The effect of diet on rat liver nuclear DNA-dependent RNA polymerase

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The response of the rat liver cytoplasm to changes in the protein content of the diet has been well documented and some of these changes have been reviewed in this symposium. The equally profound changes which occur in the nucleus are less well known but have been investigated by several workers.

Lagerstedt (1949) was probably first to investigate the histological changes occurring in the nucleus of rat liver after changes in the diet. He showed that starvation for 24 h produced a 50% decrease in the size of the nucleolus and he also observed a decrease in the number of nucleoli per nucleus. The nuclear size was also reduced. Starvation for a further 4 d did not cause further changes in size. Giving a low-(4%) protein diet produced less rapid changes which were similar to the starvation pattern. Giving a high-(22%) protein diet to these animals led to a very rapid increase (+100%) in nucleolar size within 3 h. Lagerstedt also noticed that the number of nucleoli per nucleus increased to the pre-starvation number, and that the nuclear size also increased. In the same year Stowell (1949), working in Caspersson's laboratory in Stockholm, published a short account of less extensive observations of the effect of high- and low-protein diets on nucleolar size. He described an increase in nucleolar mass after giving a low-(5%) protein diet.

Because of the discrepancy between Lagerstedt's (1949) and Stowell's (1949) results on the effects of high- and low-protein diets on nucleolar size, Stenram reinvestigated the problem: he found that animals fed for 10 d with 0% or 3% protein diet had larger nucleoli than the animals given the high-(25%) protein diet for a similar period (Stenram, 1953).

In a later series of experiments he studied the time-course of the effect of giving the high-protein diet (Stenram, 1956*a*). Giving a high-protein diet to rats which had been starved for 5 d resulted in an increase of nucleolar size over the next 3 d, which then decreased in size over the following 15 d to reach the size attained 6 h after feeding. In the group of rats fed on a diet without protein the nucleolus slowly increased in size so that after 3 d on the diet the nucleolar size exceeded that produced by either starvation or high-protein feeding.

Stenram (1956*b*) further investigated the effect of the protein-free diet. He used a nutritionally adequate mixture of essential amino acids and found that the single omission of any one of lysine, isoleucine, methionine, threonine, tryptophan or valine produced changes in nucleolar size similar to those with the protein-free

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diet. However, the omission of histidine, leucine or phenylalanine from the amino acid mixture did not produce differences in nucleolar size when compared with the effect of giving the complete amino acid mixture.

Other workers have observed nucleolar changes after giving diets deficient in one amino acid. Thus Spector (1948) and Spector & Adamstone (1950) found that after 2 d of forcibly giving a tryptophan-deficient diet, a single, huge nucleolus developed in liver cells. Dick, Hall, Sydenstricker, McCollum & Bowles (1952) found that in threonine-deficient rats there tended to be one prominent nucleolus instead of several nucleoli and similar changes have been found in lysine-deficient rats.

The nucleolus is now known to be the site of synthesis of ribosomal precursor RNA in rat liver (Muramatsu, Hodnett, Steele & Busch, 1966) and Widnell & Tata (1966) have shown that, in rat liver, nuclear, Mg^{2+}-activated DNA-dependent RNA polymerase (*EC* 2.7.7.6) synthesizes an RNA with a ribosome-like base ratio. This enzyme activity is known to be preferentially located in the nucleolus (Siebert, Villalobos, Ro, Steele, Lindenmayer, Adams & Busch, 1966; Roeder & Rutter, 1969, 1970; Chesterton & Butterworth, 1971*a*) and it is therefore of interest to follow the changes in activity of this enzyme in a variety of dietary states.

Shaw & Fillios (1968) studied the activity of both the Mg^{2+}- and Mn^{2+}-activated DNA-dependent RNA polymerases (Widnell & Tata, 1966) in rat liver after giving a 0, 5 or 20% protein diet over a period of from 7 to 42 d. Only the Mg^{2+}-activated enzyme responded to changes in the level of dietary protein. Taking the activity of the enzyme in the animals fed with the 20% protein diet as 100% (and this remained constant), they found that the 5% protein diet gave about 140% of this enzyme activity after 7 d feeding—which fell to the 100% level of activity after 28 d feeding. In animals fed with the 0% protein diet the enzyme activity was about 150% after 7 d feeding and remained about 120% activity even after the end of the study. If we thus equate nucleolar size with Mg^{2+}-activated DNA-dependent RNA-polymerase activity then the observations of Shaw & Fillios (1968) tend to support the findings of Stowell (1949) and Stenram (1953).

Rickwood & Klemperer (1970) studied the effect of starvation on the activities of rat liver Mg^{2+}- and Mn^{2+}-activated RNA polymerases. Within 1 d of starvation the Mg^{2+}-activated polymerase had decreased by 30% and this activity continued to decrease further over the next 3 d. By contrast, the activity of the Mn^{2+}-activated enzyme fell by 50% but only during days 3 and 4 of starvation. Lagerstedt (1949) noted the reduction of nucleolar size after overnight starvation; so here again we have an association between enzyme activity and nucleolar size.

Henderson (1970) also described a reduction in nuclear, Mg^{2+}-activated RNA polymerase of rat liver after overnight starvation, and found an extremely rapid response to giving a nutritionally complete amino acid mixture by stomach-tube. Several animals appeared to respond within 5 min of feeding but the maximum response was obtained after about 1 h when the enzyme activity had doubled. Increased activity was still apparent 2 h after feeding. It is of interest that Lagerstedt (1949) described a 50% increase in nucleolar size occurring within 3 h of giving a high-protein diet.

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The rapid response of RNA-polymerase activity to feeding was abolished if tryptophan was omitted from the amino acid mixture, and the liver tryptophan levels were found to correlate extremely well with the RNA-polymerase activity for both diets. It was also found that the polymerase response to the complete amino acid mixture could be abolished by pretreatment of the animal with cycloheximide or puromycin in doses which did not affect the enzyme activity in starved animals. This observation suggests that an RNA polymerase may be synthesized after feeding with a complete amino acid mixture.

Why should liver RNA polymerase activity increase after feeding anyway? The decrease in the total liver content of RNA on starvation (Kosterlitz, 1947; Bernhard, Haguenau, Gautier & Oberling, 1952) suggests that refeeding should result in an increase in the synthesis of liver RNA. Stenram (1956*a*) did in fact observe a rapid increase in the size of rat liver nucleoli within 6 h after giving a high-protein diet; Kosterlitz (1947) found an increase in the total RNA content of rat liver and Bernhard *et al.* (1952) observed a rapid regeneration of endoplasmic reticulum of liver cytoplasm. This work indicates that the increased RNA synthesis in liver consequent on refeeding is accompanied by a very rapid response of the Mg^{2+}-activated DNA-dependent RNA polymerase.

Our knowledge of the biochemistry of rat liver DNA-dependent RNA polymerase has been considerably advanced by the discovery of multiple forms of the enzyme (Roeder & Rutter, 1969, 1970). Initially, these authors described two RNA polymerases (polymerase I and II) from rat liver, designated by the order in which they were eluted from DEAE-Sephadex, but in the following year polymerase III was recognized (Lindell, Weinberg, Morris, Roeder & Rutter, 1970). Polymerase I is located in the nucleolus, and polymerases II and III in the nucleoplasm. Only polymerase II is sensitive to α-amanitin (a toxic substance from the mushroom, *Amanita phalloides*). More recently it has become clear that there is further complexity. Chesterton & Butterworth (1971*b*) have shown that polymerase I can be separated, on phosphocellulose, into two components present in equal amounts (forms Ia and Ib) and that form Ib may be derived from form Ia or from a previously inactive source (Chesterton & Butterworth, 1971*c*). Polymerase II has also been separated into at least two components (Chesterton & Butterworth, 1971*d*; Mandel & Chambon, 1971). It is necessary to point out that these two groups of workers now use a different nomenclature to describe the several forms of RNA polymerase (Kedinger, Nuret & Chambon, 1971). The enzymes insensitive to α-amanitin are class A and those sensitive to its action are class B polymerases. All class B enzymes are nucleoplasmic in location and they correspond to Roeder and Rutter's (1969, 1970) type II polymerase. AI and AII correspond to forms Ia and Ib, both being located in the nucleolus. AIII is located in the nucleoplasm and is similar to polymerase III which elutes last from DEAE-Sephadex.

Recently, we have been using the procedure of Roeder & Rutter (1969, 1970) to separate the multiple forms of RNA polymerase from nuclei of rat liver to investigate further the effects on nuclear function of feeding. We have shown that overnight starvation decreases all the main bands of activity and that giving the

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complete amino acid mixture previously described leads, within 1 h, to a specific increase in activity in the region of polymerase III. Giving the amino acid mixture lacking tryptophan does not produce this pattern (Spencer and Henderson, unpublished observations). We had previously shown, on the basis of temperature effects on whole nuclear RNA-polymerase activity, that there appears to be a component present in rats fed with the amino acid mixture that is not present in the starved animals (Spencer & Henderson, 1971), and the present findings lend support to this suggestion.

None the less, these findings do appear to be paradoxical. All the previous evidence points to rapid nucleolar changes, yet the first change which we have been able to record is a rapid increase in a nucleoplasmic RNA polymerase. We suggest, therefore, that the function of the nucleolus is regulated by a type of RNA formed in the extranucleolar compartment. Such an RNA could be messenger RNA or transfer RNA and it is worthy of note that Zylber & Penman (1971) have suggested that polymerases II and III from HeLa cell nuclei synthesize nuclear heterogeneous RNA which may serve as a precursor to cytoplasmic messenger RNA (Darnell, Pagoulatos, Lindberg & Balint, 1970). Also, Jacob, Muecke, Sajdel & Munro (1970) have suggested that there is extranucleolar control of nucleolar RNA synthesis on the basis of in vivo α-amanitin inhibition of nucleolar function.

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