INCORPORATION OF URACIL-C^{14} INTO VARIOUS LIVER RNA FRACTIONS AND INTO NUCLEIC ACIDS OF CERTAIN ORGANS OF BURNED RABBITS

T. A. Borisova

UDC 617.166-07:/616.36 + 616.74/-008.939.633.2-073.916

Utilization of uracil-C^{14} by the tissues is more intensive in burned rabbits than in healthy animals. The level of radioactivity of high-polymer and low-polymer cytoplasmic liver RNA after burns is increased, whereas in the nuclear fraction of liver RNA it remains unchanged.

* * * *

Burns cause severe disturbances of metabolic processes. The nuclear metabolism is particularly disturbed after burns [1, 6-8, 20]. My earlier investigations [1, 8] showed that in these circumstances the RNA content in the liver and the RNA and DNA in the skeletal muscles are increased. However, the actual processes responsible for producing quantitative changes in the nucleic acids in these tissues remained unknown.

The object of the present investigation was to ascertain whether the changes in the rate of nucleic acid synthesis taking place in these organs after burns is one of the causes of the disturbances previously demonstrated.

Uracil-C^{14} was used as nucleic acid precursor. The impression was gained in the literature that synthesis of pyrimidine nucleotides in the body during RNA biosynthesis takes place mainly not as a result of the use of ready-made bases, but via the synthesis of orotic acid from amino acids and their breakdown products. Work has also been published showing that uracil is incorporated into the RNA of animal tissues [9, 12, 15, 17, 18]. Admittedly, the rate of uracil incorporation into normal tissues was much slower than the rate of incorporation of pyrimidine nucleotides and of orotic acid [11, 14]. The cytoplasmic fraction of rat liver has been shown to contain uridine phosphorylase and uridine kinase, enzymes capable of synthesizing uridine-5-phosphate from uracil via uridine [10, 19]. It has also been found both in vivo and in vitro that incorporation of labeled uracil into rat liver RNA is directly dependent on the concentration of precursor in the medium and inversely dependent on the presence of enzymes splitting uracil in that tissue [11]. The difference between the ability of rat and mouse liver and Ehrlich's ascites tumor to utilize uracil has been shown to depend on differences in the activity of enzymes participating in uracil conversions in these tissues. A much higher level of enzymes participating in synthetic processes directed toward utilization of uracil in RNA synthesis was found in extracts of ascites tumor, whereas the enzymes of uracil catabolism, present in rat and mouse liver, were absent from the tumor.

Faced with these facts, it was decided to use uracil-2-C^{14} as precursor of the nucleic acids to examine their biosynthesis after burns. Another reason for this decision was that in burns, as in the other pathological processes [14, 16, 21], the utilization of uracil is more intensive than in intact animals.

EXPERIMENTAL METHOD

Experiments were performed on rabbits. An intraperitoneal injection of 1.2 μCi uracil-2-C^{14} in 0.85% NaCl solution (specific activity 16 μCi/g) was given to 4 control animals and to 4 burned animals with signs of burn exhaustion on the 33rd day after injury. The animals were sacrificed 4 h after injection of the isotope by decapitation, and samples of liver, heart, and skeletal muscle, and blood were quickly taken. These tissues were homogenized and then treated successively with cold 5% $HClO_4$ solution to remove acid-soluble nucleotides, and with lipid solvents and dried with ether. The dry residues were applied to discs

A. V. Vishnevskii Institute of Surgery, Academy of Medical Sciences of the USSR, Moscow (Presented by Active Member of the Academy of Medical Sciences of the USSR A. A. Vishnevskii). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 65, No. 2, pp. 42-45, February, 1965. Original article submitted September 30, 1966.

TABLE 1. Incorporation of Uracil-C^{14} into Nucleic Acids of Organs of Normal and Burned Rabbits (pulses/min/mg; mean values)

Experimental conditions	Liver	Blood	Heart*	Intact skeletal muscle
Control	81±9	27±3	32±4	9±2
Burned	153±14	41±5	41±10	15±3,5

*Radioactivity determined with a 4 π gas-flow counter.

TABLE 2. Radioactivity of Different Fractions of Liver RNA from Intact and Burned Animals (pulses/min/mg; RNA; mean data)

Experimental conditions	HP-RNA	LP-RNA	Nuclear RNA
Control	32±2,0	13±1,5	38±3,2
Burned	63±5,2	96±3,5	41±2,5

Legend: HP – high-polymer; LP – low polymer.

and their radioactivity determined. Fractions of high- and low-polymer cytoplasmic RNA were obtained from the liver by phenolic fractionation [3], and total nuclear RNA was isolated by treating unpurified phenolic nuclei* in a system of phenol (pH 6.0) – 0.14 M NaCl at 60-65° [4, 5].

The high-polymer cytoplasmic and nuclear fractions were reprecipitated three times with 2.5 M NaCl to purify them completely from DNA. The radioactivity of the resulting fractions was determined by a Geiger-Mueller counter and the RNA concentration in each sample was determined spectrophotometrically after alkaline hydrolysis [13].

EXPERIMENTAL RESULTS

As Table 1 shows, incorporation of uracil-C^{14} into total nucleic acids was observed in all investigated tissues of both control and burned animals, and after burns the incorporation of uracil was intensified.

The specific activity of the various liver RNA fractions from intact and burned animals is given in Table 2. Incorporation of uracil-C^{14} into high- and low-polymer cytoplasmic fractions of liver RNA after burns took place more intensively than into the same fractions of liver RNA from intact animals. The nuclear fractions of liver RNA of the control and burned rabbits possessed the same level of radioactivity.

It may be concluded from the preliminary data thus obtained for incorporation of uracil-C^{14} into various fractions of liver RNA and also into the nucleic acids of the investigated organs of intact and burned rabbits that utilization of uracil-C^{14} by the animal tissues takes place for nucleic acid formation. In the burned animals this process takes place more intensively than in the intact animals in all organs investigated. In the liver after burns intensification of uracil-C^{14} incorporation into high- and low-polymer cytoplasmic RNA fractions is observed. The results described above suggest that burn trauma is associated with intensification of nucleic acid synthesis in the investigated tissues. This is evidently one cause of the increased content of RNA in the liver and of RNA and DNA in intact skeletal muscle discovered in my previous experiments.

LITERATURE CITED

1. T. A. Borisova, Éksper. Khir., No. 1, 43 (1968).
2. V. A. Gvozdev, Dokl. Akad. Nauk SSSR, 153, No. 3, 714 (1963).
3. G. P. Georgiev, et al., Biokhimiya, No. 3, 472 (1959).
4. G. P. Georgiev and V. L. Mant'eva, Biokhimiya, No. 1, 143 (1960).
5. G. P. Georgiev, Biokhimiya, No. 6, 1095 (1961).
6. P. D. Demidova, in the book: Problems in Radiobiology [in Russian], No. 2, Moscow (1957), p. 137.
7. P. G. Demidova, Biokhimiya, No. 1, 47 (1958).
8. T. L. Zaets and T. A. Borisova, Dokl. Akad. Nauk SSSR, 150, No. 3, 677 (1963).
9. E. S. Canellakis, Fed. Proc., 14, No. 1, 324 (1955).
10. E. S. Canellakis, J. Biol. Chem., 227, 329 (1957).
11. E. S. Canellakis, J. Biol. Chem., 227, 701 (1957).
12. A. Cantarow, T. L. Williams, and K. E. Paschkis, Fed. Proc., 15, No. 1, 30 (1956).

*The intermediate layer between the aqueous and phenolic phase obtained after treatment of the cell suspension three times with phenol (pH 6.0) – 0.14 M NaCl.

13. A. Fleck and H. N. Munro, Biochim. Biophys. Acta, 55, 571 (1962).
14. C. Heidelberger, K. C. Liebman, E. Harbers, et al., Cancer Res., 17, 399 (1957).
15. U. Lagerkvist, P. Reichard, B. Carlsson, et al., Cancer Res., 15, 164 (1955).
16. K. C. Leibman and C. Heidelberger, Fed. Proc., 14, 243 (1955).
17. A. Pileri and L. Ledoux, Biochim. Biophys. Acta, 26, 209 (1957).
18. P. Reichard, Acta Chem. Scand., 9, 1275 (1955).
19. P. Reichard and O. Sköld, Biochim. Biophys. Acta, 28, 376 (1958).
20. J. S. Roth, Am. J. Physiol., 176, 471 (1954).
21. R. J. Rutman, A. Cantarow, and K. E. Paschkis, Cancer Res., 14, 119 (1954).