THE EFFECT OF LEVEL OF ENERGY INTAKE ON THE METABOLISM OF RIBONUCLEIC ACID AND PHOSPHOLIPIN IN DIFFERENT PARTS OF THE LIVER CELL

by

T. W. WIKRAMANAYAKE*, F. C. HEAGY** AND H. N. MUNRO
Department of Biochemistry, University of Glasgow (Scotland)

INTRODUCTION

Previous experiments (MUNRO, NAISMITH AND WIKRAMANAYAKE[1]) have demonstrated that the absolute rate of incorporation of phosphorus into liver ribonucleic acid (RNA) in the rat depends on the level of energy provided by the diet. The effect this has on the amount of RNA in the liver depends on the protein content of the diet. With diets containing adequate amounts of protein a rise in energy intake results in an increase in the total amount of RNA in the liver. In the case of diets free from protein an increment in energy intake does not greatly affect the total amount of RNA in the liver but it increases the rate of replacement of phosphorus atoms in the RNA.

This picture was obtained with whole liver. It seemed desirable, however, to investigate the effect of the level of energy intake on the metabolism of RNA in different parts of the cell, since several investigators have shown that the rate of synthesis of RNA varies in different fractions of the liver cell[2-6]. In the experiments to be reported, the rate of replacement of phosphorus atoms in the RNA of different cellular fractions was investigated in the livers of rats given different amounts of a protein-free diet. As a control procedure, the rate of incorporation of phosphorus into the phospholipin of the same cell fractions was examined, (*cf.* WIKRAMANAYAKE, MUNRO, NAISMITH AND HUTCHISON[7]). In addition, we investigated the total amounts of RNA, phospholipin and protein in these cell fractions when the animals had been fed on the protein-free or protein-containing diets at different levels of energy intake.

EXPERIMENTAL

Animals, diet and management. The general conditions were similar to those described by MUNRO AND NAISMITH[8]. Male albino rats weighing close to 180 g after fasting overnight were housed in individual cages under thermostatic conditions. A diet containing adequate amounts of protein or a protein-free diet was first fed at a level of 1200 kg cal/day/sq.m body surface area for one week. Then for a 4-day period energy intake was altered by varying the amount of carbohydrate fed, some animals receiving approximately 800 kg cal./sq.m and other 1400–1700 kg cal. At the end of this period the rats were killed under ether anaesthesia. The livers were rapidly perfused with 0.9%

* Present address: Faculty of Medicine, University of Ceylon, Colombo, Ceylon.
** British Empire Cancer Campaign Exchange Fellow. Present address: Department of Biochemistry, University of Western Ontario, London, Ontario, Canada.

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saline and then removed. In the experiments with radioactive phosphorus, 20 μc. of ^{32}P per 100 g body weight in the form of inorganic phosphate was injected intramuscularly at 2, 4 or 18 hours before death.

Fractionation of the liver. The isotonic sucrose technique of SCHNEIDER[9] was employed to separate different parts of the liver cells. Livers from single rats in the quantitative studies or from groups of 3 to 6 rats on the same level of protein and energy intake in the radioactivity studies were homogenized with ice-cold 0.25 *M* sucrose in a POTTER-ELVEHJEM[10] homogenizer. The suspension produced was examined microscopically to ensure that the majority of the cells had disintegrated and a portion was taken for analysis ("whole homogenate"). A knife-point of NaF was dissolved in the rest of the homogenate to inhibit enzymic breakdown of RNA during subsequent fractionation which was performed in a refrigerated centrifuge. The homogenate was first spun at 600 g to remove nuclei and unbroken cells ("nuclear fraction"). The precipitate was washed twice with 5 ml portions of 0.25 *M* sucrose and the washings were added to the supernatant. A portion of the supernatant was taken for analysis ("cytoplasmic fraction") and the remainder centrifuged at 8500 g for 10 min to precipitate the mitochondria and other large granules of the cytoplasm ("mitochondrial fraction"). The precipitate was washed once with 2.5 ml of 0.25 *M* sucrose. The supernatant and washings then were centrifuged at 18,000 g for 60 min to give a precipitate ("microsome fraction") and a residual fraction ("cell sap"). In the experiments in which the total amounts of RNA, phospholipin and protein in different fractions were estimated, the washing of the mitochondrial fraction was omitted. The adequacy of these fractionation procedures, as carried out in our laboratory, has been fully established by SMELLIE *et al.*[6].

In the experiments with ^{32}P, the nuclear fraction was purified further by suspending it in 0.05 *M* citric acid and stirring in a Nelco blendor, then centrifuging at 600 g for 10 min. The precipitate was resuspended in 0.01 *M* citric acid and centrifuged for 5 min. This procedure was repeated 3 or 4 times, until microscopical examination showed the nuclei to be relatively free from contaminating cytoplasmic material. As a further check on the uptake of ^{32}P by nuclear RNA, in some experiments a portion of the liver was stored overnight at $-10°$ and then the nuclei were isolated directly in citric acid as described by MIRSKY AND POLLISTER[11]. Nuclei prepared in this way exhibited the same effect of level of energy intake as nuclei prepared by the first method.

In one experiment the liver cytoplasm was fractionated by the saline technique of CLAUDE[12]. The livers of 5 rats were pooled and homogenized in ice-cold 0.85% (w/v) NaCl and then centrifuged twice at 1500 g for 10 min to remove unbroken cells and nuclei. The supernatant fraction was then spun at 18,000 g for 90 min to separate the granules (mitochondria and microsomes) from the rest of the cytoplasm.

Analytical procedures. All the fractions isolated were treated with 10% trichloroacetic acid. To estimate the radioactivity of the liver inorganic phosphate and the uptake of ^{32}P by RNA and phospholipin, the procedures described before[1,7] were used, except that in the case of RNA the lipid-free trichloroacetic acid precipitate was extracted with hot 10% (w/v) NaCl, as recommended by DAVIDSON AND SMELLIE[13], in order to remove radioactive contaminants more effectively before isolating the ribonucleotides by ionophoresis. The radioactivity of the phosphorus of the ribonucleotides, phospholipin and inorganic phosphate was expressed as the specific activity (counts per minute per 100 μg P) and from these data the relative specific activities of the ribonucleotide phosphorus and phospholipin phosphorus were derived (specific activity of ribonucleotide phosphorus or phospholipid P as a percentage of the specific activity of liver inorganic phosphorus).

In the quantitative experiments the slightly modified Schmidt-Thannhauser procedure employed previously[8] was used. Measurements of ultraviolet absorption at 260 mμ in a Beckman spectrophotometer showed that the ratio of ultraviolet-absorbing material to phosphorus in the alkaline digest was not altered by protein deficiency or change in energy intake in any of the fractions and this justifies the use of phosphorus as a measure of RNA in the alkaline digests from the cytoplasmic fractions.

RESULTS

Experiments with radioactive phosphorus. In Tables I, II, III and IV are reported the relative specific activities of the RNA phosphorus and phospholipin phosphorus of the liver cell fractions isolated 2, 4 or 18 hours after injection of labelled phosphorus into rats receiving different amounts of a protein-free diet. Since changes in the level of energy intake had essentially the same effect on ^{32}P uptake by each of the nucleotides, the data from the four nucleotides were averaged and are shown as the activity of the RNA. In order to compare different experiments, changes in relative specific activity produced by an increase in energy intake of 1000 kg cal./sq.m body surface area are

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expressed as a percentage of the relative specific activity corresponding to an energy intake of 1200 kg cal./sq.m.

In agreement with previous experiments on rats receiving protein-free diets[1], the relative specific activity of the RNAP in the liver cell as a whole increased when energy intake was raised (Tables I, II and III). At 2 h after ^{32}P injection, when nuclear RNAP shows about 10 times the radioactivity of cytoplasmic RNAP, the influence of energy intake is mainly confined to the nuclear RNA. At 4 and 18 h after injection an increased uptake of ^{32}P in response to the increment in energy supply is now shown by all cell fractions. There is, however, a quantitative difference in the response of the nucleus, mitochondria and microsomes on the one hand, and the response of the cell sap on the other, the uptake by the cell sap being less influenced by the plane of energy intake.

TABLE I

THE INFLUENCE OF LEVEL OF ENERGY INTAKE OF A PROTEIN-FREE DIET ON UPTAKE OF ^{32}P BY LIVER RIBONUCLEIC ACID AND PHOSPHOLIPIN IN DIFFERENT CELL FRACTIONS ISOLATED IN ISOTONIC SUCROSE 2 h AFTER INJECTION OF LABELLED PHOSPHORUS. (MEAN ACTIVITIES OF NUCLEOTIDES FROM POOLED LIVERS OF 4 RATS AT EACH ENERGY LEVEL. THE CHANGE PRODUCED BY AN INCREASE OF 1000 kg cal./sq.m SURFACE AREA HAS BEEN EXPRESSED AS A PERCENTAGE OF THE RELATIVE SPECIFIC ACTIVITY CORRESPONDING TO AN ENERGY INTAKE OF 1200 kg cal.)

Fraction	*Ribonucleic acid Relative specific activity*			*Phospholipin Relative specific activity*		
	810 kg cal./sq.m	*1750 kg cal./sq.m*	*Change/1000 kg cal.*	*810 kg cal./sq.m*	*1750 kg cal./sq.m*	*Change/1000 kg cal.*
Whole homogenate	2.04	2.41	+18%	13.9	13.1	−6%
Nucleus	10.2	13.3	+29%	14.0	11.1	−24%
Cytoplasm	1.41	1.27	−11%	14.9	13.3	−12%
Mitochondria	0.57	0.57	0%	13.0	11.4	−14%
Microsomes	0.57	0.65	+14%	15.2	14.2	−7%
Cell sap	2.45	2.07	−18%	15.5	14.5	−7%

TABLE II

THE INFLUENCE OF LEVEL OF ENERGY INTAKE OF A PROTEIN-FREE DIET ON UPTAKE OF ^{32}P BY LIVER RIBONUCLEIC ACID AND PHOSPHOLIPIN IN DIFFERENT CELL FRACTIONS ISOLATED IN ISOTONIC SUCROSE 4 h AFTER INJECTION OF LABELLED PHOSPHORUS. (MEAN ACTIVITIES OF NUCLEOTIDES FROM POOLED LIVERS OF 6 RATS AT EACH ENERGY LEVEL. THE CHANGE PRODUCED BY AN INCREASE OF 1000 kg cal./sq.m SURFACE AREA HAS BEEN EXPRESSED AS A PERCENTAGE OF THE RELATIVE SPECIFIC ACTIVITY CORRESPONDING TO AN ENERGY INTAKE OF 1200 kg cal.)

Fraction	*Ribonucleic acid Relative specific activity*			*Phospholipin Relative specific activity*		
	830 kg cal./sq.m	*1800 kg cal./sq.m*	*Change/1000 kg cal.*	*830 kg cal./sq.m*	*1800 kg cal./sq.m*	*Change/1000 kg cal.*
Whole homogenate	5.7	8.0	+36%	35.5	35.4	0%
Nucleus	28.4	34.9	+22%	28.4	26.8	−6%
Cytoplasm	4.1	5.6	+33%	36.9	36.6	−1%
Mitochondria	2.8	4.1	+40%	38.8	32.0	−19%
Microsomes	3.1	4.2	+34%	37.8	42.8	+13%
Cell sap	7.1	8.2	+15%	48.3	52.6	+9%

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TABLE III

THE INFLUENCE OF LEVEL OF ENERGY INTAKE OF A PROTEIN-FREE DIET ON UPTAKE OF ^{32}P BY LIVER RIBONUCLEIC ACID AND PHOSPHOLIPIN IN DIFFERENT CELL FRACTIONS ISOLATED IN ISOTONIC SUCROSE 18 h AFTER INJECTION OF LABELLED PHOSPHORUS. (MEAN ACTIVITIES OF NUCLEOTIDES FROM POOLED LIVERS OF 6 RATS AT EACH ENERGY LEVEL. THE CHANGE PRODUCED BY AN INCREASE OF 1000 kg cal./sq.m SURFACE AREA HAS BEEN EXPRESSED AS A PERCENTAGE OF THE RELATIVE SPECIFIC ACTIVITY CORRESPONDING TO AN ENERGY INTAKE OF 1200 kg cal.)

Fraction	*Ribonucleic acid Relative specific activity*			*Phospholipin Relative specific activity*		
	780 kg cal./sq.m	*1440 kg cal./sq.m*	*Change/1000 kg cal.*	*780 kg cal./sq.m*	*1440 kg cal./sq.m*	*Change/1000 kg cal.*
Whole homogenate	28.9	33.2	+ 20 %	99	104	+ 8 %
Nucleus	32.1	38.5	+ 27 %	98	98	0 %
Cytoplasm	26.6	33.5	+ 34 %	104	103	— 2 %
Mitochondria	22.7	28.1	+ 31 %	99	100	+ 2 %
Microsomes	23.4	29.0	+ 32 %	106	101	— 7 %
Cell sap	34.6	37.5	+ 12 %	102	101	— 2 %

The above data were obtained with the isotonic sucrose technique of SCHNEIDER[9]; in order to verify this difference in the behaviour of the cell sap from the rest of the cell, a further experiment was carried out in which the liver was fractionated by CLAUDE'S[12] isotonic saline procedure. The rats were killed 4 h after injection of ^{32}P and the cytoplasm was separated into a granule fraction (mitochondria and microsomes together) and the cell sap. Once more, the effect of raising energy intake was greater in the RNA of the granular fraction than in the RNA of the cell sap (Table IV). The phospholipin data given in Tables I, II and III show no changes in ^{32}P uptake comparable in magnitude to those revealed by RNA when energy intake was altered.

TABLE IV

THE INFLUENCE OF LEVEL OF ENERGY INTAKE OF A PROTEIN-FREE DIET ON UPTAKE OF ^{32}P BY LIVER RIBONUCLEIC ACID IN DIFFERENT CELL FRACTIONS ISOLATED IN 0.85 % NaCl 4 h AFTER INJECTION OF LABELLED PHOSPHORUS. (MEAN ACTIVITIES OF RIBONUCLEOTIDES FROM POOLED LIVERS OF GROUPS OF 5 RATS AT EACH ENERGY LEVEL. THE CHANGE PRODUCED BY AN INCREASE OF 1000 kg cal./sq.m HAS BEEN EXPRESSED AS A PERCENTAGE OF THE RELATIVE SPECIFIC ACTIVITY CORRESPONDING TO AN ENERGY INTAKE OF 1200 kg cal.)

Fraction	*Ribonucleic acid Relative specific activity*		
	780 kg cal./sq.m	*1530 kg cal./sq.m*	*Change/1000 kg cal.*
Whole homogenate	5.75	8.41	+ 46 %
Granules	2.83	3.59	+ 31 %
Cell sap	5.35	5.78	+ 10 %

Quantitative studies. These experiments were intended to provide a quantitative picture of changes in the total amounts of RNA, protein and phospholipin in the different parts of the liver cell when the energy content of the protein-containing or the protein-free diet was altered. Unfortunately, the nuclei did not separate quantitatively but were contaminated by unbroken cells. The extent of the contamination

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can be judged from the observation that the nuclear fractions of the four dietary groups shown in Figs. 1–3 contained 39, 45, 36 and 40% respectively of the total cellular protein. These figures, together with comparable data for RNA and phospholipin, suggest that the degree of cell contamination of the nuclear fractions was fairly uniform for the four dietary groups studied. This is reasonable, since all four groups were treated in the same manner during the separation procedures. Under the circumstances, we have limited ourselves to a consideration of the cytoplasmic fractions and have assumed that the recoveries of cytoplasm on all four diets were proportionately the same. The mitochondrial fraction may be somewhat in error, since there is reason to believe[14] that SCHNEIDER's[9] method of separating nuclei may result in some loss of mitochondria into the nuclear fraction. However, in view of the high centrifugal force (8500 *g*) needed

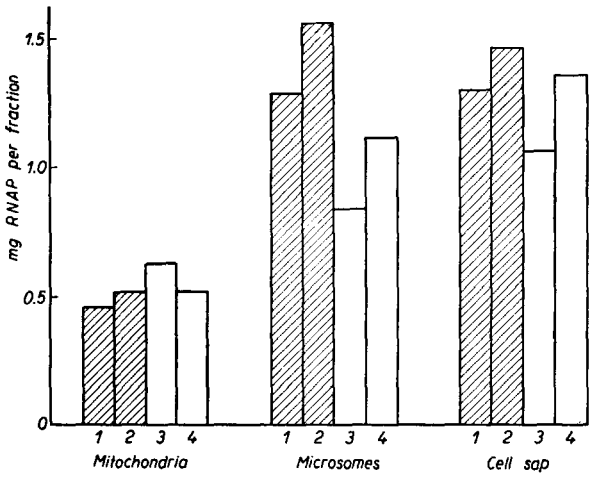

Fig. 1. The effect of different levels of protein and energy intake on the amount of ribonucleic acid in different cytoplasmic fractions of the liver. Animals receiving a protein-containing diet are indicated by shaded columns; those on a protein-free diet are shown by open columns. The levels of energy intake in kg cal./sq.m body surface area are indicated by the numbers below the columns: 1, 760 kg cal.; 2, 1660 kg cal.; 3, 790 kg cal.; 4, 1660 kg cal.

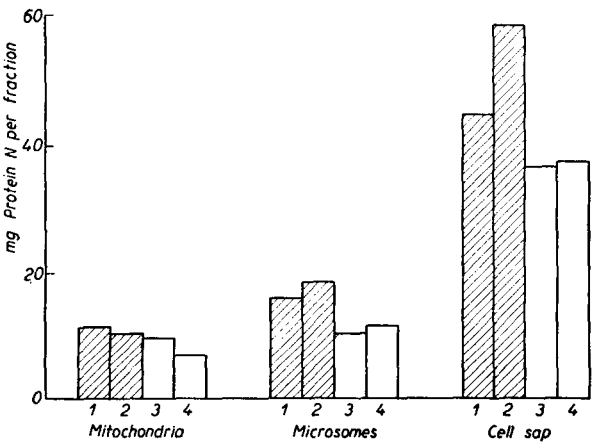

Fig. 2. The effect of different levels of protein and energy intake on the amount of protein in different cytoplasmic fractions of the liver. Protein and energy levels are indicated by shading and numbers as in Fig. 1.

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to sediment all the mitochondria, the loss from this fraction at 600 *g* is not likely to be large.

The data (Figs. 1, 2 and 3) allow us to compare the effect of varying the energy intake when the diet contains protein and when it is free from protein. In the case of RNA (Fig. 1), the total amount in the mitochondrial fraction showed little variation with diet. The total amount of RNA in the microsomes was considerably reduced by removal of protein from the diet, whereas the amount in the cell sap was only slightly affected. On the other hand, the RNA in both the microsomal and cell sap fractions was increased by raising the level of energy intake, and this effect was of the same magnitude irrespective of whether the diet contained protein or not.

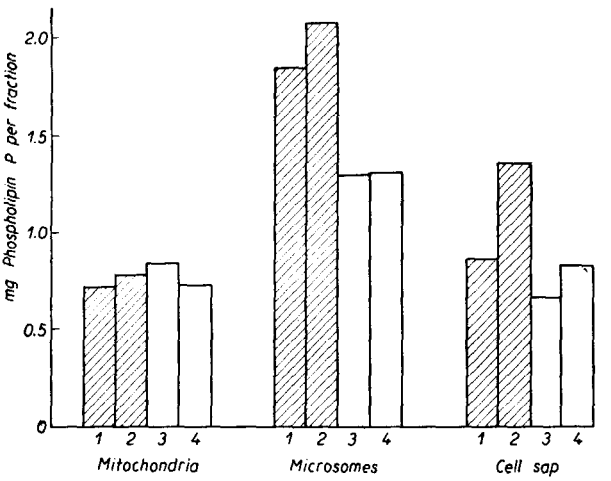

Fig. 3. The effect of different levels of protein and energy intake on the amount of phospholipin in different cytoplasmic fractions of the liver. Protein and energy levels are indicated by shading and numbers as in Fig. 1.

In the case of protein (Fig. 2), the mitochondrial fraction showed a reduction on the protein-free diet, and also with increments in energy intake. None of these changes was considerable. By contrast, both the microsomal and cell sap fractions were definitely affected by removal of protein from the diet. In the case of the microsomal fraction, increments in energy intake caused a small increase in total protein at both levels of protein intake. The amount of protein in the cell sap was considerably increased by a rise in energy intake when the diet contained protein, but was not appreciably altered by raising energy intake on the protein-free diet.

In the case of phospholipin (Fig. 3), the mitochondria again showed only slight variations with diet. The amounts in both the microsomes and the cell sap were influenced by protein level, and in each case a change in energy intake had more effect when the diet contained protein.

DISCUSSION

Previous experiments have demonstrated that addition of carbohydrate to a protein-free diet causes an increase in the uptake of phosphorus by liver RNA, accompanied by only minimal changes in the total amount of liver RNA[1]. The primary

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object of the present series of experiments has been to determine in what fraction or fractions of the liver cell this increased phosphorus uptake occurs. The results obtained for the whole cell at different time-intervals after ^{32}P injection show the same response to increased energy intake as was previously found. During the first 2 h after injection of the labelled phosphate this is almost entirely due to an increased uptake by the nucleus (Table I), an observation which is consistent with the established greater rate of RNA synthesis by the nucleus. At 4 h and at 18 h (Tables II and III) all fractions now show an increased phosphorus uptake into RNA with raised energy intake, but in all these experiments the level of energy intake had less effect on the RNA of the cell sap than on the nucleus and granules, and even at the 2 h interval the cell sap fraction is less favourably affected than the other cellular fractions when energy intake is increased.

In an attempt to throw light on this peculiarity of the cell sap fraction, we studied quantitative changes occurring in certain constituents of the cellular fractions. This revealed that the RNA of the cell sap, unlike the protein and phospholipin of this fraction, was not appreciably diminished by removal of protein from the diet. In this respect it differed from the microsome fraction in which all 3 constituents were reduced in total amount by transferring the animals to a protein-free diet, and from the mitochondrial fraction, in which none of the constituents was consistently changed by varying the protein level. Confirmation of these findings can be obtained by recalculating the data of MUNTWYLER, SEIFTER AND HARKNESS[14] on the intracellular distribution of RNA at different levels of protein intake (Table V). In this calculation the total amount of RNA for each cell fraction can be obtained by using the deoxyribonucleic acid content of the liver as a reference standard[15]; this has the advantage of expressing the amount of RNA in terms of a liver constituent which is proportional to the number of liver cells and is unaffected by the protein-free diet. It is apparent (Table V) that removal of protein from the diet affects only the RNA of the microsomes but not of the mitochondria or cell sap. Recalculation of other experiments reported by these authors[16] also shows this picture. VENDRELY AND VENDRELY[17] have reached a similar conclusion in a study of protein depletion.

TABLE V

RECALCULATION OF THE DATA OF MUNTWYLER *et al.*[14] TO SHOW THE TOTAL AMOUNT OF RIBONUCLEIC ACID IN VARIOUS LIVER CELL FRACTIONS AT DIFFERENT LEVELS OF PROTEIN INTAKE. (THE RIBONUCLEIC ACID IN EACH FRACTION HAS BEEN RELATED TO THE DEOXYRIBONUCLEIC ACID OF THE WHOLE CELL AS A REFERENCE STANDARD)

Fraction	*mg ribonucleic acid phosphorus in fraction per 100 mg deoxyribonucleic acid phosphorus in whole homogenate*		*Change on Protein-free diet*
	Protein-containing diet	*Protein-free diet*	
Nucleus	42	31	−26%
Mitochondria	16	18	+13%
Microsomes	155	114	−26%
Cell sap	114	123	+ 8%

When we consider the radioactivity and quantitative data together, it is thus apparent that the cell sap differs from the other fractions in two respects, the rate of ^{32}P uptake is less affected by energy level on the protein-free diet, and the total amount

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of RNA in the fraction is sensitive to energy intake but not to protein intake. It will be noted that, although the total RNA of the mitochondrial fraction is also little influenced by protein level, it is not appreciably altered in amount by energy level. The reason for the peculiarities of RNA metabolism in the cell sap is not at all clear; it may be that the RNA and protein contained in the cell sap react differently to variations in protein intake because of some function of RNA in this fraction other than protein synthesis. In support of this, it is to be noted that the microsomes appear to play a major role in protein synthesis[18] and in this fraction RNA and protein behave similarly towards variations in protein intake.

ACKNOWLEDGEMENTS

We are much indebted to Dr. R. M. S. SMELLIE for advice on liver fractionation procedures. The work was carried out with the aid of a grant for expenses from the Medical Research Council of Great Britain to one of us (H.N.M.).

SUMMARY

1. Rats receiving protein-free diets of differing energy content were killed at various time-intervals after injection with ^{32}P. The livers were then separated into nuclear, mitochondrial, microsomal and cell sap fractions. An increase in the level of energy intake eventually caused a greater incorporation of ^{32}P into the ribonucleic acid of all fractions, but the effect was less marked in the case of the cell sap. The uptake of ^{32}P by the phospholipins was not increased in any cell fraction.

2. Studies of the amount of ribonucleic acid, protein and phospholipin in different fractions of the liver cell cytoplasm were made in relation to the amount of protein and energy in the diet. Only the microsome fraction showed definite changes in the amount of ribonucleic acid when protein was withdrawn from the diet. The quantitative changes found in the ribonucleic acid of different cell fractions are discussed in relation to the rate of ^{32}P incorporation.

RÉSUMÉ

1. Des rats qui avaient reçu un régime non-protéique avec des quantités différentes d'énergie ont été tués à divers temps après l'injection de ^{32}P. Les foies ont été alors séparés en diverses fractions cellulaires — noyaux, mitochondries, microsomes et suc cellulaire. L'incorporation de ^{32}P dans l'acide ribonucléique de toutes ces fractions était plus rapide quand le niveau d'énergie s'élevait, mais cet effet était moins marqué dans le cas de l'acide ribonucléique du suc cellulaire. L'incorporation de ^{32}P dans les phospholipides n'augmentait dans aucune fraction.

2. Des études sur la quantité de l'acide ribonucléique, de protéines et de phospholipides dans les différentes fractions cytoplasmiques du foie ont été faites en relation avec la quantité d'énergie et de protéines dans le régime. Seulement la fraction microsomique a manifesté des changements dans la teneur en acide ribonucléique pendant le jeûne protéique. On a discuté les changements dans la teneur en acide ribonucléique des fractions en relation avec la vitesse d'incorporation de ^{32}P.

ZUSAMMENFASSUNG

1. Mit proteinfreier Nahrung unterschiedlichen Energieinhalts ernährte Ratten wurden nach Injektion mit ^{32}P nach verschieden langen Zeitabständen getötet. Die Leber wurde dann in eine Kern-, eine Mitochondrien-, eine Mikrosom- und eine Zellsaftfraktion aufgeteilt. Eine Erhöhung der Energieaufnahme verursachte einen erhöhten Einbau des ^{32}P in die Ribonucleinsäure bei allen Fraktionen, jedoch war die Wirkung im Falle des Zellsaftes am wenigsten ausgeprägt. Im Phospholipin wurde die ^{32}P-Aufnahme in allen Zellfraktionen dadurch nicht erhöht.

2. Es wurden Untersuchungen angestellt, in wie weit der Ribonucleinsäure-, der Protein- und der Phospholipingehalt der verschiedenen Fraktionen des Leberzellcytoplasmas in Beziehung zum Protein- und Energiegehalt der Nahrung steht. Nur in der Mikrosomenfraktion wurde eine deutliche Veränderung des Ribonucleinsäuregehaltes bei Proteinentzug in der Nahrung beobachtet. Die gefundenen quantitativen Veränderungen des Ribonucleinsäuregehaltes der verschiedenen Zellfraktionen wurden in Bezug zu der Geschwindigkeit des ^{32}P-Einbaues besprochen.

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Received April 20th, 1953