Metabolism of Nucleic Acids during Regeneration of Wound Tissue*

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The formation of regenerating wound tissue involves not only production of new cells but also synthesis of relatively large amounts of protein (1–5). From these facts, it may be inferred that while this new tissue is being formed, nucleic acid metabolism is probably different from that observed in normal animals. There has been some indication that this may be the situation for ribonucleic acid during limb regeneration in amphibians (6). The problem of the formation and metabolism of both types of nucleic acid during regeneration of liver has been investigated extensively (7–13). As the liver is a more versatile and active tissue than the skin, in the metabolic sense, it might be expected that the metabolism of nucleic acids in regenerating skin tissue should be on a different level from that in regenerating liver. As yet, no basis for comparison exists, inasmuch as there appears to be no literature even on the nucleic acid content of such regenerating tissue. However, there are several reports indicating that specific nucleotides affect the formation of regenerating wound tissue (14–18).

The earliest work on nucleic acids was in connection with exudates from regenerating wound tissue (19). More recent work on such exudates still does not definitely establish whether the nucleic acids originate in the damaged cells, extraneous body tissue, leukocytes, or by synthesis in cells of the regenerating tissue (20–22).

The demonstration of great changes in the protein metabolism of injured animals as compared to normal animals (1, 2, 23, 24) points to the possibility that there also may be some change in the nucleic acid metabolism of the wounded animals. It seems quite probable that some further clue to the metabolism of the nucleic acids may be obtained from consideration of the nucleotide content of the regenerating tissue. The present report considers the metabolism of nucleic acids in regenerating wound tissue and in the nonregenerating tissues of wounded animals.

EXPERIMENTAL PROCEDURE

The following experiments were carried out on female albino rats weighing 200 ± 20 g at the start of the experiment. In every case, the animals were maintained on a protein-free diet (1) for 3 days before the beginning of the experimental period. In order to keep all the animals on the same caloric intake, they were offered 8 g of diet per day. This amount of food had been found to be completely consumed before the next daily feeding.

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Water was permitted ad libitum. This regime was used because it had been shown that the rate of tissue regeneration is greatly affected by dietary protein intake (23, 25). Not only did the animals serve as their own supply of nitrogen, but it was expected also that the formation of protein and nucleic acids would be minimal except in the regenerating wound tissue.

On the 4th day, while under Nembutal anesthesia, the rats were wounded as previously described (29), being given a standard circular wound 4 cm in diameter by excision of the skin on the back of the neck down to the loose fascia. At various intervals thereafter, several of the animals were killed and samples of liver, kidney, and regenerating tissue taken. The tissue samples were immediately homogenized in the cold. Although liver and kidney samples were readily homogenized with a glass and Teflon homogenizer, the regenerating wound tissue could be homogenized satisfactorily only by being ground repeatedly in a mortar with sand and small amounts of cold water. In several experiments, essentially the same results were obtained from the analysis of samples of the same liver or kidney when homogenized either in glass or by grinding with sand.

After withdrawal of an aliquot of the homogenates for the determination of total nitrogen (micro-Kjeldahl), the remaining material was treated with 10% trichloroacetic acid to precipitate the proteins and nucleic acids. The supernatant fluid was considered to be the “acid-soluble phosphate” fraction. The precipitate was then fractionated to separate the RNA from the DNA by a modification of the technique described by Schmidt and Thannhauser (26, 27). The phosphate content of each fraction was measured by the method of Fiske and SubbaRow (28) as modified by Leloir and Cardini (29). Analysis of the RNA fraction showed that 2.9 ± 1.0% of the total phosphate in the samples was inorganic, probably arising from hydrolysis of part of the phosphoproteins during the fractionation procedure (30).

In those instances where sufficient tissue was available, the nucleic acid content of the RNA and DNA of the homogenized tissues was also determined by measuring the pentose in these fractions. Deoxyribose was determined by a modification of the diphenylamine method of Dische (31, 32), with deoxyadenosine as the standard. Ribose was measured by the orcinol method of Mejbaum (33), with AMP as the standard. These methods have been reported to measure only the pentoses contained in purine nucleotides (34). Analysis of the nucleic acid fractions from the wound tissue showed that it contained approximately 2 moles of phosphate per mole of pentose.

After determination of the level of nucleic acid in the regenerat-
ing wound tissue, a study was undertaken to determine the relative rate of formation of the nucleic acids during different stages of regeneration. Rats which had been kept on a protein-free diet were wounded as described above. Two hours before the wounded rats were killed for the collection of tissue samples, 80 μc of disodium phosphate-P³² were administered subcutaneously to each one. After separation of the DNA and RNA fractions of the tissue samples, the phosphate content was measured and the activity of the P³² in the fractions determined by means of an end window G-M counter tube assembly.

RESULTS AND DISCUSSION

The amount of DNA contained in regenerating tissue at various intervals after excision of the skin is shown in Fig. 1. The data from three separate and consecutive experiments are included. It was found particularly difficult to obtain valid data from analysis of samples collected before the 5th day after wounding, because usually the regenerating tissue formed up to this time was insufficient for replicate measurements. The amount of DNA per milligram of nitrogen in the regenerating tissue appeared to increase for about 8 days and thereafter gradually decreased. This decrease may have been more apparent than actual, because the nitrogen content of this tissue had been shown to increase as regeneration progressed (1, 3, 20). Thus, on the basis of tissue weight, the DNA appeared to remain almost unchanged once it had reached the maximal level.

The amount of RNA per milligram of nitrogen in the regenerating tissue, shown in Fig. 2, also reached its maximum at about 8 days after wounding. At this time, there was more than twice as much RNA as DNA, on the basis of phosphorus, in the regenerating tissue. To some extent, the very great decrease in the RNA level was, as in the situation with DNA, a reflection of the increasing nitrogen content of the regenerating tissue. However, even on the basis of tissue weight, there appeared to be an appreciable decrease in the amount of RNA in the newly formed tissue after the 8th day.

The data shown in Table I were obtained from the analysis of the P³² incorporated into the nucleic acids of the regenerating tissue, 2 hours after the administration of the radiophosphorus. The incorporation of P³² into the RNA fraction indicated a rapid production of this nucleic acid during the early stages of tissue formation. Thereafter, the rate of RNA formation decreased very considerably to a much lower but relatively constant level. This reduced rate of RNA formation may be another factor which contributes to the decrease in the RNA level in the regenerating wound tissue shown in Fig. 2.

In terms of specific activity, the most rapid formation of DNA, indicative of the greatest mitotic activity and cell replication (35), appeared to occur about 8 days after wounding; within 2 weeks, the uptake of P³² by the DNA fraction became practically zero. In this way, the metabolism of the DNA of the regenerating wound tissue eventually assumed the characteristics of that in nonregenerating tissue with respect to production and turnover (13). The specific activity of the acid-soluble phosphate fraction, which contained not only nucleotides but also other organic and inorganic phosphates of low molecular weight, remained approximately unchanged throughout almost the entire experimental period. The very great increase observed at about 15 days may be connected with the fact that at this time there was relatively little RNA and essentially no DNA production, and therefore greatly reduced phosphate utilization.

It has been shown in many experiments (2, 23, 24, 35–37) that the stimulus of wounding results in very great changes in protein metabolism. To find if this stimulus also affects nucleic acid metabolism, liver and kidney tissue samples from normal and wounded animals were fractionated and the nucleic acid content measured. In all experiments, it was found that the DNA level of these tissues remained unchanged through the entire period of observation. However, the amount of RNA in these tissues appeared to fluctuate widely during the course of tissue regeneration (Table II). In the liver, the concentration of RNA began to rise rapidly and reached a peak level in about 5 to 6 days. After a precipitous decrease to a level equal to, or even below, that found in the liver of normal animals, the RNA concentration began to rise rapidly again. The second period of RNA deposi-
tion began about 10 days after the injury was made. The same situation was found to hold with regard to the level of RNA in the kidney with the exception that the first period of RNA accretion was so small that it is not statistically significant. These same changes were regularly observed in several other experiments.

The uptake of P³² by the nucleic acids of nonregenerating tissues, 2 hours after the administration of radiophosphorus, is presented in Table III. The specific activity of the P³² in the RNA and acid-soluble phosphate fractions of both the liver and kidney in the wounded rats was much below that found in the control animals. This decreased ability to take up P³² appeared to continue until after the maximal utilization of nucleotides by the regenerating tissue for the formation of nucleic acids. After this time, the rate of formation of nucleotides and RNA in the nonregenerating tissues began to return to the level found in the unwounded animals.

The inhibition of P³² uptake by the liver during the early phases of regeneration may be a partial explanation of the greater increase of RNA formation in the liver and kidney between the 10th and 15th days as compared to the 1st to 6th days after wounding (Table II). It seems possible also that the stimulus of wounding may affect the formation of nucleotides rather than the synthesis of RNA from nucleotides, since the decreased uptake of P³² by the RNA and by the acid-soluble phosphate fractions appears to follow the same pattern of change. The over-all increase in the level of RNA in the nonregenerating tissues may be presumed to be a part of the mechanism to replace the protein utilized to supply the excess sulfur amino acids required by the regenerating wound tissue proteins and the residual nitrogen to make up the negative nitrogen balance (2, 23, 24). Inasmuch as the formation of RNA is markedly decreased below normal during the period of tissue regeneration (see Table III), the increase of RNA in the nonregenerating tissues must be attributed to an inhibition in the breakdown of this nucleic acid.

Although there appears to be no significant change in the acid-soluble phosphate fraction of the kidney, this fraction increases very rapidly in the liver after wounding and then gradually begins to return to the normal level. It seems possible that these changes are related to the great increase in the production of urea in the liver since they coincide approximately with the period of excessive protein catabolism and nitrogen excretion usually observed after injury.

**SUMMARY**

During the study of nucleic acid metabolism in wounded rats, it was found that almost three times as much ribonucleic acid as deoxyribonucleic acid is deposited in the regenerating wound tissue. On the basis of nitrogen content, both nucleic acids reach a maximal concentration in about 8 days, followed by a rapid and marked decrease. This decrease appears to stem largely from the increase in nitrogen content of the regenerating tissue as regeneration progresses. From the uptake of radiophosphorus, it appears that ribonucleic acid is formed most rapidly during the very early phases of regeneration, whereas deoxyribonucleic acid is formed most rapidly at about 8 days. Shortly thereafter, deoxyribonucleic acid synthesis ceases. The accumulation of ribonucleic acid in the liver and kidney of wounded rats is inferred to be the result of an inhibition of ribonucleic acid breakdown. The course of accretion of ribonucleic acid in these tissues appears to be interrupted by a short period during which the concentration is greatly reduced followed by a return to a level even higher than normal.

**REFERENCES**

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