INFLUENCE OF TOTAL BODY X-RAY IRRADIATION ON METHIONINE AND CHOLINE LEVELS IN RAT LIVERS

U. S. KUMTA, S. U. GURNANI AND M. B. SAHASRABUDHE

Biology Division, Dept. of Atomic Energy, Indian Cancer Research Centre, Bombay-12

ONE of the earliest events in the sequence of reactions, that result after radiation exposures, is the formation of free radicals like H, OH, HO_2, etc.[1,2] These radicals being very reactive and consequently of a very unstable nature combine with some of the essential metabolites of the system and thus bring about radiation damages like lowering of RBC and WBC counts, inducing hæmorrhages, etc., by blocking one system or another. Among the many compounds that are normally present in the metabolic pool, SH compounds appear to be more sensitive to the action of the free-radicals. Various SH containing compounds such as cysteine, BAL, thiourea, glutathione, etc., have therefore been tried therapeutically to counteract the radiation damages without much success.[3-5] Apparently the changes in the metabolic pool are of such a nature that they cannot be reversed by any of the SH containing compounds tested so far. Experiments with micro-organisms[3,6] tissues,[7] animals,[8,9] etc., have shown that these compounds do show some amount of protection if administered prior to radiation. This probably implies that the added compounds successfully compete with substances in the metabolic pool in their avidity for combination with free radicals.

Although quite a large amount of information is available about the lability of SH containing compounds to radiation exposures,[10-15] very little appears to be known about the fate of other sulphur containing metabolites in the body. Methionine which contains a $S\text{-}CH_3$ group calls for special attention for two reasons: (*i*) it is an essential amino acid, and (*ii*) it takes part in the biological transfer of methyl groups. Experiments have therefore been carried out to study the effect of total body irradiation on the levels of methionine in rat livers. Since choline is one of the first products formed as a result of transfer of methyl group, levels of choline have also been studied in irradiated animals and are reported in this communication.

EXPERIMENTAL

Wister rats, 2½-3 months old, weighing between 200-250 g., have been used in the present investigation. The animals were irradiated with X-rays using 230 KV Westinghouse machine with 1 mm. Al and 0·5 mm. cu. filters. Whole body irradiation of 600 r (L.D. 50 for rats in 30 days) was given at a rate of 50 r per minute by exposing the dorsal and ventral sides to 300 r each. Methionine levels were studied at 24 hours after irradiation whereas choline levels were determined at 24 and 48 hours after irradiation. Independent sets of experiments were carried out for studying the levels of methionine and choline. All the animals, controls as well as experimental, were fasted for 24 hours before sacrifice. Animals were killed by dislocation of the cervical vertebræ, and the livers were immediately dissected out and processed for the assay of methionine or choline as the case may be.

For the estimations of methionine, the livers were homogenised in 0·013 N acetic acid and the homogenate boiled for a couple of minutes. This procedure extracts the free amino acids and at the same time coagulates the tissue proteins, which are subsequently removed by filtration. The filtrates are further deproteinised by adding 1 ml. of 10% tungstic acid per 5 g. of tissue taken according to the method of Solomon *et al.*[16] for the estimation of free amino acids. The deproteinised filtrate is adjusted to pH 6·8 and aliquots taken for the microbiological assay of methionine using the organism *Leuconostoc mesenteroides* P-60.[17]

For the assay of choline the micromethod of Erickson *et al.*[18] was followed. The liver tissue is extracted with $CH_3OH \cdot CHCl_3$ (1 : 1) and the extract is concentrated on a water-bath. The residue is hydrolysed with 5 ml. of saturated $Ba(OH)_2$ and then neutralised with 10% HCl and filtered. Suitable aliquots of the filtrate are treated with iodine-iodide reagent in cold to precipitate choline as its enneaiodide. The precipitate is oxidised by bromine in acetate solution and the iodate thus formed is directly titrated against standard sodium thiosulphate.

The results are given in Table I.

It will be seen from the table that methionine levels drop to 25% of the levels in control animals at 24 hours after irradiation. Choline levels on the other hand drop to 66 and 57% of the control values at 24 and 48 hours after irradiation respectively. This clearly shows that methionine containing an active

S-methyl group is very radio-labile and our unpublished results (not included here) further suggest that the cleavage is probably taking place at S-C linkages.

TABLE I

Effect of total body irradiation (600 r) *on free-methionine and choline levels in rat livers at 24 and 48 hours after irradiation*

Group	Amount of methionine µg/g. of liver	Amount of choline mg./g. of liver
Control ..	86·16 ± 5·83 (10)	9·31 ± 0·064 (4)
24 hr. after irradiation	18·75 ± 0·79 (11)	6·22 ± 0·26 (4)
48 hr. after irradiation	..	5·44 ± 0·19 (4)

Figures in paranthesis indicate the number of animals used. The standard errors have been calculated using the formula $[\Sigma d^2/n(n-1)]^{\frac{1}{2}}$.

Grateful thanks are due to Dr. V. R. Khanolkar and Dr. A. R. Gopal Ayengar for helpful suggestions and criticisms, and to Drs. Mody and Vohra of Tata Memorial Hospital and Dr. M. S. Waravdekar of Bombay Hospital for providing the facilities of irradiating the animals.

1. Weiss, J., *Trans. Faraday, Soc.*, 1947, **43**, 314.
2. Guzman Barron, E. S., *Ann. N.Y. Acad. Sci.*, 1955, **59**, 574.
3. Hollander, A. and Stapleton, G. E., *Phy. Rev.*, 1953, **33**, 77.
4. Brues, A. M. and Patt, H. M., *Ibid.*, 1953, **33**, 85.
5. Ord, M G. and Stocken, L. A., *Ibid.*, 1953, **33**, 357.
6. Burnett, W. I., Stapleton, G. E. and Morse, M. L. *Proc. Soc. Exp. Biol. Med.*, 1951, **77**, 636.
7. Straube, R. L., Patt, H. M. and Smith, D. E., *Canc. Res.*, 1950, **10**, 243.
8. Patt, H. M., Blackford, M. E. and Straube, R. L., *Proc. Soc. Exp. Biol. Med.*, 1952, **80**, 92.
9. Brues, A. M., "*Biological Antioxidants*, 1950, p. 41, Josiah Macy. Jr. Foundation.
10. Barron, E. S. C. and Flood, V., *J. Gen. Physiol.*, 1950, **33**, 229.
11. Kinsey, V. E., *J. Biol. Chem.*, 1935, **110**, 551.
12. Dale, W. M. and Davies, J. V., *Biochem. J.*, 1951, **48**, 129.
13. Barron, E. S., Dickman, G. S., Munitz, J. A. and Singer, T. P., *J. Gen. Physiol.*, 1949, **32**, 537.
14. —, *Ibid.*, 1949, **32**, 595.
15. Barron, E. S. G. and Johnson, P., *Arch. Biochem. Biophys.*, 1954, **48**, 149.
16. Soloman, J. D., Johnson, C. A., Sheffner, A. L. and Bergium, O., *J. Biol. Chem.*, 1951, **189**, 629.
17. Barton-Wright, E. C., "*Microbiological Assay of Vitamins and Amino-Acids*, 1952, London, Sir Isaac Pitman Publications.
18. Erickson, B. N., Avrin, I., Teague, M. and Williams, H. H., *J. Biol. Chem.*, 1940, **135**, 671.

THE ANTI-PROTON

THE Atomic Energy Commission and the University of California have jointly announced the discovery of a new atomic particle, the anti-proton, which may open the way to a fuller understanding of basic nuclear processes.

The anti-proton, or negative proton, is not a part of the atomic nucleus, which consists of only protons and neutrons, but is created after some event such as a high energy collision of nuclear particles. The main reason why the anti-proton was not discovered earlier is that it occurs only at high energy, and until the construction of the great "bevatron" on the University of California's campus at Berkeley it was impossible to create nuclear bombardments of sufficient energy.

The "bevatron", which was built and operated by the A.E.C. enabled protons to be accelerated to 60,000 million electron volts, at which point they were directed at a target of copper inside the "bevatron" chamber. The collision of one of the protons with a neutron of copper produced not only the original proton and neutron but also a new set of heavy particles—another proton, and an anti-proton. In the collision, a part of the bombarding proton energy is converted into mass, according to Einstein's theory.

In a vacuum the anti-proton is stable and will not disappear spontaneously, but in contact with a proton both particles immediately decay into mesons and disappear; this has led some reports to describe the anti-proton as the "annihilator of matter". To identify the anti-proton a "maze" was devised through which only anti-protons could pass.

The term "reverse matter", which has also been used in some reports, is a reference to the further speculation that elsewhere in the universe there might exist matter in which all the protons were negative and all the electrons positive—a sort of "looking glass" world. The discovery confirms earlier reports by Dr. M. Schein of Chicago University and Professor Bruno Rossi of the Massachusetts Institute of Technology, who, however, had less satisfactory evidence.