SOME OBSERVATIONS ON PROTEIN METABOLISM IN CHROMOSOMES OF NON-DIVIDING CELLS

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In cells of the liver and pancreas of mature animals (in which few dividing cells are present) the bulk of protein synthesis occurs in the cytoplasm (1–4 and 6). The experiments to be described in this paper show that under certain circumstances syntheses of protein in the cytoplasm and in the chromosomes are correlated. In these experiments, which were done on mice, protein metabolism was varied by fasting and subsequent feeding, rates of protein synthesis being low in a period of fasting and rising when feeding was resumed.

Changes in protein metabolism were followed by injecting glycine labelled with N^{15}. Both rates of uptake of N^{15} by proteins of the cell and also of retention of N^{15}, previously incorporated, were measured. Each protein fraction takes up more and more N^{15}, reaching a maximum, and then gradually loses what it has taken up. Uptake of N^{15}-glycine is considered to involve protein synthesis, although the possibility of exchange reactions should also be considered. Retention of N^{15}-glycine may simply be a measure of loss of labelled protein by the cell, but under certain conditions it may also be an indication of protein synthesis.

Tissue proteins of both nucleus and cytoplasm were examined for their N^{15} contents. Cytoplasmic proteins were obtained from sucrose homogenates. Unbroken cells, nuclei, and mitochondria were discarded, and two fractions were retained: the pellet material which sediments by centrifugation at high speed, in which much of the cytoplasmic nucleoprotein is found (5); and the protein remaining in the supernate after high-speed centrifugation, a fraction which, in the pancreas, is rich in the secretory enzymes (6). To obtain nuclear proteins, the nuclei were first isolated by the citric acid procedure (7). In the course of isolation by this procedure certain proteins are unfortunately extracted from nuclei and lost. Histone and "residual protein," both constituents of chromosomes, are, however, retained in the nuclei, and these proteins were isolated.

It should first be noted in a general way how the various protein components compare in their uptake of N^{15}. These differences in rate of uptake have already been described in previous papers (1–4, 6, and 8) and they can be seen in any of the tables of this paper. In the pancreas cytoplasmic ribonucleoprotein has

by far the most rapid rate of uptake for the 1st hour, and afterwards the extent of N^{15} incorporation into this fraction is equalled and then exceeded by the supernatant protein of the cytoplasm. The ribonucleoprotein of the pancreas, however, can be further fractionated by ribonuclease digestion. One fraction obtained in this way (see entries in Table II for "pellet fragment") reaches a far higher level of activity at early times than does "supernatant" protein, and satisfies the requirements of a precursor material (6). In liver and kidney the cytoplasmic ribonucleoprotein has a higher level of activity than all other fractions considered for the time interval under investigation. Rates of N^{15} incorporation into histone and residual chromosome protein are lower, that for histone being the lowest of any of the protein components considered in the present investigation, and that for residual protein coming closer to the over-all rate for cytoplasmic protein.

In the first series of the present experiments mice were subjected to a prolonged fast and then divided into two groups in both of which uptake of N^{15}-glycine was measured. In one group N^{15}-glycine was injected into animals immediately after being fed and in the other group while they were still fasting. The N^{15} uptake determinations of the various protein components, cytoplasmic and nuclear, are given in Table I for three organs—the pancreas, liver, and kidney.

Considering first the pancreas, the figures given in the top rows of the first columns show that in the first 1½ hours feeding caused a sharp increase in N^{15} uptake by the mixed tissue proteins and by the nucleoprotein and supernatant protein of the cytoplasm. For the corresponding times there is also a marked increase in N^{15} uptake by chromosomal proteins—histone and residual protein.

The increases in N^{15} uptake due to feeding observed in the pancreas are not found in liver and kidney. The effect of feeding on these tissues is, on the contrary, either to leave N^{15} uptake by proteins almost unchanged or to produce a slight decrease; and, it should be noted, the unchanged or diminished N^{15} uptake is found in the chromosomal proteins as well as in the cytoplasmic. The decrease in uptake is probably to be attributed to the glycine derived from the food diluting the injected N^{15}-glycine and so competing with it for incorporation into proteins. Feeding probably causes an increase in protein synthesis in the liver and kidney, as well as in the pancreas, but in the former organs N^{15}-glycine uptake is impeded by the dilution effect to such an extent that an increase in synthesis is obscured. It should be emphasized that whatever the explanation of the difference due to feeding in N^{15} uptake by liver and kidney on the one hand and by pancreas on the other, in all tissues the effects observed in cytoplasmic proteins are paralleled by those in chromosomal proteins.

In another series of experiments the effect of feeding on the retention of N^{15} by proteins was studied. The N^{15} uptake experiments yield significant

TABLE I

Effects of Feeding and Fasting upon Glycine-N^{15} Incorporations into Nuclear and Cytoplasmic Proteins of Pancreas, Liver, and Kidney

The N^{15} concentration in the various protein fractions is expressed relative to that of administered glycine-N^{15} taken as 100. (Actual N^{15} concentration of injected glycine 33.3 atom per cent N^{15} excess.)

All animals were fasted for 4 days, then divided into two groups. Animals in the first group, data for which are listed in the "fasted" columns of the table, were given N^{15}-glycine intraperitoneally at zero time, and killed at the time intervals recorded.

Animals in the second group were fed 30 minutes prior to administering N^{15}-glycine (at $t = 0$), then killed at the successive intervals recorded in the table.

Time	Atom per cent N^{15} excess $\times \frac{100}{33.3}$									
	Homogenate		Cytoplasmic proteins				Nuclear proteins			
			Ribonucleoprotein pellet		Supernate		Histone		Residual chromosome	
	Fasted	Fed	Fasted	Fed	Fasted	Fed	Fasted	Fed	Fasted	Fed
					(*a*) Pancreas					
hrs.										
0.5	0.288	0.429	0.579	0.778	0.258	0.472	0.054	0.084	0.114	0.184
1.0	0.460	0.597	0.781	1.138	0.569	1.138	0.072	0.097	0.250	0.331
1.5	0.703	1.063	1.114	1.229	1.045	2.162	0.096	0.180	0.423	0.541
3.0	1.129	1.343	0.889	1.166	2.161	2.103		0.195		0.667
	1.407	1.382					0.167			
4.5	1.164	1.057	1.174	0.709	1.871	2.194				
6.0	1.081	1.510	1.268	1.114	1.624	2.330				
					(*b*) Liver					
0.5	0.150	0.159	0.264	0.306	0.105	0.111	0.066	0.102	0.114	0.125
	0.162	0.159					0.094	0.122		
1.0	0.250	0.269	0.463	0.478	0.197	0.197	0.091	0.084	0.219	0.203
1.5	0.309	0.255	0.679	0.564	0.285	0.297	0.120	0.120	0.285	0.276
3.0	0.631	0.553	0.823	0.768	0.622	0.607	0.198	0.192	0.625	0.520
4.5	0.664	0.661	0.883	0.789	0.682	0.631				
6.0	1.087		1.223	1.081	1.084	0.858				
					(*c*) Kidney					
0.5	0.084						0.054			
1.5	0.144	0.150	0.241	0.252	0.156	0.162	0.063	0.063	0.132	
3.0	0.367	0.324	0.501	0.466	0.423	0.406	0.126	0.126		
4.5	0.436	0.393	0.594	0.532	0.501	0.453				
6.0	0.595	0.552	0.871	0.859	0.775					

results within several hours. In the experiments on N^{15} retention animals were studied for periods up to 90 hours. Mice were injected with a single dose of N^{15}-glycine and then divided into two groups: one was fed for the next 90

hours; and the other was given nothing but glucose during this time. In Tables II, III, and IV determinations are given of the retention at varying time intervals of N^{15} by cytoplasmic and chromosomal proteins of pancreas, liver, and kidney. The main result of these experiments can be stated briefly: the effect of feeding is a marked decrease in retention of N^{15} by *both* cytoplasmic proteins and the histone and residual protein of chromosomes. Changes in N^{15} concentration in liver and kidney proteins due to feeding which were not found in the uptake experiments are observed in the retention studies.

Decreased retention of N^{15}, loss of N^{15}, caused by feeding in these experiments can be attributed to an increased rate of synthesis of unlabelled protein and to a more rapid loss of the originally labelled material. In the pancreas, particularly, feeding will obviously increase the secretion of digestive enzymes and so induce an increased synthesis of protein within the gland. It is noteworthy that along with the over-all rise in protein synthesis in the pancreas there is also an increase in the rate of histone synthesis. This correlation in the pancreas between synthesis of digestive enzymes and synthesis of histone was also found in experiments on N^{15}-glycine uptake. The top three rows of Table I show that an increase caused by feeding in the N^{15} content of supernatant cytoplasmic protein (which is a fraction rich in the digestive enzymes) is accompanied by a sharp rise in the N^{15} content of histone.

Variations in uptake and retention of N^{15} by histones that are correlated with over-al changes of protein synthesis in tissues show that synthesis of histone is iln some way connected with synthesis of other proteins; and yet the N^{15} uptake rate for histone is far lower than those for the other proteins considered in the present investigation. It is possible that the relatively low N^{15} uptake of histone is due to variations in activity of histones in different chromosomal regions. This would mean that certain histone fractions have even lower N^{15} uptakes than those that have been determined and that a small fraction would show a high activity. In this connection it should be mentioned that fractionation experiments on histones have already shown histone to be a complex mixture and that there is a group of histones, exceedingly rich in lysine (9). The lysine-rich histones do not precipitate in the range about pH 10.5, and so would not have been included in the present determinations. Low N^{15} uptake by histones may, on the other hand, be due to the fact that they are combined with DNA, a substance showing an exceedingly low turnover in non-dividing cells (8, 10–12).

The experiments described in this paper show clearly that under certain conditions uptake and retention of N^{15} by cytoplasmic and chromosomal proteins are correlated. These experiments do not disclose just how the correlation is accomplished. In a general way, however, it may be said that feeding mice brings about changes first of all in the cytoplasm of pancreas, liver, and kidney cells and that the changed condition of the cytoplasm in some way

TABLE II

Time Course of N^{15} Incorporation into Nuclear and Cytoplasmic Proteins of Mouse Pancreas Tissue

N^{15} concentration in protein expressed relative to that of administered glycine taken as 100. (N^{15} concentration in glycine administered 33.3 atom per cent N^{15} excess.)

(*a*) Uptake observed after intraperitoneal injection of N^{15}-glycine at time zero. Animals fasted during the experiment and for 20 hours prior to glycine administration.

(*b*) N^{15} retention in pancreas proteins of mice during prolonged fasting. Each animal received N^{15}-glycine intraperitoneally at time zero. Animals had access to a glucose-vitamin solution, but no other food was supplied.

(*c*) N^{15} retention in pancreas proteins of fed mice. Each animal received N^{15}-glycine intraperitoneally at time zero. Animals fed their regular diet throughout the duration of the experiment.

The pellet fragment comprises the proteins which are released by ribonuclease digestion of the total ribonucleoprotein pellet (6).

Time	Atom per cent N^{15} excess $\times \frac{100}{33.3}$					
	Homogenate	Cytoplasmic proteins			Nuclear proteins	
		Ribonucleoprotein pellet	Pellet fragment	Supernate	Histone	Residual chromosome
(*a*) Glycine-N^{15} uptake in pancreas protein following a 20 hour fast						
hrs.						
0.5	0.315	0.550		0.201		
1.0	0.834	1.320		0.765		
1.5	1.119	1.596	3.426	1.635	0.189	0.562
2.0	1.254	1.314		1.779		
3.0	1.086	1.017	2.240	1.248	0.233	0.638
4.0	0.924	0.894		1.131		
4.5	0.860	0.820		1.130		
5.0	0.834	0.762		1.134	0.197	0.655
6.0	0.939				0.203	0.673
(*b*) N^{15} retention in pancreas proteins of fasted animals						
17.5	0.568	0.875	0.657	0.762		
18.25	0.503				0.200	0.525
41.5	0.472	0.353	0.403	0.641		
42.0	0.428				0.178	
89.0	0.409	0.338		0.525		
90.0	0.350				0.175	0.425
(*c*) N^{15} retention in pancreas proteins of fed animals						
1.0	0.722				0.111	0.348
24.0	0.322				0.131	
49.0	0.252					
91.0	0.178				0.078	

TABLE III

Time Course of N^{15} Incorporation into Nuclear and Cytoplasmic Proteins of Mouse Liver Tissue

N^{15} concentration in protein expressed relative to that of administered glycine taken as 100. (N^{15} concentration in glycine administered 33.3 atom per cent N^{15} excess.)

(*a*) Uptake observed after intraperitoneal injection of N^{15}-glycine at time zero. Animals fasted during the experiment and for 20 hours prior to glycine administration.

(*b*) N^{15} retention in liver proteins of mice during prolonged fasting. Each animal received N^{15}-glycine intraperitoneally at time zero. Animals had access to a glucose-vitamin solution, but no other food was supplied.

(*c*) N^{15} retention in liver proteins of fed mice. Each animal received N^{15}-glycine intraperitoneally at time zero. Animals fed their regular diet throughout the duration of the experiment.

Time	Atom per cent N^{15} excess $\times \frac{100}{33.3}$				
	Homogenate	Cytoplasmic proteins		Nuc'ear proteins	
		Ribonucleo-protein pellet	Supernate	Histone	Residual chromosome
(*a*) Glycine-N^{15} uptake in liver protein following a 20 hour fast					
hrs.					
0.5	0.177	0.321	0.147		
1.0	0.330	0.630	0.300		
1.5	0.399	0.669	0.366	0.207	0.388
2.0	0.519	0.777	0.471		
3.0	0.551	0.810	0.567	0.255	0.534
4.0	0.588	0.729	0.528		
4.5	0.620	0.805	0.570		
5.0	0.696	0.819	0.579	0.222	0.647
6.0				0.243	0.702
(*b*) N^{15} retention in liver proteins of fasted animals					
17.5	0.850	0.983	0.875		0.768
18.25	0.778			0.241	0.741
41.5	0.609	0.697	0.697		0.597
42.0	0.682			0.265	
89.0	0.516	0.587	0.578	0.206	0.503
90.0	0.528			0.231	0.497
(*c*) N^{15} retention in liver proteins of fed animals					
1.0	0.339			0.132	0.291
24.0	0.538			0.234	0.553
49.0	0.434			0.203	0.466
91.0	0.316			0.122	0.291

produces a change in the nucleus. Modifications in chromosomal proteins may, therefore, be regarded as a response to an altered cytoplasm. Since it is well

TABLE IV

Time Course of Glycine-N^{15} Incorporation into Nuclear and Cytoplasmic Proteins of Mouse Kidney Tissue

N^{15} concentration in protein expressed relative to that of administered glycine taken as 100. (N^{15} concentration in glycine administered 33.3 atom per cent N^{15} excess.)

(*a*) Uptake observed after intraperitoneal injection of N^{15}-glycine at time zero. Animals fasted during the experiment and for 20 hours prior to glycine administration.

(*b*) N^{15} retention in kidney proteins of mice during prolonged fasting. Each animal received N^{15}-glycine intraperitoneally at time zero. Animals had access to a glucose-vitamin solution, but no other food was supplied.

(*c*) N^{15} retention in kidney proteins of fed mice. Each animal received N^{15}-glycine intraperitoneally at time zero. Animals fed their regular diet throughout the duration of the experiment.

Time	Atom per cent N^{15} excess $\times \frac{100}{33.3}$			
	Homogenate	Cytoplasmic proteins	Nuclear proteins	
		Ribonucleoprotein pellet	Histone	Residual chromosome
(*a*) Glycine-N^{15} uptake in kidney proteins following a 20 hour fast				
hrs.				
0.5	0.075	0.138		
1.0	0.153	0.261		
1.5	0.225	0.330	0.111	0.171
2.0	0.297	0.385		
3.0	0.330	0.487	0.137	0.260
4.0	0.334	0.451		
4.5	0.364	0.507		
5.0	0.394	0.528	0.137	0.332
6.0	0.482		0.156	
(*b*) N^{15} retention in kidney proteins of fasted animals				
17.5	0.657			
18.25	0.606		0.184	
41.5	0.509			
42.0	0.491		0.141	
89.0	0.457			
90.0	0.443		0.122	
(*c*) N^{15} retention in kidney proteins of fed animals				
1.0	0.183			
24.0	0.384		0.119	
49.0	0.316			
91.0	0.256		0.088	

known that chromosomes influence activities in the cytoplasm, it may be supposed that modifications in chromosomal proteins, especially those com-

bined with DNA, will affect the way in which the chromosomes influence the cytoplasm. There can be little doubt that we are here dealing in a fragmentary way with interactions between cytoplasm and chromosomes, and that the pattern of interaction here considered—the cytoplasm, changed by external conditions, producing modifications in the chromosomes and these modifications reacting upon the cytoplasm—holds for physiological changes in non-dividing cells and also for the differentiation that occurs in the course of development.

EXPERIMENTAL

Adult animals, both male and female, of the Rockefeller Institute colony of Swiss mice were used in these experiments. They were kindly given to us by the Laboratories of the Division of Medicine and Public Health of The Rockefeller Foundation. The mice were 6 or 7 months old and just past their prime with respect to breeding, though still capable of reproduction. In the experiments summarized in Table I, the animals were subjected to a prolonged fast of 4 days before the injection of glycine. A fast of 4 days is extreme for mice. At the end of the fast 15 per cent of the animals were dead or moribund while the survivors had lost about 25 per cent of their body weight. The livers and pancreases of these mice had lost about one-third of their weight. The survivors were divided into two groups: one group was fed whole milk to which 3 per cent casamino acids (Difco) and 0.03 per cent *l*-tryptophane were added. 30 minutes after the mice had begun to feed, they were injected with N^{15}-glycine (33.3 atom per cent N^{15} excess). The other group was injected with glycine while still fasting. The injections of glycine were given intraperitoneally, the animals receiving 1.0 mg. glycine per 0.75 gm. body weight.

In the series of experiments in which the retention of N^{15} by proteins was studied, the animals were injected with N^{15}-glycine (1.0 mg. per gm. body weight) and then divided into two groups: one group was fed its regular diet of bread, milk, and Purina fox chow; the other was fed a nitrogen-free diet consisting of mammalian Ringer's solution containing 10 per cent glucose and 0.3 ml. per liter poly-vi-sol (Mead Johnson and Co.) for the period of the experiment.

All animals were killed by decapitation after ether anesthesia. The organs were removed and placed immediately into a container chilled in ice or directly into an ice cold sucrose or citric acid solution. All further operations were carried out in the cold except when otherwise indicated. For each preparation of nuclear proteins, the organs of about 50 mice were required, while for the preparation of pellet protein 8 mice were used.

Preparation of the Pellet Material.—Tissue samples were homogenized in either a glass tube fitted with a teflon pestle or a Waring blendor run at low speed (35 v). For pancreas a solution of 0.25 M sucrose containing 0.01 M citrate (to inhibit desoxyribonuclease) and 0.1 mg./ml. soy bean trypsin inhibitor was used. Liver and kidney were homogenized in 0.25 M sucrose.

Homogenates were centrifuged at 5000 × *g* for 10 minutes, the residues washed once with sucrose solution, and the combined supernatants and washings centrifuged again for 10 minutes at 5000 × *g*. The sediments contained nuclei, mitochondria, and unbroken cells, and were discarded. The final supernatant was centrifuged in the

Spinco model L ultracentrifuge for 30 minutes at 40,000 R.P.M. (105,400 × *g*). The pellet was washed by resuspension in sucrose and resedimented in the ultracentrifuge.

The pellet material was resuspended and treated with ribonuclease to remove RNA; the protein was precipitated with 5 per cent trichloroacetic acid, washed several times with trichloroacetic acid, hot alcohol, and ether, and dried for 10 minutes at 110°C.

The protein remaining in the supernatant obtained after sedimenting the pellet material was also prepared for N^{15} analysis. The supernatant solution was brought to pH 5.4 by the addition of acetic acid in order to precipitate nucleoprotein which did not sediment in the ultracentrifuge. The supernatant proteins were then precipitated with trichloroacetic acid and washed with trichloroacetic acid, hot alcohol, and ether and dried.

Preparation of Nuclear Proteins.—Nuclei were isolated by the citric acid procedure (7). Tissues were placed in the Waring blendor with a mixture of ice and a large volume of 1 per cent citric acid and blended for 8 minutes. The homogenate was strained through gauze and flannelette, and then centrifuged. By repeated washings with 0.2 per cent citric acid and by gradually diminishing the speed and time of centrifugation, microscopically clean preparations of liver and pancreas nuclei were obtained. Good preparations of kidney nuclei could not be obtained by this procedure, but they were satisfactory for the preparation of histone.

Histone was prepared by extracting one portion of the suspension of nuclei with 0.25 N H_2SO_4 (final concentration) in the cold for 10 minutes. The extract was dialyzed against water, and the histone precipitated by the addition of alkali to pH 10.6. To insure complete precipitation of histone it was necessary to allow the suspension to stand for one-half hour at room temperature or slightly above. The suspension was centrifuged and the histone redissolved in H_2O with the addition of acid. The histone was reprecipitated at pH 10.6, washed with hot alcohol and ether, and dried.

To isolate the residual protein another portion of nuclei was used because in the procedure just described not all the histone is removed. The nuclei were extracted overnight with a solution of 1 M NaCl in 1 per cent citric acid (final concentration) and washed twice more with this solution. In this manner all the histone is removed. The residue was washed with water, with 0.1 M phosphate buffer pH 7.6, and then treated with ribonuclease and desoxyribonuclease to remove nucleic acid. The protein remaining is the residual protein. This was washed with trichloroacetic acid, alcohol, and ether and dried.

N^{15} Analyses.—The N^{15} concentrations of the proteins were determined in the mass spectrometer (Process and Instruments Company model) after the usual conversion of organic nitrogen to gaseous nitrogen by Kjeldahl digestion and treatment with hypobromite. N^{15} concentrations of the samples were determined to within plus or minus 2 per cent (average deviation) and compared with tank nitrogen as standard.

SUMMARY

1. The metabolism of chromosomal proteins has been studied in the pancreas, liver, and kidney of adult mice (*a*) by measuring the rates of glycine-N^{15} incorporation into histones and residual chromosome fractions, and (*b*) by measuring the extent to which N^{15}, once incorporated into chromosomal proteins, is retained.

2. The uptake of isotopic nitrogen by these nuclear constituents was compared with that of protein fractions prepared from the cytoplasm by differential centrifugation in sucrose solutions. One such fraction, which comprises the bulk of the ribosenucleoprotein of the cell sediments as a pellet on high speed centrifugation. The supernatant remaining after this centrifugation is a fraction which, in the pancreas, is rich in the secretory enzymes synthesized by the cell.

3. A comparison of the rates of glycine-N^{15} uptake shows that cytoplasmic ribonucleoprotein is the most active of the protein fractions analyzed. In the pancreas it meets the conditions required of a precursor for the secretory enzymes of the supernate.

4. In all tissues considered the rates of glycine-N^{15} uptake into histone and residual chromosome fractions are lower, that for histone being the lowest of any of the protein components considered and that for residual protein approximating the over-all rate for cytoplasmic protein.

5. The effects of feeding and fasting upon glycine-N^{15} incorporation have been studied. In the pancreas, feeding causes a sharp increase in N^{15} uptake by the mixed tissue proteins and by the nucleoprotein and supernatant protein of the cytoplasm. There is a parallel increase in N^{15} uptake by the chromosomal constituents—histone and residual protein.

6. A parallelism between N^{15} uptake in cytoplasmic and chromosomal proteins is also observed in the liver and kidney when over-all protein metabolism is altered by feeding and fasting.

7. The responsiveness of the histones and residual proteins to changes in the environment has also been demonstrated in N^{15} retention experiments. The loss of isotope once incorporated into chromosomal proteins is much more rapid in fed than in fasted animals.

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