum during 6 to 9 hours after irradiation. On the contrary, the activity of the enzyme in the liver, spleen and kidney was less than one twentieth that of the small intestine, suggesting a prediction that these organs are not attributable to the increased

deoxycytidineuria.

A maximum deoxycytidineuria was exhibited at 9-12 hours period, attributed a large amount of CdR to the small intestine, which might correlate with the change in the CdR-aminohydrolase activity. Radiation-induced CdR seems to be liberated from the small intestine into the blood when the CdR-aminohydrolase activity declines abruptly. Then, the CdR is rapidly subjected to a filtration in the kidney without undergoing a further degradation pathway in the blood.

REFERENCES

Beltz, R.E., V. Lancker and V.R. Potter, 1957. Nucleic acid metabolism in regenerating liver: IV. The effect of X-radiation of the whole body on nucleic acid synthesis in vivo. *Cancer Research* **17** : 688—697.

Camiemer, G.W. and C.G. Smith, 1965. Studies of the enzymatic deamination of cytosine arabinoside: I. Enzyme distribution and species specificity. *Biochem. Pharmacol.* **14** : 1405.

Chen, I. W., J. G. Kereiakes, B. I. Friedman and E.L. Saenger, 1968. Colorimetric analysis of deoxycytidine in urine after separation by ion-exchange column chromatography. *Anal. Biochem.* **23** : 230—240.

Creasey, W. A., 1963. Studies on the metabolism of 5-iodo-2-deoxycytidine in vitro: Purification of nucleoside deaminase from mouse kidney. *J. Biol. Chem.* **238**(5) : 1772—1776.

Guri, C.D., K.F. Swingle and L.J. Cole, 1967. Urinary excretion of deoxycytidine in rats after X-irradiation: Dose-response and effect of age. *Intern. J. Radiat. Biol.* **12** : 355—365.

Guri, C.D., K.F. Swingle and L.J. Cole, 1968. Plasma deoxycytidine: Increased levels after X-irradiation. *Proc. Soc. Exper. Biol. Med.* **129** : 31—34.

Kang, M. S., 1972. Radiosensitivity of various tissues of the rat with special regard to deoxycytidine-2-^{14}C metabolism in vitro. *Korean J. Zool.* **15** : 1—14.

Kang, M.S., J. G. Rhee and J.M. Cho, 1974. Metabolism of pyrimidine deoxyribonucleoside and heat-resistivity of CdR-aminohydrolase in the mouse small intestine. *Korean J. Zool.* **17** : 107—116.

Lipkin, M., H. Quastler and F. Muggia, 1963. Protein synthesis in the irradiated intestine of the mouse. *Radiat. Res.* **19** : 277—285.

Maley, G.F. and F. Maley, 1959. Nucleotide interconversion in embryonic and neoplastic tissues: I. The conversion of deoxycytidylic acid to deoxyuridylic acid and thymidylic acid. *J. Biol. Chem.* **234** : 2975—2980.

Maley, F. and G.F. Maley, 1960. Nucleotide interconversion: II. Elevation of deoxytidylate deaminase and thymidylate synthetase in regenerating rat liver. *J. Biol. Chem.* **235** (10) : 2968—2970.

Maley, G.F. and F. Maley, 1964. The purification and properties of deoxycytidylate deaminase from chick embryo extracts. *J. Biol. Chem.* **239**(4) : 1168—1176.

Miletic, B., Z. Kucan and D. Novak, 1964. Effect of repeated X-irradiation on the process of degradation of deoxyribonucleic acid. *Nature* **202** : 106—107.

Nygaard, O.F. and R.L. Potter, 1960. Effect of X-radiation on DNA metabolism in various tissues of the rat: II. Recovery after sublethal doses of irradiation. *Radiat. Res.* **12** : 120—130.

Parizek, J., M. Arient, Z. Dienstbier and J. Skoda, 1958. Deoxycytidine in urine as an indicator of changes after irradiation. *Nature, Lond.* **182** : 1721—1722.

Pollard, E.C. and P.M. Achey, 1966. The action of ionizing radiation on postirradiation synthesis and degradation of DNA in *Escherichia coli* 15T-L. *Radiat. Res.* **27** : 419—433.

Rotherham, S. and W.C. Schneider, 1960. Deoxycytidine, deoxyuridine and 5-methyldeoxycytidine in rat urine. *Biochem. Biophys. Acta* **41** : 344—345.

Rothman, L.K., E.D. Zanjani, A.S. Gordon and R. Silber, 1970. Nucleoside deaminase: An enzymatic marker for stress erythropoiesis in the mouse. *J. Clinical Invest.* **49** : 2051—2027.

Scarano E., L. Bonaduce and B.D. Detrocellis, 1962. The enzymatic aminohydrolysis of 4-aminopyrimidine deoxyribonucleotides: III. Purification and properties of 2-deoxyribosyl 4-aminopyrimidone-2, 5′-phosphate aminohydrolase from monkey liver. *J. Biol. Chem.* **237**(12) : 3742—3751.

Shejbal, J., 1970. Microbiological determination of deoxyribose derivatives in urine. *Ann. 1st Super. Sanita.* **6** : 106—112.

Shejbal, J., 1970. Metabolism and postirradiation excretion of deoxycytidine. *Ann. 1st Super. Sanita.* **6** : 46—61.

Silber, R., 1967. Regulatory mechanisms in the human leukocyte: I. The feedback control of deoxycytidylate deaminase. *Blood* **29**(6) : 896—905.

Solle, M. and J. Shejbal, 1968. Determination of deoxyribonucleosides in urine: Suitability of the Dische-Stumpf reaction. *Biochem. Med.* **2** : 164—173.

Stuy, J.H., 1961. Studies on the radiation inactivation of microorganisms. VII. Nature of the X-ray-induced breakdown of deoxyribonucleic acid in *Haemophilus influenzae*. *Radiat Res.* **14** : 56—65.

Sugino, Y., E.P. Frenkel and R.L. Potter, 1963. Effect of X-radiation on DNA metabolism in various tissues of the rat: V. DNA metabolism in regenerating thymus. *Ratiat. Res.* **19** : 682—700.

Tsubouchi, S. and T. Matsuzawa, 1973. Recovery of function and cell population in the intestinal epithelium of X-irradiated mice. *Radiat. Res.* **56** : 345—353.

Zicha, B. and L. Buric, 1969. Deoxycytidine and radiation response: Exceedingly high deoxycytidine aminohydrolase activity in human liver. *Science* **63** : 191—192.