The nutritional regulation of ribonucleic-acid metabolism in the liver cell

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The impact of nutrition on the metabolism of individual cells is illustrated by the way in which the metabolic activities of the liver are affected by the protein content of the diet. When rats are fed on a protein-deficient diet the liver cell rapidly loses a part of its protein, of its ribonucleic acid and of its phospholipid (Kosterlitz, 1947; Campbell & Kosterlitz, 1948, 1952). This loss suggests that the protein level in the diet exerts a controlling influence over the metabolism of each of these cellular components. In the present review an attempt has been made to explore the mechanism by which the protein content of the diet regulates the metabolism of ribonucleic acid (RNA) in the liver cell. The influence of the energy content of the diet on liver RNA metabolism (Munro, Naismith & Wikramanayake, 1953) will not be considered here.

Protein intake and incorporation of labelled precursors into ribonucleic acid

It might be supposed that the loss of RNA from the liver during protein deficiency is due to a decline in the rate of RNA synthesis. However, there is evidence to show that it is not so (Campbell & Kosterlitz, 1948, 1952; Munro *et al.* 1953). When rats are fed on a protein-free diet for several days and are then injected with labelled orthophosphate, uptake of ^{32}P by liver RNA (relative specific activity) is considerably raised above the level observed in control animals fed on a diet containing protein. The diminished amount of RNA in the livers of the protein-depleted animals thus develops a compensatory increase in replacement, so that the absolute rate of RNA synthesis remains unaffected by lack of protein in the diet.

Since protein deficiency does not reduce the rate of RNA synthesis in the liver, we must look elsewhere for an explanation of the fall in liver RNA content. More detailed study of the effects of protein depletion on RNA metabolism in the liver has provided evidence of a relationship between protein intake and the breakdown of RNA (Munro *et al.* 1953; Clark, Naismith & Munro, 1957). When rats are transferred from a normal diet to a regimen free from protein, the amount of RNA in the liver falls very rapidly during the 1st day, but from about the 2nd or 3rd day onwards it tends to level off (Fig. 1). However, the compensatory increase in ^{32}P uptake described above does not take place immediately on deprivation of the rat of protein in its diet. On the contrary, there is a considerable depression in isotope uptake on the 1st day of protein deficiency, and it coincides with the period during which the RNA content of the liver is falling precipitously (Fig. 1). The low level of ^{32}P uptake at this time can be readily explained if we assume that nucleotides, released as the result of RNA breakdown, enter the pool of free nucleotides from which RNA is synthesized and can thus be re-utilized (Fig. 2). If the withdrawal of protein from the diet causes a sudden acceleration in RNA breakdown, large quantities of

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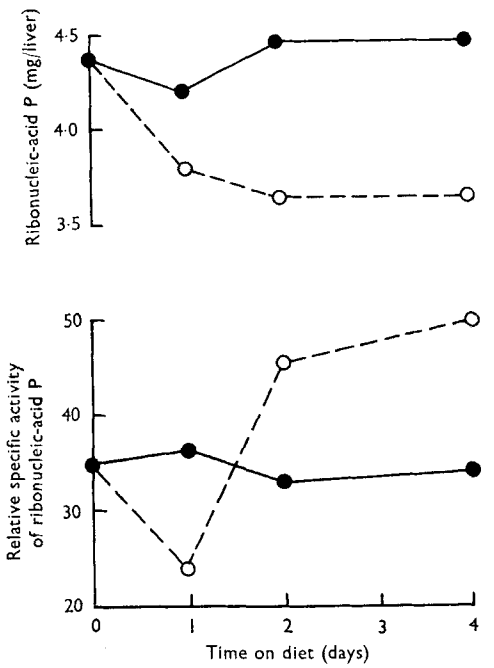

Fig. 1. Influence of protein intake on the amount of ribonucleic acid in the liver (upper two curves) and on its uptake of radioactive phosphorus (lower two curves). The rats were given either a diet containing protein (●——●) or a similar diet free from protein (○ - - - ○), and were injected with ^{32}P as orthophosphate 24 h before killing. Each point is the mean of observations on three rats. (Reproduced from Munro *et al.* 1953).

unlabelled free nucleotides would be released and in consequence would dilute the radioactivity of the nucleotides which serve as precursors of RNA. Thus adenine nucleotides released from RNA would dilute labelling in the adenine nucleotide pool from which new RNA is synthesized, and so on (Fig. 2). This interpretation has been greatly strengthened by findings with rats injected with [^{14}C]glycine (Clark *et al.* 1957). When protein was suddenly withdrawn from the diet, uptake of [^{14}C]glycine by the purine bases of RNA was almost completely obliterated. It was shown that this phenomenon coincided with a similar reduction in the labelling of the purine bases present in the acid-soluble fraction of the liver from which RNA is presumed to be synthesized. The changes in labelling which follow withdrawal of protein from the diet are thus compatible with a temporary increase in RNA breakdown leading to the sudden release of breakdown products and consequent dilution of isotopic labelling among the precursors of RNA.

The evidence accordingly suggests that part of the RNA of the liver cell immediately becomes unstable when protein is withdrawn from the diet. If this interpretation of the findings is correct, the feeding of protein to animals undergoing this loss of RNA should restrain breakdown of RNA and should for this reason swiftly terminate dilution of labelling among its precursors. It has turned out to be so. Rats that had been subjected to deprivation of dietary protein overnight, were then given

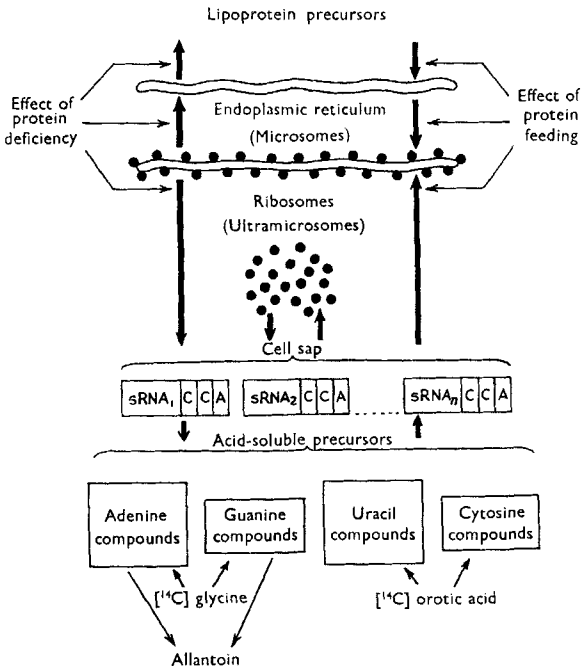

Fig. 2. Schematic representation of the influence of protein intake on ribonucleic-acid metabolism in liver-cell cytoplasm. sRNA, cell sap ribonucleic acid; C, cytidylic acid; A, adenylic acid; the subscript numbers (1, 2, . . . *n*) denote separate species of sRNA corresponding to different amino acids.

casein to eat. A marked rise in isotope uptake both by RNA and by the acid-soluble pool of purine-precursor compounds became evident within 3 h of giving protein (Clark *et al.* 1957). Independent evidence of a reduction in the rate of RNA breakdown was obtained by measurement of allantoin excretion (Fig. 2), which fell significantly after the meal of protein.

Intracellular source of the ribonucleic acid lost during protein deficiency

The various locations of RNA in the cytoplasm of the liver cell are shown schematically in Fig. 2. Mitochondrial RNA has been omitted for the sake of simplicity, although there is recent persuasive evidence (Rendi, 1959) that RNA in mitochondria exists as a functionally independent form. In the cell sap there is a type of RNA of low molecular weight (sRNA) which accepts amino acids after they have been activated (Hoagland, Zamecnik & Stephenson, 1957). It is believed (Smith, Cordes & Schweet, 1959) that there are separate species of sRNA corresponding to the different amino acids ($sRNA_1$, $sRNA_2$, and so on in Fig. 2). However, all sRNA molecules appear to terminate with the same sequence of three nucleotides, namely two of cytidylic acid and one of adenylic acid (Fig. 2) (Hecht, Zamecnik, Stephenson & Scott, 1958). The sRNA molecules transfer their amino acids to the ribonucleoprotein of the particulate fractions of the cell where protein synthesis takes place. In the liver cell, particulate RNA occurs in two forms. Some are ribonucleoprotein

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particles lying free in the cytoplasm; these are the ribosomes (the ultramicrosome fraction obtained by differential centrifugation of disintegrated cells). Other particles are attached to the endoplasmic reticulum (the microsome fraction obtained on centrifugation). Studies made both in vivo (Shigeura & Chargaff, 1958) and in vitro (von der Decken & Hultin, 1958; Bosch, Bloemendal & Sluyser, 1959) suggest that sRNA is the precursor of the RNA in the particles, but in none of these experiments was microsomal RNA distinguished from ultramicrosomal RNA. We thus do not know whether the sRNA is first assembled in the form of ribosomes which subsequently become attached to the endoplasmic reticulum, or whether the RNA of each particulate fraction arises independently from sRNA, as shown arbitrarily in Fig. 2.

When the RNA content of the liver is reduced by the giving of a protein-free diet for a few days, it has been found by differential centrifugation of disintegrated liver cells that the loss of RNA is essentially confined to the microsome fraction (Wikramanayake, Heagy & Munro, 1953; Munro, 1954). At the time these experiments were carried out the ultramicrosomal RNA and cell sap RNA could not be separated. The results obtained in them thus show that the combined RNA of these two fractions does not diminish during a short period of protein deficiency.

The predominant loss of RNA from the microsome fraction of homogenates is in good agreement with the results of electron microscopy of intact liver cells. Two independent studies with the electron microscope (Fawcett, 1955; Bernhard & Rouiller, 1956) have demonstrated a gross reduction of endoplasmic reticulum in the liver cells of animals starved for several days. Regeneration of reticulum began within a few hours of a meal rich in protein. The protein component of the diet appears to have been responsible for this change, since a diet low in protein was much less effective in restoring reticulum (Fawcett, 1955). At first, the growth of new reticulum occurred in the form of filaments, followed by the appearance of ribonucleoprotein particles attached to them. The ribonucleoprotein particles are thus not the focus from which the new reticulum originated, but on the contrary their appearance on the reticulum is a sequel to its formation (Fig. 2). Furthermore, the nucleoprotein particles appearing on this new reticulum do not seem to be synthesized directly from the free acid-soluble nucleotides of the cell sap, since administration of protein to starved animals does not preferentially increase uptake of isotopes by microsomal RNA (Clark *et al.* 1957). Consequently, the RNA deposited during the formation of new reticulum must arise by translocation of preformed RNA from other parts of the liver cell. The most likely source of this transferred RNA is sRNA (Fig. 2). Evidence supporting this conclusion is given below.

Connexion between ribonucleic-acid breakdown in the liver and protein synthesis

If the giving or withholding of protein causes alterations in RNA metabolism as the result of variations in the amount of endoplasmic reticulum, does the omission of a single essential amino acid from the diet affect the mechanism, as would be expected if the phenomenon has some connexion with protein synthesis? Evidence

in favour of this view was obtained when rats were fed with an amino-acid mixture from which a single essential amino acid could be deleted. Although the action of amino-acid mixtures on liver RNA metabolism occurs to some extent indirectly through stimulation of the adrenal cortex (Munro & Mukerji, 1958, and unpublished), it was possible to show under our experimental conditions that removal of tryptophan from the amino-acid mixture greatly reduced the uptake of [^{14}C]glycine by the purine bases and of [^{14}C]orotic acid by the pyrimidine bases of liver RNA (Munro & Clark, 1959*a*). These findings indicate that liver RNA metabolism is sensitive to omission of a single essential amino acid from the diet, presumably because the incomplete amino-acid mixture is no longer able to sustain endoplasmic reticulum formation in the liver cells.

These experiments have also provided some information about the chemical structure of the RNA involved in these metabolic changes. The omission of tryptophan from the amino-acid mixture had a greater effect on uptake of radioactivity by adenine and by cytosine than on uptake by guanine and by uracil. This finding is significant because the sRNA has terminal adenylic- and cytidylic-acid residues (Fig. 2). Enzymic breakdown of RNA in homogenates appears to occur preferentially at the ends of chains (Schneider & Potter, 1958), so that variations in the amount of sRNA broken down would produce greater changes in the labelling of the precursor pools of adenylic and cytidylic acids than in those of guanylic and uridylic acids. Direct examination of the acid-soluble adenine and guanine compounds confirmed that omission of tryptophan did in fact cause the expected changes in the labelling of the precursor pools.

Thus sRNA has the necessary chemical qualifications to be the species of RNA whose breakdown is affected by the nutritive properties of the amino-acid mixture given. There are probably separate sRNA molecules corresponding to each amino acid, but the change in isotope uptake produced by omitting tryptophan from the mixture of amino acids is too large to be confined to the degradation of one such species, and must represent a general effect on sRNA metabolism. Accordingly, the fate of sRNA as a whole must be determined at some site where the amino acids are assembled into proteins and where deficiency of a single amino acid will affect the utilization of all. This conclusion is consistent with the picture already presented (Fig. 2), in which the supply of amino acids for endoplasmic reticulum (microsome) formation determines the course of the reactions

$$\text{acid-soluble precursor compounds} \rightleftharpoons \text{sRNA} \rightleftharpoons \text{microsomal RNA}.$$

Protein intake and RNA metabolism in malignant liver cells

Recently, we have examined the influence of dietary protein level on RNA metabolism in malignant liver cells (Munro & Clark, 1959*b*). The main experiments were carried out on rats bearing a transmissible hepatoma implanted subcutaneously, which permitted comparison of the response of RNA metabolism in the tumour with that in the (normal) liver of the same animal. When protein intake was varied the RNA of the normal liver cells showed the same sensitivity to diet previously

observed with healthy rats, but the tumour did not show this response. The protein and RNA contents of the tumour cells were uninfluenced by the level of dietary protein, and the uptake of isotopes by the RNA of the tumour did not respond in the manner characteristic of normal liver cells. It is therefore significant that electron microscopy of the hepatoma used in these studies showed, in agreement with the findings of others (Howatson & Ham, 1955; Novikoff, 1957), that the cells of this type of tumour lack the endoplasmic reticulum found in normal liver cells. The absence of a response of RNA metabolism to dietary protein level can thus be correlated with a deficiency of endoplasmic reticulum.

Conclusions

The RNA content of the normal liver cell varies according to the protein level in the diet. This change seems to occur primarily in the RNA associated with the endoplasmic reticulum of the liver cell. We do not yet, however, understand why the endoplasmic reticulum of the hepatocyte comes and goes so rapidly with changes in the supply of protein in the diet. The malignant liver cell is deficient in endoplasmic reticulum and for this reason its RNA metabolism seems to be insensitive to dietary protein level. The picture we have evolved to explain the detailed steps in RNA metabolism is shown in Fig. 2. It is only a partial picture, since it takes no account of nuclear RNA, which also has been shown to vary in amount with the dietary intake of protein in normal but not in malignant liver cells (Stenram, 1958). Furthermore, nuclear RNA may be the source of cytoplasmic RNA (Richter, 1959).

REFERENCES

Bernhard, W. & Rouiller, C. (1956). *J. biophys. biochem. Cytol.* **2**, Suppl. p. 73.
Bosch, L., Bloemendal, H. & Sluyser, M. (1959). *Biochim. biophys. Acta*, **34**, 272.
Campbell, R. M. & Kosterlitz, H. W. (1948). *J. biol. Chem.* **175**, 989.
Campbell, R. M. & Kosterlitz, H. W. (1952). *Biochim. biophys. Acta*, **8**, 664.
Clark, C. M., Naismith, D. J. & Munro, H. N. (1957). *Biochim. biophys. Acta*, **23**, 587.
Fawcett, D. W. (1955). *J. nat. Cancer Inst.* **15**, 1475.
Hecht, L. I., Zamecnik, P. C., Stephenson, M. L. & Scott, J. F. (1958). *J. biol. Chem.* **233**, 954.
Hoagland, M. B., Zamecnik, P. C. & Stephenson, M. L. (1957). *Biochim. biophys. Acta*, **24**, 215.
Howatson, A. F. & Ham, A. W. (1955). *Cancer Res.* **15**, 62.
Kosterlitz, H. W. (1947). *J. Physiol.* **106**, 194.
Munro, H. N. (1954). *Proc. Nutr. Soc.* **13**, 115.
Munro, H. N. & Clark, C. M. (1959*a*). *Biochim. biophys. Acta*, **33**, 551.
Munro, H. N. & Clark, C. M. (1959*b*). *Brit. J. Cancer*, **13**, 324.
Munro, H. N. & Mukerji, D. (1958). *Biochem. J.* **69**, 321.
Munro, H. N., Naismith, D. J. & Wikramanayake, T. W. (1953). *Biochem. J.* **54**, 198.
Novikoff, A. B. (1957). *Cancer Res.* **17**, 1010.
Rendi, R. (1959). *Exp. Cell Res.* **17**, 585.
Richter, G. (1959). *Biochim. biophys. Acta*, **34**, 407.
Schneider, J. H. & Potter, V. R. (1958). *J. biol. Chem.* **233**, 154.
Shigeura, H. T. & Chargaff, E. (1958). *J. biol. Chem.* **233**, 197.
Smith, K. C., Cordes, B. & Schweet, R. S. (1959). *Biochim. biophys. Acta*, **33**, 286.
Stenram, U. (1958). *Acta path. microbiol. scand.* **44**, 239.
von der Decken, A. & Hultin, T. (1958). *Exp. Cell Res.* **15**, 254.
Wikramanayake, T. W., Heagy, F. C. & Munro, H. N. (1953), *Biochem. biophys. Acta*, **11**, 566.