Nuclear Protein in the Liver in Protein-Calorie Deficiency

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1. The effect of protein-calorie deficiency on nuclear proteins was studied in growing and adult rats by using chemical and electrophoretic methods of fractionation. 2. After 7 days on protein-deficient diets, the amount of neutral-soluble proteins increased, and their electrophoretic pattern changed, with the appearance of a new component. 3. The histone fraction of the liver nuclei in rapidly growing rats was lower than that in either deficient or adult animals. 4. The possible role of nuclear proteins in relation to synthesis of the proteins and nucleic acids of the other parts of the cells is discussed.

It has been known for a long time that liver metabolism and function are affected in protein-depleted animals and human subjects. Glycogen, fat, proteins and nucleic acids are components of the liver which are most sensitive to dietary changes. Many extensive studies have established the view that the liver of animals which have been fed on protein-free or low-protein diets loses protein to a greater extent than any other organ (Addis, Poo & Lew, 1936; Addis, Lew & Poo, 1940; Kosterlitz, 1947; Munro, 1964). The rate of loss of protein is greatest during the first few days of protein depletion and is accompanied by a parallel decline in the RNA content of the liver cells (Allison, Wannemacher, Banks, Wunner & Gomez-Brenes, 1962; Cooper, 1953; Mendes & Waterlow, 1958; Munro, 1964; Wannemacher, Russel & Allison, 1963; Williams, 1961).

It is evident that the changes take place through a decrease in the amount of cytoplasm, rather than in the total number of cells. This is shown by a loss in stainable protoplasm (Elman, Smith & Sachar, 1943; Kosterlitz, 1947; Platt, Heard & Stewart, 1964). Detailed studies involving chemical or electrophoretic separation of liver protein have not indicated preferential losses of any particular fractions (Demling, 1954; Luck, 1936; Smetana, Hruza & Korinek, 1957). Similarly determination of the chemical composition of subcellular components of the liver in protein deficiency has shown a rather uniform diminution of protein content in mitochondria, microsomes and cell-sap, with the exception that the decrease in RNA occurs only in the microsomes (Muntwyler, Seifter & Harkness, 1950; Seifter, Muntwyler & Harkness, 1950;

Wikramanayake, Haegy & Munro, 1953; Munro, 1964).

Recently it has been demonstrated that the effects of low-protein diets are not only confined to the cytoplasm. Stenram (1958, 1963), using a histochemical technique, has drawn attention to the interrelationship between the amount of dietary protein and the size of the nucleolus, which may increase in size, sometimes twofold, during protein depletion. In protein-calorie-deficient pigs histological changes have been found in the nuclei of the liver cells, which are enlarged and hyperchromatic, and the number of double-nucleated cells is increased (Platt *et al.* 1964). The importance of the nucleus in relation to the biosynthesis of proteins has been reviewed by Bush (1963). This subcellular component not only stores genetic material but also takes part in the control mechanism of cellular metabolism. There is some evidence based on experiments *in vitro* that nuclear proteins might be involved in regulation of the rate of DNA synthesis (Allfrey & Mirsky, 1962, 1963; Lehnert, 1964), act as 'gene modifiers' (Stedman & Stedman, 1952) or influence messenger-RNA synthesis, thus indirectly affecting protein synthesis (Allfrey, Faulkner & Mirsky, 1964).

It is now generally accepted by nutritionists that, because of the interrelationship that exists between protein and energy metabolism, it is impossible in any experiment to separate the effects of protein and/or calorie deficiency. Because of this, the meeting of the joint Food and Agriculture Organization of the United Nations-World Health Organization Expert Committee on Nutrition (Food and Agriculture Organization of the United Nations, 1962) proposed the use of the term

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protein-calorie deficiency and this has been used to categorize the experiments discussed in this paper.

In the present study, the technique of electrophoresis on polyacrylamide gels has been used to study changes resulting from protein-calorie deficiency in protein fractions obtained from the nuclei of the liver cells of both young and adult rats. The protein and nucleic acid contents of the other intracellular components have also been examined.

EXPERIMENTAL

Animals and procedure

Male hooded rats were used both for the pilot trial and the main experiments. Before experiments started the animals were kept in a temperature-controlled room and maintained on stock diets for 5 days.

In the pilot trial, five pairs of adult rats of approximately similar weights were fed *ad libitum* on diets of 'net dietary-protein kcal. per cent' 10 and 3. This value for a diet is a measure of the proportion of the dietary energy which is available to an animal as protein completely utilizable for anabolism (Platt, Miller & Payne, 1961). Protein values in this paper refer to this expression. Pairs of animals were killed after 5, 10, 14, 28 and 42 days and liver samples taken for chemical analysis.

The main experiments were done on young animals weighing 54.0–62.0 g. at 22 days of age and on adult animals weighing 249.8–282.5 g. Animals of each age were divided into three groups and fed *ad libitum* with diets having protein values of 10, 3 and 0 (protein-free). The composition of these diets is shown in Table 1. The rats were killed after 7 and 14 days of feeding on the different diets and material was taken for chemical determinations.

One group of the adult animals was maintained on a protein-free diet for 14 days and then was re-fed for 7 days with a diet having a protein value of 10; the animals were then killed.

Analytical methods

Livers were perfused *in vivo* with 0.25 M-sucrose solution containing 1.6 mM-CaCl₂ and 1 mM-MgCl₂. Under light ether anaesthesia thin polyvinyl tubes were inserted into the aorta, vena cava and portal vein and perfusion was per-

formed until all traces of blood were removed. Then the livers were excised, small samples taken for total nitrogen and nucleic acid determination and the remainder immediately placed in ice-cold sucrose solution (composition as described above).

Isolation of the nuclei. The nuclei were isolated by the method of Chauveau, Moulé & Rouiller (1956) as modified by Wilczok & Chorazy (1960) and S. Waddington (personal communication). The tissues were disrupted, macerated and then suspended in 10 vol. of the sucrose solution. Initial homogenization was carried out in a Potter-Elvehjem homogenizer with eight strokes of the pestle rotating at about 1000 rev./min. The homogenate was filtered through nylon gauze and spun at 65g for 5 min. in an MSE refrigerated centrifuge (at 0°) to remove most of the large debris and unbroken cells. Thereafter the supernatant was layered over 0.34 M-sucrose–2 mM-CaCl₂–1 mM-MgCl₂ and centrifuged at 1000g. The sediment was resuspended in 1 vol. of 0.25 M-sucrose–2 mM-CaCl₂–1 mM-MgCl₂ and floated on to the surface of 9 vol. of 2.31 M-sucrose–2 mM-CaCl₂–1 mM-MgCl₂, which gave a resultant concentration of 2.2 M-sucrose. The suspension obtained was centrifuged at 25000g for 1½ hr. (at 0–1°).

In the pilot experiments the yellow nuclear sediment was resuspended in 5 vol. of 0.14 M-NaCl, homogenized with 15 strokes of the pestle and subsequently frozen and thawed six times to disrupt the nuclear membrane.

In the main experiments the nuclear sediment was separated into eight fractions by chemical extraction, as described by Steele & Bush (1963). In particular, the soluble nuclear-protein fractions were prepared by extracting the nuclear sediment twice with 10 vol. of 0.14 M-NaCl and once with 5 vol. of 0.1 M-tris-HCl buffer, pH 7.6, in that order, homogenizing and stirring each time, for 20 min. at room temperature. A precipitate was obtained in each case by centrifugation at 6000g for 10 min.

A separate sample of homogenized liver was used to isolate the subcellular components by centrifugal fractionation in 0.25 M-sucrose–2 mM-CaCl₂ solution (Schneider, 1948; Wikramanayake *et al.* 1953). The supernatant, after centrifuging at 700 rev./min. for 10 min., was further centrifuged at 6500g for 5 min. The sediment was taken to be the 'mitochondrial fraction'. The supernatant was spun once more at 10000g for 1 hr. and the sediment obtained was referred to as the 'heavy-microsomal fraction'; the final supernatant, which consisted of a mixture of light microsomes and the cell sap, was named the 'cytoplasmic fraction'.

Chemical analysis

Protein. The total protein content was determined in all the soluble fractions by the method of Lowry, Rosebrough, Farr & Randall (1951). Insoluble nuclear proteins were determined gravimetrically after washing with 2 vol. of 95% ethanol-ether, twice with 5% trichloroacetic acid and finally twice with chloroform-methanol (1:3, v/v). The material was dried overnight at 105° to constant weight.

Total serum protein and albumin were measured by the biuret method (King & Wootton, 1959).

Deoxyribonucleic acid and ribonucleic acid. These were determined in whole-liver homogenates, isolated subcellular particles and nuclear fractions by the method of Schmidt & Tannhauser (Schmidt, 1957) and expressed in mg. of DNA and RNA phosphorus respectively.

Table 1. *Composition of the diets*

'Protein value' in Tables 1–4 refers to the net dietary-protein kcal. per cent (see the Experimental section).

Protein value.....	Percentage composition		
	10	3	0
Rolled oats	45	45	—
Starch	—	25	45
Casein	25	—	—
Dripping	25	25	15
Sucrose	—	—	35
Salt mixture*	4	4	4
Vitamin mixture	1	1	1

* Osborne & Mendel (1918).

Electrophoresis techniques. In the pilot experiments soluble nuclear proteins were separated by starch-gel electrophoresis, by the technique of Smithies (1959), with a glycine-NaOH buffer (pH 8.0; 0.02M). By this procedure the soluble nuclear proteins were separated into 15 or 16 fractions (see Fig. 1).

In the main experiments polyacrylamide-gel electrophoresis (Ornstein & Davis, 1962) was applied to the separation of nuclei, mitochondrial, heavy microsomes and cytoplasmic proteins. This procedure required much smaller amounts of material and gave better resolution than starch-gel electrophoresis.

Preparation of the gel. Solution A [acrylamide (Eastman Kodak) 30.0g., bisacrylamide (*NN'*-methylenebisacrylamide; Eastman Kodak) 0.8g., Temed (*NNN'*-tetramethylethylenediamine; Eastman Kodak) 1.0ml., water up to 100ml.] was mixed with solution B [glycine-NaOH buffer, pH 7.6 (50ml. of 0.2M-glycine + 3.0ml. of 0.2M-NaOH made up to 200ml. with water)] and solution C (ammonium persulphate, 1.0g., made up to 100ml. with water) in the proportions 1:2:1.

To separate the basic proteins (histones), instead of glycine-NaOH buffer, 0.1M-acetic acid-4M-urea buffer (pH 4.1) was used. The solutions, after being mixed, were immediately poured on to several microscope slides previously placed in a glass dish 3mm. deep. Then the gel was covered very carefully with a Perspex plate so as to avoid formation of air bubbles. After polymerization, which took place in about 15–30min., the Perspex plate was removed and the slides were separated with a sharp razor. The size of the gel slab obtained was 80mm. $\times$ 40mm. $\times$ 1.5mm.

Application of samples. Narrow slots, 1.1mm. long, were cut about 2.5cm. from the ends of the slabs with a sharp blade; protein samples in an amount not exceeding 250 μ g. of protein were applied with a micropipette on to strips of Whatman 3MM filter paper, which were then placed in the slots.

Electrophoresis. This was performed with either borate buffer, pH 8.6, or 0.1N-HCl solution for histone separation. The gel was connected to the electrode compartment by pieces of lint soaked in the buffer. The duration of electrophoresis was 3hr., with a voltage gradient 25v/cm. Under these conditions the most rapidly moving fraction migrated about 35–40mm. from the starting line and the soluble proteins of the subcellular components were separated into seven to eight fractions with good resolution.

Gels were stained overnight in 0.025% solution of Amido Black 10 B in solvent (methanol-acetic acid-water, 5:1:5, by vol.) and destaining was performed by a second electrophoresis, with 10% acetic acid solution. This procedure was completed in about 1½ hr. with a voltage gradient 25v/cm.

Quantitative estimation of the individual fractions was performed by the elution of the stained fractions. The stained sections were cut out and placed in stoppered tubes, macerated and eluted with 2.0ml. of 0.1N-NaOH. The tubes were kept at 37° for 6hr. The volumes were made up to 4.0ml. and the extinction was determined at 620 m μ against a blank derived from an unstained portion of the gel, and the relative amount of each fraction was calculated.

Recovery of the nuclei was calculated as the percentage of DNA phosphorus content of isolated nuclei as a proportion of the DNA phosphorus content of the whole-liver homogenate. These values were used to calculate the

protein and RNA contents of the nuclear components of whole livers.

Since the view has been generally accepted that the DNA phosphorus content of individual liver cells remains relatively constant during growth and protein depletion (Harrison, 1953; Mendes & Waterlow, 1958), and may therefore serve as a standard of reference, the results have also been expressed per unit of DNA phosphorus.

RESULTS

The results of the pilot experiments showed that during the initial stages of protein-calorie deficiency the total nitrogen content of the liver diminished from an average of 215mg./100g. body wt. to 160mg./100g. body wt. after 42 days of feeding with the diet of protein value 3. However, the total nitrogen content of the liver-cell nuclei remained constant at about 32mg./liver/100g. body wt. The liver-cell nuclei were homogenized as previously described and extracted with neutral salt solution. This extract was subjected to electrophoresis on starch-gel medium. After only

Fig. 1. Starch-gel electrophoresis of the soluble liver nuclear proteins extracted with 0.14M-NaCl solution from the protein-deficient rat. 1, Diet of protein value 3; 2, diet of protein value 10.

5 days on the low-protein regimen a fast-moving band appeared on the electrophoretogram which was completely absent from the control animals (Fig. 1). The new fraction was unaffected by changes in pH or ionic strength of the buffer and increased with increased duration of feeding on the low-protein diet. The liver cells obtained from animals in the second experiment were fractionated according to the method given by Steele & Bush (1963). Very marked changes were observed in both the ratio of protein to DNA phosphorus and the electrophoretic pattern of nuclear protein extracted with 0.14M-sodium chloride and with 0.1M-tris buffer, pH7.6. Tables 2 and 3 show that in both young and adult rats the ratio of protein to DNA phosphorus in the 0.14M-sodium chloride extract increased with time on low-protein diets. Also a new fraction appeared during electrophoresis on polyacrylamide gel; this new fraction increased with time and was larger in the young animals, amounting to up to 2% of the total soluble nuclear protein after 14 days on a protein-free diet. Precipitation of the proteins from the 0.14M-calcium chloride extract by acidifying with either sulphuric acid or hydrochloric acid, washing and redissolving in neutral solution did not change the electrophoretic pattern. Table 2 also shows that re-feeding of adult animals for 7 days with a diet of protein value 10 after depletion for 14 days on a protein-free diet restores the electrophoretic pattern to that of the control groups.

The histone fraction showed a significant rise in total protein expressed in relation to DNA phosphorus, from the lowest value of 24mg./mg. of DNA phosphorus in young, rapidly growing rats on the diet of protein value 10 to 32mg. after 14 days on a protein-free diet; in adult rats this ratio remained constant at about 28mg./mg. of DNA phosphorus in all the groups. On electrophoresis, eight distinct fractions were obtained; however, there were no differences in the proportions of these in any of the experimental groups.

No differences in total protein or electrophoretic pattern were found in any of the other nuclear-protein fractions.

The RNA contents of the nuclear fractions soluble in 0.1M-tris and 0.05M-sodium hydroxide were determined. Expressed in DNA phosphorus units, these showed significant increases in both young and adult animals on the low-protein regimens (Table 4); in particular, the RNA content of the 0.05M-sodium hydroxide-soluble fraction from the livers of young rats was very high after 7 days on the diet, but fell to a lower value after 14 days.

Measurements were made of total protein, RNA and electrophoretic components of the mitochondrial, heavy-microsomal and cytoplasmic cell fractions, also of blood plasma, and the changes

Table 2. *Effect of the protein-calorie deficiency on the 0.14M-sodium chloride-soluble fraction of the liver nuclear proteins in adult rats*

Results are mean percentages $\pm$ s.d. and changes marked * are statistically significant ($P < 0.01$). Each group of animals consisted of five rats. Changes in tris buffer-soluble fractions are identical in trend.

Electrophoretic fractions	Protein value.....				After 7 days				After 14 days				After re-feeding for 7 days	
	10				3				10				10	
	0				0				0				0	
Protein/DNA phosphorus (mg./mg.).....	17.7 $\pm$ 0.70				19.3 $\pm$ 0.80*				19.9 $\pm$ 0.70*				16.3 $\pm$ 2.06	
I	7.4 $\pm$ 0.91	7.3 $\pm$ 0.88	6.0 $\pm$ 1.01	6.4 $\pm$ 0.96	7.3 $\pm$ 0.88	6.0 $\pm$ 1.01	6.6 $\pm$ 1.06	6.2 $\pm$ 0.96	6.4 $\pm$ 0.96	7.7 $\pm$ 0.60	7.7 $\pm$ 0.60	7.7 $\pm$ 0.60	7.7 $\pm$ 0.60	7.7 $\pm$ 0.60
II	—	1.7 $\pm$ 1.00*	1.9 $\pm$ 1.03*	2.2 $\pm$ 1.00*	1.7 $\pm$ 1.00*	1.9 $\pm$ 1.03*	—	2.2 $\pm$ 1.00*	3.0 $\pm$ 1.12	—	—	—	—	—
III	4.9 $\pm$ 1.60	5.1 $\pm$ 1.10	5.4 $\pm$ 1.19	5.7 $\pm$ 1.16	5.1 $\pm$ 1.10	5.4 $\pm$ 1.19	5.0 $\pm$ 0.96	5.6 $\pm$ 1.12	5.7 $\pm$ 1.16	5.3 $\pm$ 1.00	5.3 $\pm$ 1.00	5.3 $\pm$ 1.00	5.3 $\pm$ 1.00	5.3 $\pm$ 1.00
IV	11.0 $\pm$ 0.96	10.3 $\pm$ 1.07	9.9 $\pm$ 1.09	10.5 $\pm$ 1.26	10.3 $\pm$ 1.07	9.9 $\pm$ 1.09	11.6 $\pm$ 1.06	10.9 $\pm$ 1.06	10.5 $\pm$ 1.26	11.2 $\pm$ 1.20	11.2 $\pm$ 1.20	11.2 $\pm$ 1.20	11.2 $\pm$ 1.20	11.2 $\pm$ 1.20
V	14.5 $\pm$ 1.30	12.6 $\pm$ 1.27	13.6 $\pm$ 1.19	15.2 $\pm$ 1.16	12.6 $\pm$ 1.27	13.6 $\pm$ 1.19	14.3 $\pm$ 1.00	15.8 $\pm$ 1.21	15.2 $\pm$ 1.16	13.5 $\pm$ 1.00	13.5 $\pm$ 1.00	13.5 $\pm$ 1.00	13.5 $\pm$ 1.00	13.5 $\pm$ 1.00
VI	16.3 $\pm$ 1.36	15.6 $\pm$ 1.50	15.5 $\pm$ 1.29	16.8 $\pm$ 1.21	15.6 $\pm$ 1.50	15.5 $\pm$ 1.29	16.5 $\pm$ 0.96	16.6 $\pm$ 1.06	15.8 $\pm$ 1.19	16.8 $\pm$ 1.21	16.8 $\pm$ 1.21	16.8 $\pm$ 1.21	16.8 $\pm$ 1.21	16.8 $\pm$ 1.21
VII	27.5 $\pm$ 1.09	27.1 $\pm$ 1.30	26.8 $\pm$ 1.19	26.4 $\pm$ 1.06	27.1 $\pm$ 1.30	26.8 $\pm$ 1.19	27.8 $\pm$ 0.99	26.2 $\pm$ 1.12	26.4 $\pm$ 1.06	26.9 $\pm$ 1.20	26.9 $\pm$ 1.20	26.9 $\pm$ 1.20	26.9 $\pm$ 1.20	26.9 $\pm$ 1.20
VIII	18.6 $\pm$ 1.36	20.5 $\pm$ 1.26	20.2 $\pm$ 1.09	17.4 $\pm$ 1.21	20.5 $\pm$ 1.26	20.2 $\pm$ 1.09	18.2 $\pm$ 1.00	18.3 $\pm$ 1.19	17.4 $\pm$ 1.21	18.7 $\pm$ 1.46	18.7 $\pm$ 1.46	18.7 $\pm$ 1.46	18.7 $\pm$ 1.46	18.7 $\pm$ 1.46

Table 3. *Effect of the protein-calorie deficiency on the 0.14M-sodium chloride-soluble fraction of the liver nuclear proteins in young rats*

Results are mean percentages $\pm$ S.D. and changes marked * are statistically significant ($P < 0.01$). Each group of animals consisted of five rats. Changes in tris buffer-soluble fractions are identical in trend.

Protein value.....	After 7 days			After 14 days		
	10	3	0	10	3	0
Protein/DNA phosphorus (mg./mg.).....	17.9 $\pm$ 1.30	20.2 $\pm$ 1.76*	24.7 $\pm$ 1.30*	17.1 $\pm$ 1.16	20.6 $\pm$ 1.60*	23.5 $\pm$ 1.37*
Electrophoretic fractions						
I	5.4 $\pm$ 0.26	4.9 $\pm$ 0.70	5.7 $\pm$ 0.93	3.9 $\pm$ 0.91	5.4 $\pm$ 0.66	4.5 $\pm$ 0.49
II	—	5.3 $\pm$ 1.06*	5.3 $\pm$ 0.96*	—	7.1 $\pm$ 1.00*	8.2 $\pm$ 1.36*
III	6.0 $\pm$ 1.00	5.7 $\pm$ 1.06	5.7 $\pm$ 0.86	6.1 $\pm$ 1.12	5.3 $\pm$ 1.30	5.3 $\pm$ 1.66
IV	11.3 $\pm$ 1.45	10.2 $\pm$ 0.71	10.3 $\pm$ 0.70	12.0 $\pm$ 1.90	10.0 $\pm$ 1.96	10.7 $\pm$ 1.66
V	15.0 $\pm$ 0.95	14.7 $\pm$ 0.97	14.2 $\pm$ 1.27	14.6 $\pm$ 1.09	14.3 $\pm$ 1.70	14.0 $\pm$ 1.60
VI	16.5 $\pm$ 0.90	15.3 $\pm$ 1.06	14.7 $\pm$ 1.03	16.8 $\pm$ 0.91	14.5 $\pm$ 1.11	15.0 $\pm$ 1.66
VII	27.5 $\pm$ 0.94	25.6 $\pm$ 1.03	25.6 $\pm$ 1.26	27.5 $\pm$ 1.06	25.4 $\pm$ 1.74	25.7 $\pm$ 1.43
VIII	18.3 $\pm$ 3.27	18.7 $\pm$ 2.19	18.5 $\pm$ 2.90	17.8 $\pm$ 1.90	17.9 $\pm$ 1.66	16.6 $\pm$ 1.97

observed resulting from protein-calorie deficiency were similar to those reported by other workers.

DISCUSSION

The data presented show that protein-calorie deficiency produces a complex pattern of changes in liver cells. Thus an increase was found in the nuclear proteins soluble in 0.14M-sodium chloride and in tris buffer and in acidic medium (histones). The RNA, soluble in tris buffer and 0.05M-sodium hydroxide, was also increased; no changes were found in any of the other nuclear fractions examined. The protein and RNA contents of most other protoplasmic components diminished. Muntwyler *et al.* (1950) and Seifter *et al.* (1950), using rather crude techniques for isolation of nuclei, found a uniform loss of protein from all cellular fractions; however, the findings of Stenram (1958, 1963) are in general agreement with those presented here.

Electrophoresis of the 0.14M-sodium chloride and tris buffer-soluble fractions shows that most of the increase is associated with the appearance of a new fraction moving faster than serum albumin through acrylamide gel and thus presumably of low molecular weight. This fraction which appears and disappears rapidly on depletion and repletion cannot be classified as a histone since it was shown to be insoluble in acid. Zbarsky & Georgiew (1959) showed that weak neutral salt solutions extract extra-chromatin protein chiefly from the nuclear and nucleolar saps; it seems likely therefore that the 0.14M-sodium chloride extract in which the new component arises is derived from these structures.

The functional significance of the new fraction is a matter for speculation. Allfrey, Mirsky & Osawa

(1957) have shown very rapid rates of incorporation of labelled amino acids into proteins extracted with weak neutral phosphate buffers. Also, Evans, Holbrook & Irwin (1962) and Holbrook, Evans & Irwin (1962) obtained evidence that in regenerating liver the total amount of protein extracted with weak citrate solutions decreased markedly, just at the time when incorporation of labelled amino acids into cytoplasmic proteins was maximal. These experiments, taken in conjunction with the present work, point to the existence of a labile low-molecular-weight fraction, whose absence is associated with protein synthesis and whose presence is associated with an inhibition of synthesis, and it is noteworthy that Barr & Butler (1963) have shown that a low-molecular-weight non-specific compound such as polylysine can form complexes with DNA which inhibit RNA-polymerase activity and hence inhibit RNA and protein synthesis.

It is also of interest that the protein content of the histone fraction obtained from the livers of young, rapidly growing animals on a diet of protein value 10 was significantly lower than that of any of the other groups, but that, after 7 days on a low-protein or protein-free diet, the content had risen to a value similar to that found in the adult rats. There is now good evidence that histones play an important part in the regulation of protein synthesis; thus Allfrey & Mirsky (1963) and Allfrey *et al.* (1964) have demonstrated the formation of complexes between histones and the DNA needed as a primer for the synthesis of RNA. Also, Hindley (1963) has demonstrated that RNA polymerase can be inhibited by histones. Similarly several authors have been able to show an inhibitory action of histones on nuclear ATP synthesis and on the

Table 4. Effect of the protein-calorie deficiency on the ribonucleic acid, phosphorus/deoxyribonucleic acid phosphorus content of some nuclear fractions of the liver in rats

Results are mean values (mg.) $\pm$ s.d. and changes marked * are statistically significant ($P < 0.01$). Each group of animals consisted of five rats.

	Protein value.....	After 7 days			After 14 days			After re-feeding for 7 days
		3			3			
		10	0	0	10	0	0	
Adult animals	0.1 M-tris fraction	0.074 $\pm$ 0.005	0.079 $\pm$ 0.005	0.078 $\pm$ 0.004	0.070 $\pm$ 0.006	0.084 $\pm$ 0.007*	0.083 $\pm$ 0.009*	0.069 $\pm$ 0.007
	0.05 N-NaOH fraction	0.076 $\pm$ 0.006	0.089 $\pm$ 0.006	0.096 $\pm$ 0.007*	0.078 $\pm$ 0.005	0.091 $\pm$ 0.004*	0.097 $\pm$ 0.006*	0.067 $\pm$ 0.009
Young animals	0.1 M-tris fraction	0.083 $\pm$ 0.004	0.082 $\pm$ 0.004	0.105 $\pm$ 0.005*	0.075 $\pm$ 0.005	0.088 $\pm$ 0.004*	0.098 $\pm$ 0.006*	
	0.05 N-NaOH fraction	0.077 $\pm$ 0.018	0.142 $\pm$ 0.018*	0.130 $\pm$ 0.021*	0.073 $\pm$ 0.005	0.089 $\pm$ 0.009*	0.096 $\pm$ 0.006*	

activity of other oxidative enzymes (Person & Fine, 1960; Fenster, Allfrey & Mirsky, 1962; Huang & Bonner, 1962; McEwen, Allfrey & Mirsky, 1963).

At first sight, the rise in RNA found in the tris- and 0.05M-sodium hydroxide-soluble fractions from the livers of depleted rats seems to be in conflict with the evidence of reduced protein synthesis. However, the work of Zbarsky & Georgiew (1959) indicates that this material is probably derived from the nucleolus. Stenram (1958, 1963) has also found histochemical evidence of an increase in RNA in the nucleolus during protein depletion; this has been discussed by Munro & Clark (1960) and Munro (1964), who speculate that the RNA necessary for the structure of the membranes of microsomes may be accumulated in the nucleolus during periods when microsomal activity in the cytoplasm is reduced.

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