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CELLULAR DESTRUCTION AND PROTEIN BREAKDOWN INDUCED BY EXPOSURE TO X-RAYS

II. FURTHER STUDIES USING THE CONCEPT OF THE DYNAMIC GLYCINE POOL*

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The first paper of this series[1] was concerned with an assessment of tissue breakdown subsequent to exposure to X-irradiation by means of measuring the extent of dilution of ^{14}C-activity in the "free glycine fraction"[1] of rats given glycine-2-^{14}C after exposure to X-rays. The present communication deals with another approach to the problem of tissue breakdown, using again the concept of the dynamic glycine pool as defined by ARNSTEIN AND NEUBERGER[2]. In the present study, however, the rate of release of radioactive glycine into this pool is used as a measure of breakdown of proteins labeled extensively with glycine-2-^{14}C prior to exposure of rats to X-rays. In adopting the release of ^{14}C-labeled amino acid residues from tissue proteins as an index of radiation damage to tissue it is assumed that tissue proteins liberated from cells destroyed by ionizing radiation are rapidly catabolized by intracellular proteases or other catabolic reactions. In this way, increased amounts of ^{14}C-glycine and of other ^{14}C-labeled glycine precursors should appear in the "free glycine pool" assessible by means of the isotope concentration in the glycine moiety of urinary hippuric acid. Furthermore, it is assumed that ^{14}C-labeled glycine originating from tissue proteins is the only major source contributing ^{14}C-activity to glycine in urinary hippuric acid. This assumption appears justified in view of the fact that at the time of irradiation,

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i.e., eight hours after the last injection of glycine-2-^{14}C, other potential contributors of radioactivity to urinary hippuric acid, such as the "free glycine fraction" present at the time of irradiation[3] or glutathione[4] can be expected to contain only negligible amounts of ^{14}C. Moreover, the glycine moiety of hippuric acid is not likely to be derived from glutathione[4]. In addition, glycine was isolated from some of the tissue proteins in order to permit a comparison of the specific ^{14}C-activity of tissue glycine with that of hippuric acid as well as to obtain information about radiation-induced changes in total glycine-^{14}C activity in the tissues studied. An attempt will be made to evaluate in the light of the data to be presented the known changes in protein nitrogen partitioning in general and of protein content of the liver in particular[5]. Possible radiation-induced alterations in the metabolism of glycine will also be considered.

EXPERIMENTAL

Design of the isotope experiment

Eight daily doses of 2 ml of glycine-2-^{14}C containing 337 μg of glycine and 337,280 counts per minute (c.p.m.) were injected intraperitoneally into each of ten Sprague-Dawley rats (189 $\pm$ 0.5 g in weight), which had access to food *ad libitum*. Eight hours after the last injection of glycine-2-^{14}C, six rats were exposed to 756 r of 250 kV X-rays and four rats were given a sham exposure. The radiation factors have been described elsewhere[6]. Immediately after the exposure or sham exposure, all ten rats were injected intraperitoneally with 10 mg of benzoic acid in 10 ml of 0.9% NaCl and then were placed in individual confining cages for the collection of urine. After 24 hours another intraperitoneal injection of 10 mg of benzoic acid in 10 ml of 0.9% NaCl was given and the collection of urine was resumed for another 24 hours. The animals were starved during the entire period of urine collection and sacrificed at the end of this period. Various organs were removed, frozen quickly in liquid air and lyophilized.

Chemical procedures

Hippuric acid was determined according to previously published paper chromatographic procedures[7,8]. The radioactivity of hippuric acid was determined directly on the paper strip which had been placed under a thin-window Geiger-Müller counter. The combined "free" and "bound"* glycine was isolated from the lyophilized tissues as 2,4-dinitrophenylglycine (DNP-glycine) and this derivative of glycine was purified by chromatography on buffered celite[9,10]. As a rule two or three chromatographic runs were necessary to achieve radiochemical purity. The chemical purity of DNP-glycine so isolated was ascertained further by paper chromatography in *n*-butanol/acetic acid/water, 4:1:1 (v/v), and *n*-amyl alcohol/pyridine/water, 95:35:30 (v/v). The radioactivity of DNP-glycine was determined finally by placing an "infinitely thin" layer of the compound on planchets 2 cm^2 in area and counting the activity with a thin window Geiger-Müller tube. The counts obtained on paper strips have been corrected so as to make them directly comparable with those obtained on planchets.

* "Free" glycine is defined here as that fraction of the total body glycine which exists in the body in the dynamic steady state and which, therefore, is available for conjugation with benzoic acid to form hippuric acid. "Bound" glycine is that fraction of the total body glycine which has been incorporated into protein, glutathione, etc. and which is not immediately available for hippuric acid formation.

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RESULTS AND DISCUSSION

As is evident from the data presented in Table I, there is a significant increase in the isotope concentration of hippuric acid excreted by the rats exposed to X-rays but no concomitant net increase in hippuric acid excretion. This increase in the isotope concentration of hippuric acid noted even during the first period of urine collection (0–6 hours) was most marked during the second period of urine collection (6–24 hours). The average total ^{14}C-activity excreted as hippuric acid during the 48-hour period was 8,900 c.p.m. and 13,341 c.p.m. for control and irradiated rats, respectively.

TABLE I

EXCRETION OF N-BENZOYL-GLYCINE-2-^{14}C AFTER EXPOSURE TO X-RAYS

Period of urine collection hours	*Experimental group*	*Number of animals*	*Hippuric acid excreted, mg*	*Specific ^{14}C-activity* of N-benzoyl-glycine*	*Total ^{14}C-activity excreted as benzoyl-glycine, c.p.m.*
0–6	Control	4	6.30 (5.78–6.96)	1227 ± 54**	4664 ± 373**
	X-irradiated	6	6.57 (5.25–8.00)	1676 ± 42	6516 ± 1525
6–24	Control	4	0.71 (0.60–0.88)	3862 ± 1845	1412 ± 966
	X-irradiated	6	0.56 (0.37–0.60)	7023 ± 1260	2260 ± 533
24–48	Control	4	2.77 (2.40–3.00)	1692 ± 294	2824 ± 470
	X-irradiated	6	3.04 (2.21–3.40)	2570 ± 536	4565 ± 387

* Specific ^{14}C-activity = c.p.m. per mg glycine carbon atoms ($C_a + C_b$).
** ± = Standard deviation.

For reasons mentioned earlier, it seems most likely that the ^{14}C-labeled tissue proteins are the sole source of ^{14}C-glycine available—primarily in the liver—for hippuric acid synthesis. The injection of benzoic acid in amounts reported not to cause mobilization of glycine outside the "free" glycine pool[2] serves to provide this reactant in the formation of hippuric acid in a non-limiting concentration. The increased specific activity of the glycine moiety of hippuric acid observed in the animals exposed to X-rays is therefore interpreted as an indication of an increased flow of ^{14}C-glycine into the "free" glycine pool.

The experimental data presented in Tables I and II suggest that relatively labile tissue protein fractions with higher than the average ^{14}C-activities shown in Table II constitute the major sources supplying the excess ^{14}C-activity in hippuric acid excreted by the X-irradiated rats. The evidence for this lies primarily in the finding that the specific activity of the glycine moiety of hippuric acid exceeds several fold the specific activity of tissue glycine. The difference between these two glycine fractions is greater in the irradiated rats. Since the tissue proteins were not subjected

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TABLE II

^{14}C-ACTIVITY OF TISSUE GLYCINE

Organ	*Dry weight of organs*, ***		*^{14}C-activity*, ***		*Total ^{14}C-activity in "bound" glycine****	
	Control g	*X-irradiated* g	*Control* c.p.m.	*X-irradiated* c.p.m.	*Control* c.p.m.	*X-irradiated* c.p.m.
Liver	1.70 (1.60–2.04)	2.23 (2.09–2.63)	741 (724–764)	782 (733–890)	14,300	19,100
Muscle	17.0§	16.0§§	535	467	107,000§	87,500§§
Kidney	0.197 (0.192–0.202)	0.200 (0.180–0.220)	778	869	790	895
Spleen	0.085 (0.070–0.102)	0.043 (0.035–0.052)	932	817	58.5	22.1
Thymus	0.047 (0.030–0.065)	0.11 (0.006–0.013)	706	508	24.7	3.5

* The figures in parentheses represent the ranges of individual values.
** ^{14}C-activity is expressed as c.p.m. per mg of glycine carbon.
*** The glycine content of tissue proteins was obtained from various reports in the literature: liver[12], muscle and kidney[13], spleen and thymus[3].
§ Calculated on the basis of a muscle mass of 45.5 % of body weight[14].
§§ Calculated on the basis of a muscle mass of 41.5 % of body weight[15].

to fractionation prior to hydrolysis and since it is known that the turnover rates of individual tissue proteins may vary considerably, the values for ^{14}C-activity of tissue glycine (Table II) must be considered averages of a wide range of specific activities. Although a variety of metabolic processes may contribute to the "free" glycine pool, the design of these experiments precludes the possibility that contributing precursors would have ^{14}C-activities exceeding the ^{14}C-activity of "bound" glycine.

It is conceivable that the increased specific activity of hippuric acid excreted by X-irradiated rats is the result of a lower isotope dilution due to partial blocking of normal metabolic pathways from sources which either are unlabeled or have a specific ^{14}C-activity much lower than that of labeled glycine. This possibility cannot be ruled out, although an increase in the total excretion of ^{14}C-hippuric acid would not be expected if this were a major factor. On the other hand, an increase in the total ^{14}C-activity contained in the "bound" glycine fraction was observed in the liver of the irradiated rats without a concomitant change in specific activity. Since the liver is thought to be the primary, physiologically important site of hippuric acid synthesis the increase in total excretion of ^{14}C-labeled hippuric acid might well be accounted for by this finding. The most important sources of glycine known are serine[3] and two-carbon compounds such as glyoxylic acid and glycolaldehyde[11]. Both of these sources should have a lower specific activity than the "bound" glycine and, therefore, a smaller contribution from serine and other sources would result in an increased specific activity of hippuric acid. This aspect of the radiation-induced changes observed cannot be evaluated at this time, but deserves further investigation.

It is of some interest to consider the possible origin of the excess ^{14}C-activity present in the livers of the irradiated animals. Since the rise in the total activity of

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the "bound" glycine fraction is of the order of 4,800 c.p.m. calculated on the basis of a glycine content of 4.3% in liver protein[12], the loss of ^{14}C-activity by the lymphatic organs would be too small to account for the observed changes after X-irradiation. However, the overall loss of ^{14}C-activity by the muscle mass is of such an order of magnitude that the muscle might be considered as the major source of the increased ^{14}C-activity in the liver of the X-irradiated animals.

If the extent of radiation-induced tissue breakdown is viewed in terms of the measurable increase in ^{14}C-activity of hippuric acid (Table I), it may be estimated that the radiation-induced protein degradation is of the order of 200% in excess of normally occurring catabolism.

SUMMARY

1. The isotope concentration was determined in the urinary hippuric acid of starved irradiated and non-irradiated control rats which previously had been given eight daily doses of glycine-2-^{14}C. In addition, the specific activity of glycine isolated from various tissues of the same rats was determined 48 hours after irradiation.

2. The specific activity of the hippuric acid excreted by the irradiated rats was increased, reaching a maximum of almost twice the normal value during the period 6–24 hours after irradiation. This is interpreted as reflecting increased ^{14}C-activity in the "free" glycine pool after irradiation owing to the release of ^{14}C-glycine from degraded tissue proteins. The increase in protein catabolism due to X-irradiation is approximately 200% during the first two days after irradiation.

3. The specific ^{14}C-activity in the glycine moiety of hippuric acid exceeded the specific activity of tissue glycine in the irradiated as well as non-irradiated animals; however, the difference was considerably greater in the case of the irradiated rats.

4. The total ^{14}C-activity in the glycine fraction of the liver was increased and the total ^{14}C-activity in the glycine fraction of muscle was decreased in the irradiated rats. The increase in ^{14}C-activity of the irradiated livers could not be accounted for by translocation of the isotope from atrophied lymphatic tissue. The ^{14}C-activity lost from the irradiated muscle could well account for the increase in ^{14}C-activity of the irradiated liver.

5. Some of the implications of the findings are discussed.

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