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Abstract

The synthesis of nuclieic acids in the liver and the lymphoid tissues of adult mice was studied during the restitution period after the 6-day starvation. The results obtained indicate that there occurs an unexpected rapid synthesis of DNA in the hepatic parenchymal cells during the restitution period without significant increase in the total amount of DNA in the liver. Most rapid DNA-synthesis in the liver appears to occur one day after refeeding. With respect to RNA in the liver as well as to both RNA and DNA in the lymphoid tissnes, on the other hand, there is a good parallelism between the rate of their synthesis and that of increase in their amounts, without apparent dissociation between both rates as seen in the liver DNA.

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NUCLEIC ACID SYNTHESIS IN THE MOUSE LIVER DURING RESTITUTION AFTER STARVATION

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A number of authors reported that starvation of an animal results in a remarkable loss of protein and ribonucleic acid from the liver (1, 3, 4, 6, 7, 13, 20). The rapid syntheses of both of the constitnents were also reported by these authors during recovery from starvation. These processes were shown to occur without significant change not only in the total cell number or in the total deoxyribonucleic acid content of the liver (2, 5, 6, 8, 12) but also in the average DNA content per cell. These findings were believed to suggest that the metabolism of liver DNA was not affected by starvation and refeeding.

In a previous experiment(14), however, a burst increase of DNA synthesis in mouse liver was found, radioautographically using tritiated thymidine during the so-called non-mitotic recovery period after starvation. The hepatic cells once labelled with tritiated thymidine were observed to persist for a considerably long period without subsequent cell division, that is, without change in the number of labelled cells.

The present study was undertaken to investigate the changes in the rate of synthesis of DNA and of RNA in liver, spleen, lymph nodes and thymus during the restitution period after starvation, by measuring the amounts of nucleic acids and the rate of the incorporation of ^{32}P into them.

MATERIALS AND METHODS

Adult mice of Db strain, weighing about 26 g, were fasted for six days, and then they were refed on the Oriental compressed diet for mice (Oriental-MF, Japan), containing 24.2% of protein. Water was given *ad libitum* throughout the experiment. At appropriate intervals in the restitution period the animals were killed in groups of ten; each mouse received 2 μC inorganic phosphate-^{32}P per gram of body weight in a single intraperitoneal injection.

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Liver, spleen, thymus and axillary and inguinal lymph nodes were removed, weighed and then homogenized in ice-chilled 5% trichloracetic acid (TCA). Each homogenate was centrifuged and the supernatant was decanted. The sediment was washed twice with cold 5% TCA, twice with 95% ethanol, and once with tris-(hydroxymethylaminoethane)-buffer, pH 7.5. Nucleic acids were extracted from protein by heating the sediment at 100°C for 20 min in 10% NaCl. The supernatant was decanted and the second extract was pooled with first one. Nucleic acids were precipitated overnight from the supernatant with 95% ethanol at room temperature. The precipitate of nucleic acids was purified by repeating twice the above extraction with NaCl. RNA was hydrolyzed by adding 0.1N-NaOH and heating at 80°C for 30 min. The samples were cooled in an icey bath, and after being neutralized with 0.3N-HCl, 25% TCA was added to each sample to give a final concentration of 5%, and then centrifuged. The supernatant containing RNA was pooled with the second extract with cold 5% TCA at 95°C for 15 min. Amounts of DNA and those of RNA were determined photometrically using diphenylamine and orcine respectively. The incorporation of ^{32}P into DNA and into RNA of each sample was counted with GM counter and the specific acivity of DNA was expressed as cpm/mg of the nucleic acids.

RESULTS

As shown in Fig. 1, the body weights of mice were reduced to less than a half of their initial values by the six-day starvation, and they recovered in ten days upon refeeding. Daily injections of ^{3}H-thymidine for these ten days resulted in a heavy labelling both of the hepatic cells and of Kupffer stellate cells as reported previously (14). Another interesting finding was that the cells which had been once labelled with tritiated thymidine during the restitution period persisted for a long period, more than 6 weeks, without apparent synthesis of DNA or mitosis.

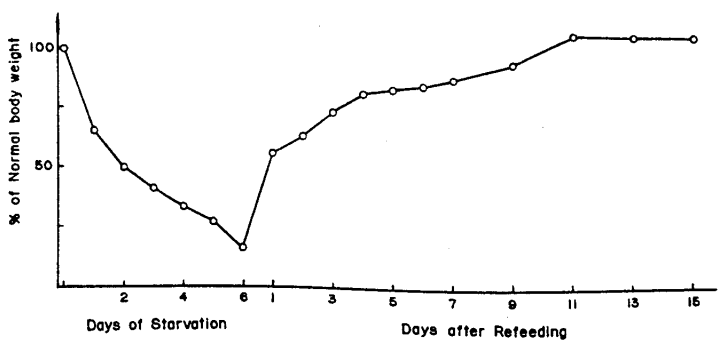

Fig. 1 Change in the body weight during starvation and refeeding

In order to know when liver DNA is most rapidly synthesized in the restitution period, the incorporation of ^{32}P into DNA was studied. Fig. 2 shows the changes in the liver weight, in its contents of DNA and RNA,

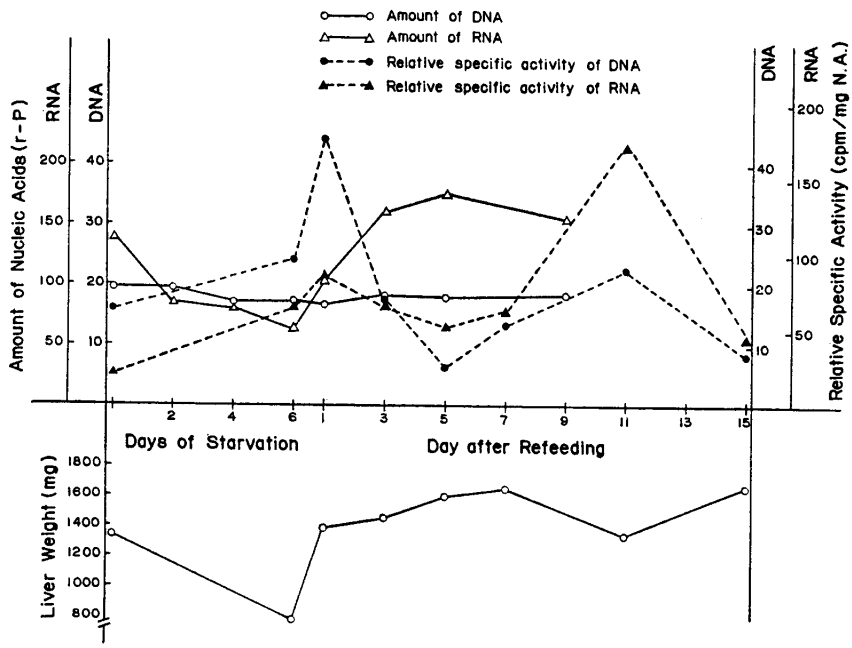

Fig. 2 Changes in the liver weight and in the amount of nucleic acids in relation to changes in the specific activity of ^{32}P incorporation into the nucleic acids in liver during starvation and refeeding period

and the change in the specific activity of ^{32}P-incorporation into DNA and into RNA during the restitution period after starvation.

The weight of the liver was reduced to about one-half of the normal value by the 6-day starvation and then rapidly recovered in a day. In contrast, the amounts of DNA did not vary significantly from normal level during the starvation nor during subsequent refeeding. The amounts of RNA, on the other hand, dropped to about a half of the initial values by starvation and then recovered rapidly by refeeding, almost parallel to the changes in the liver weight. Most rapid incorporation of ^{32}P into DNA occurred one day after refeeding. On the third day it dropped to the normal level. A peak of ^{32}P-specific activity of RNA was also observed on the first day but of much smaller magnitude as compared with that of DNA. Thereafter, a marked incorporation was observed again on the 12th day when the specific activity of DNA also rose but to a lesser

extent.

Similar studies were carried out on the lymphoid tissues. Results are shown in Figs. 3, 4, and 5 for spleen, lymph nodes and thymus respectively. In the spleen, a close parallel relation was observed among the changes in the amounts of DNA, of RNA and that of spleen weight (Fig. 4). In other words, the content of RNA per unit DNA and the splenic weight per unit DNA were nearly constant throughout the experiment. Again they were reduced to about a half of the initial values by starvation. Upon refeeding they approached the normal values on the third day. The incorporation of ^{32}P into DNA began to increase soon after the start of refeeding, reaching a peak on the fifth day. The specific activity of RNA increased also to a high level between the fifth and eleventh day. Thus the specific activity of DNA and RNA are raised almost parallel with each other.

Lymph nodes required 3 days to recover its lost weight (Fig. 5). Axillary and inguinal lymph nodes are too small in quantity to allow a precise extraction of the nucleic acids according to the present method. A rapid incorporation of ^{32}P was observed in the earlier stage of the recovery

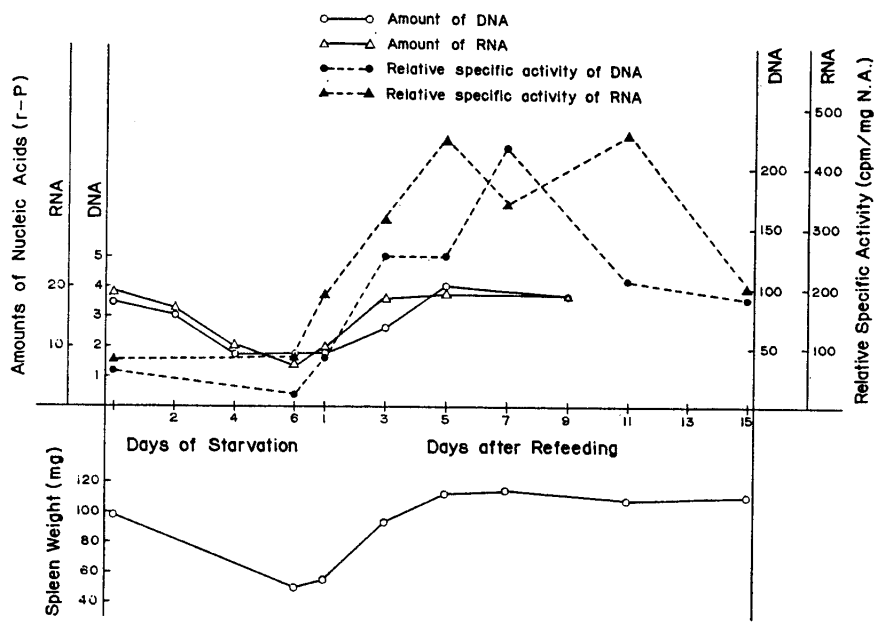

Fig. 3 Changes in the spleen weight and in the amounts of nucleic acids in relation to changes in the specific activity of ^{32}P-incorporation into the nucleic acids in spleen during starvation and refeeding period

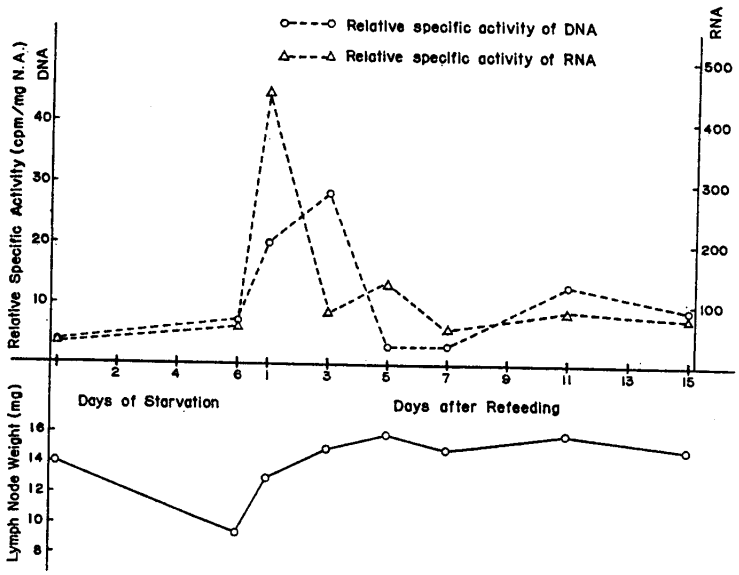

Fig. 4 Changes in the weight of axillary and inguinal lymph nodes in relation to changes in the specific activity of ^{32}P-incorporation into the nucleic acids in these nodes during starvation and refeeding

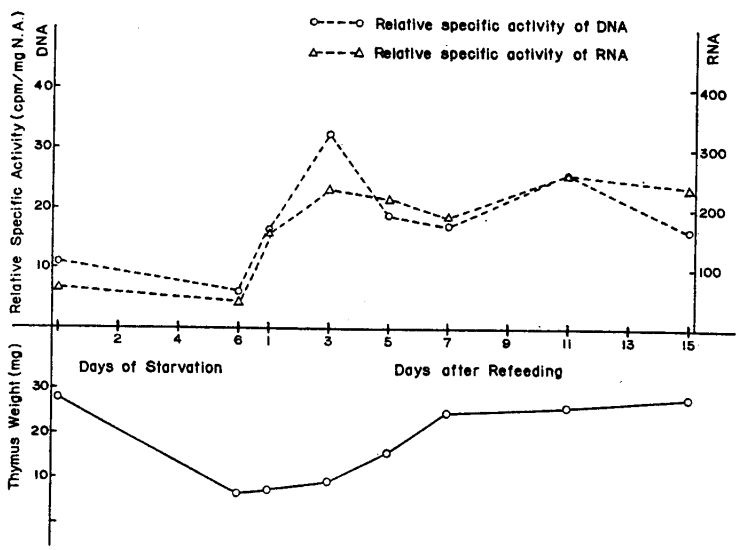

Fig. 5 Changes in the weight of thymus in relation to changes in the specific activty of ^{32}P-incorporation into the nucleic acids in thymus during starvation and refeeding

period as compared with those in spleen for both nucleic acids. Thus an enhanced synthesis of DNA occurred during the first 3 days. It dropped to the normal level after the fifth day. The specific activity of RNA abruptly rose on the first day of the recovery period, returning to normal level after the third day.

Thymus required 7 days to recover its lost weight, a much longer period than in other tissues (Fig. 6). In this tissue, again, the change in the ^{32}P-incorporation was quite proportional between DNA and RNA. The uptake of either DNA or RNA increased soon after the start of refeeding, reaching its peak on the third day. Thereafter it was maintained at high levels until the 15th day.

DISCUSSION

As outlined above, there is a good parallelism between the change in the weight and that in the RNA content of the liver, both values falling to less than a half of the initial values by starvation and recovering to normal levels in a few days by refeeding. The DNA content of the liver, on the other hand, does not change significantly throughout the starvation and recovery periods. These results indicate that in the liver the amount of DNA, or the number of cells, remains unchanged, and that the change in the liver weight is attributable to that in the amount of non-DNA components of the cell including RNA. These are in essential agreement with the results of other workers (1, 2). However, our previous experiment has demonstrated an unexpectedly intense incorporation of tritiated thymidine into liver DNA during the recovery period subsequent to starvation (14). This was confirmed by the present study. It was shown that there occurs a rapid incorporation of ^{32}P into liver DNA during the early period of restitution subsequent to starvation, a maximal incorporation being observed one day after the start of refeeding.

Although the incorporation of ^{32}P into liver DNA does not result in an increase in DNA content of the organ, it must first be considered that the mitotic activity of both hepatic cells and Kupffer cells might be enhanced by starvation and refeeding, as claimed by LEDUC (9, 10). She observed a definite increase in the number of mitotic figures of hepatic cells on the second day of refeeding with a diet, whose protein content was 23.9 per cent, after 10 day's protein depletion. As far as the present experiment is concerned, however, a definite increase in the mitotic activity of either hepatic cells or Kupffer stellate cells, which might be

responsible for a striking incorporation of ^{3}H-thymidine and ^{32}P into liver DNA, could not be confirmed. Moreover, such an enhanced cell division as claimed by LEDUC should result in a net increase in the amount of liver DNA. This was not the case in the present experiment as already emphasized.

Accordingly, a possible explanation may be that in the liver aged or exhausted cells die away (12) during the restitution after starvation and as a result replacement of the lost cells by fresh cells occurs, so that the number of cells or the amount of DNA in the liver remains unchanged. An alternate interpretation which allows explanation of the constancy of DNA content would be that cells in the non-dividing tissues such as liver of adult mammals renew their DNA as PELC and other investigators suggest (15—19), and that in the process of restitution after starvation such a renewal of DNA in the exhausted cells is promoted. A third possibility which should be considered here, is the so-called "repair of DNA" without replication, the process which is known primarily in bacteria (21).

In the spleen, lymph nodes and thymus, the changes in weight, DNA and RNA contents show no essential differences from each other. In addition, the rate of ^{32}P-incorporation into DNA and RNA is elevated almost coincident with the rise in the tissue weight and its nucleic acid contents. It is noteworthy that the specific activity of DNA is raised parallel with that of RNA in both lymph nodes and thymus, although the time of maximal synthesis of the nucleic acids is different between the two. These facts indicate that the changes in the weight of the lymphoid organs during starvation and restitution are dependent on the number of cells in the organs rather than on the amount of cellular components per cell. The above-mentioned findings in the lymphoid organs are in sharp contrast with those in the liver.

SUMMARY

The synthesis of nuclieic acids in the liver and the lymphoid tissues of adult mice was studied during the restitution period after the 6-day starvation. The results obtained indicate that there occurs an unexpected rapid synthesis of DNA in the hepatic parenchymal cells during the restitution period without significant increase in the total amount of DNA in the liver. Most rapid DNA-synthesis in the liver appears to occur one day after refeeding. With respect to RNA in the liver as well as to both RNA and DNA in the lymphoid tissnes, on the other hand, there is

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