UPTAKE OF GLYCINE-N^{15} BY COMPONENTS OF CELL NUCLEI

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An indication of the relative metabolic activities of various components of the cell nucleus is given by the rates at which they take up the nitrogen of glycine labelled with N^{15}. The nuclear components which we have examined in the work here reported are desoxyribonucleic acid (DNA) and two protein fractions, histone and residual protein. Nuclear components of two kinds of cells have been studied: those of mammalian liver and pancreas, tissues with high metabolic rates; and those of avian erythrocytes and echinoderm sperm, cells with relatively low rates of metabolism.

No other nuclear components than DNA, histone, and residual protein were studied because nuclei were isolated by the citric acid procedure, in the course of which much material is removed from nuclei (1). When nuclei are isolated from liver or pancreas with citric acid, as much as 55 per cent of their protein may be extracted. For an understanding of the role of the nucleus in the metabolism of the cell, it would be of interest to know how rapidly N^{15} is taken up by these proteins as well as by histone and residual protein. To obtain these readily extracted proteins, nuclei must be isolated in non-aqueous media. The isolation of clean nuclei by means of non-aqueous solvents is, however, far more difficult that it is with citric acid. The substances that remain in citric acid nuclei are of considerable interest so that the rate at which N^{15} of glycine is incorporated into them should be determined.

DNA and histone have been known since the work of Miescher and Kossel. Residual protein has been identified more recently (2). The residual protein of isolated chromosomes is the insoluble protein that remains after all histone has first been removed at pH 3.8 by M NaCl and DNA subsequently removed by the action of desoxyribonuclease (DNAase). It is an essential structural component of chromosomes. That it is actually protein is shown by analysis, for at least 90 per cent of it consists of various amino acids (3).

DNA, histone, and residual protein are distributed in quite different ways in the nuclei from various tissues of the same organism. Nuclei from different tissues have been found to have a constant quantity of DNA per nucleus, a constant characteristic of the species. This is clearly seen when the nuclei of liver and red cells are compared. Different as these cells are, the DNA content per nucleus is the same in both (4). The quantity of histone does not

seem to vary considerably in most cells. Under certain conditions, however, it is more variable than the DNA, for in some animals it disappears from the germ cells during spermatogenesis, its place being taken by protamine (5). When the nuclei of various cells of the same organism are compared, there is, however, a striking contrast between the distribution of DNA and residual protein. The residual protein of different nuclei varies considerably in quantity. There is, for example, far more residual protein in the nucleus of a liver cell than in that of a red cell. The quantity of residual protein in a nucleus is correlated with the mass of cytoplasm surrounding it. The residual protein may, accordingly, be expected to play a more active role in interactions with the cytoplasm than the fixed DNA.

When nuclei of thymus, liver, kidney, and pancreas are isolated by means of citric acid, much nuclear protein is extracted so that nuclear composition is close to that of isolated chromosomes (2). Citric acid nuclei isolated from calf liver, for example, have a total nucleic acid content of 25 per cent and chromosomes isolated from the same tissue contain 26 per cent nucleic acid. When the histone of these nuclei is removed with M NaCl at pH 3.8, the appearance of the chromatin fibers within the nuclei remains unchanged and even when DNA is subsequently removed by the action of DNAase, the outlines of intranuclear structure can still be seen. The protein remaining in such nuclei is essentially the same as the residual protein of chromosomes. The residual protein of liver chromosomes is 39 per cent of their mass and the protein remaining in citric acid nuclei (nuclear residual protein) after removal of histone and DNA is 42 per cent of the nuclear mass. That the nuclear residual protein is a component of chromatin is thus certain, with the reservation that it is contaminated to some extent by the material of the nuclear membrane. In this connection, a comparison of calf liver nuclei and carp erythrocyte nuclei is instructive. In both cells the ratio of nuclear membrane to chromosome mass cannot be very different, yet in liver 39 per cent of the chromosome mass is residual protein while in erythrocytes the value is only 4 per cent. Even if all the residual protein of the erythrocyte were membrane material—a most unlikely possibility—the contamination in liver residual protein would amount to no more than 10 per cent.

In the present experiments, liver and pancreas nuclei were isolated by the citric acid procedure from the tissues of white mice (6). Microscopically, all preparations were exceedingly clean. Histone was extracted with 0.25 N HCl (final concentration). After dialysis it was precipitated at pH 10.6 (5). The ribonucleic acid of these nuclei was then removed by ribonuclease. DNA was now released by the action of desoxyribonuclease. Since some of the protein material dissolved at the same time, it was necessary to separate this from the nucleotides. Purification of the nucleotides was accomplished by adsorption on a column of dowex-2 (chloride form) at pH 9–10, and elution of the nucleotides with 1 N HCl after washing the column with water and

with 0.001 N HCl until the pH of the effluent reached 6 (7). Preliminary experiments showed that all of the protein is removed from the column before elution of the nucleotides.

To isolate the residual protein, it is best to start with another batch of nuclei because in the procedure that has just been described not all of the histone and DNA are removed. When nuclei are extracted at pH 3.8 with M NaCl, all of the histone is extracted and when the nuclei are then treated with ribonuclease and finally with desoxyribonuclease, practically all the nucleic acids are removed so that what remains is residual protein.

A defect in the citric acid procedure is that, owing largely to the negative charge of nucleic acid, protein of cytoplasm may be adsorbed to nuclei. Admixture of such protein with histone is unlikely because the latter is prepared by precipitation at pH 10.6, and the possibility of admixture with residual protein is much diminished when the adhering DNA is removed from the residual protein.

Preparations of cytoplasmic protein and ribonucleic acid were made by precipitating with trichloroacetic acid the non-nuclear material obtained in the citric acid isolation of nuclei. The precipitate was washed several times with 5 per cent trichloroacetic acid to remove acid-soluble compounds, suspended in water, adjusted to pH 7.8 with dilute alkali, and treated with ribonuclease for 3 hours. The suspension was then treated with trichloroacetic acid, thus precipitating the cytoplasmic proteins and leaving the ribonucleotides in solution. The ribonucleotides were separated from the small amount of protein remaining in solution by the procedure described for the desoxyribonucleotides. As high anion concentrations interfere with the adsorption of nucleotides on the resin, the trichloroacetic acid supernatants were extracted with ether to remove most of the acid, then diluted, if necessary, before passage through the column.

Albino mice of the Swiss and Rockefeller Institute strains were used in these experiments. One group of animals was fasted for 16 hours before the administration of glycine and was allowed to have only water in the course of the injections. The animals were given four intraperitoneal injections of glycine labelled with N^{15} (33 atom per cent excess) 1 ½ hours apart. The average weight of the mice was about 37 gm. and the total amount of glycine administered per mouse was 37.5 mg. 3 hours after the last injection the animals were killed by decapitation after slight ether anesthesia. The organs were removed and cooled on a sheet of plastic over ice until all the dissections were completed. The preparation of citric acid nuclei was started immediately. The organs of 88 mice were used, and the average time between the first injection of glycine and the killing of the animals was 8 hours.

Another group of animals was fasted for 16 hours and then allowed to feed during the period in which the glycine was injected. They were given a mixture of cerelose (commercial glucose) plus 12 per cent corn oil and bacon fat, followed by bread and milk about 3 hours later. The first injection of glycine was given one half hour after the animals had begun to feed, and the rest of the procedure was the same as for the fasted group. Organs from 44 animals were used.

A third group of animals was allowed to feed on bread and milk before administration of glycine and given no food for the remainder of the experiment. The first injection of glycine was given ½ hour after feeding; the total amount of glycine

given and the average time between the first injection of glycine and the killing of the animals were the same as in the other experiments.

N^{15} Analyses.—The N^{15} concentrations of the DNA, histone, nuclear residual protein, and cytoplasmic proteins were determined in the mass spectrometer (Process and Instruments Company model) after the usual conversion of organic nitrogen to

TABLE I

Atom Per Cent N^{15} Excess in Cell Fractions

	Pancreas			Liver			Kidney
	Fasted	Fed before experiment	Continuous feeding	Fasted	Fed before experiment	Continuous feeding	Fed before experiment
DNA	0.018	0.023	0.031	0.019	0.022	0.020	0.007
RNA	0.020	0.063	0.062	0.102	0.189	0.216	0.120
Histone	0.124	0.076	0.104	0.177	0.112	0.110	0.073
Residual protein	0.234		0.281	0.348		0.341	0.182
Cytoplasmic protein	0.423	0.735	0.330	0.347	0.304	0.308	0.218

TABLE II

Uptake of Glycine-N^{15} into Cell Fractions of Chicken Erythrocytes and Arbacia Sperm

Cells	Cell component	Atom per cent N^{15} excess*
Chicken erythrocytes‡	DNA	0.000
	Histone	0.000
	Hemoglobin	0.006
Arbacia sperm§	DNA	0.005
	Basic protein	0.004
	Protein	0.006

* Atom per cent N^{15} excess over N^{15} concentration of standard tank nitrogen. Average deviation ±0.001 atom per cent.

‡ Cells incubated 24 hours at 38°C. with 1 mg. per ml. of N^{15}-glycine (33 atom per cent excess).

§ Sperm suspension incubated 14 hours at 16°C. with 0.05 M N^{15}-glycine (33 atom per cent excess).

gaseous N_2 by Kjeldahl digestion and treatment by hypobromite. N^{15} concentrations of duplicate samples were determined to within ±2 per cent (average deviation) and compared with tank nitrogen as standard. The atom per cent N^{15} excesses over standard concentration are listed for liver and pancreas in Table I, and for *Arbacia* sperm and chicken erythrocyte fractions in Table II.

Results of experiments on the incorporation of N^{15} into substances prepared from liver and pancreas are given in Table I. Incorporation into DNA is low,

into histone higher, and into residual protein much higher still, being comparable with that into mixed cytoplasmic protein. The low rate of incorporation by DNA of liver nuclei is in accord with the experience of other investigators (8–10). The rate of incorporation by the DNA of pancreas nuclei is about the same, but that of kidney is much lower, being 0.007 under the same conditions. The increase in incorporation by DNA of the pancreas when the animals were continuously fed should be noted. The possibility that the DNA of this experiment was contaminated with RNA was considered, but tests showed that no such contamination was present. It was found by previous investigators that the N^{15} of glycine is incorporated far more rapidly into the total nuclear protein of liver than into DNA (8). From the present experiments it can be seen that the rate of incorporation varies considerably in the different protein fractions of the nucleus. The activity of histone is much greater than that of DNA but considerably below that of mixed cytoplasmic protein. An interesting point is the marked difference in activity of histone depending upon whether it comes from the liver of a fasting animal or one that was being fed while the N^{15}-glycine was being administered. The most rapid uptake of N^{15} by a nuclear component is by the residual protein. In liver its rate is about the same as for mixed cytoplasmic protein; in pancreas the uptake by residual protein is higher than by histone but lower than in the mixed cytoplasmic protein.

Incorporation of N^{15} into the cytoplasmic proteins of liver and pancreas is rapid, as would be expected in actively metabolizing tissues, and the incorporation into nuclear proteins is also rapid in these tissues. When the rate of incorporation by cytoplasmic protein is low, incorporation by nuclear components is likewise sluggish. Two examples of such low rates may be cited and the data from them are given in Table II. In the nucleated red cell incorporation of N^{15} into hemoglobin is low and into globin even lower, being less than 1 per cent of the value for cytoplasmic protein of liver. Uptake of N^{15} into nuclear components, DNA and histone, of this cell was so low that it could not be measured (11).

Sperm cells are another example of sluggish incorporation of N^{15} into cell proteins and also into nuclear components. Sperm immersed in a medium containing glycine may be kept alive for a long time (12). The uptake of the N^{15} of glycine into sperm protein is exceedingly low. Uptake into the DNA and into basic protein associated with it is also very low.

Sperm of *Arbacia lixula* were used in these experiments, and the incubation of the sperm with N^{15}-glycine was carried out by Dr. Alberto Monroy at the Stazione Zoologica, Naples. The sperm were centrifuged and washed with sea water. They were then suspended in 20 ml. of sea water containing 75 mg. N^{15}-glycine (33 atom per cent excess), and equilibrated for 14 hours at 16°C. At the end of this period the sperm were motile (on dilution) and gave normal fertilization. The sperm suspension

was centrifuged and washed three times with sea water in the cold and then frozen-dried and sent to our laboratory. The dried sperm (350 mg.) were washed once with saline, suspended in dilute phosphate buffer at pH 7.6, and treated with ribonuclease for 3 hours, at room temperature. The residue was washed three times with saline in the cold, suspended in 2 M NaCl (final concentration), and allowed to stand overnight in the cold. The extraction with 2 M NaCl was repeated twice. The extracts contained practically all of the DNA and basic protein and the residue contained the other sperm proteins. The residue was washed with trichloroacetic acid, alcohol, ether, and dried *in vacuo*. The combined 2 M NaCl extracts (total volume = 13 ml.) were poured into 5 volumes of water and a fibrous precipitate was obtained. This was washed with saline, suspended in 0.25 N HCl (final concentration), allowed to stand in the cold for 15 minutes with occasional stirring, and then centrifuged. The supernatant contained the basic protein. The residue was washed with saline, suspended in dilute phosphate buffer (final pH 6.7) containing 0.01 M Mg^{++} and treated with desoxyribonuclease for 3 hours at room temperature. The suspension was treated with trichloroacetic acid (final concentration 5 per cent) in the cold and centrifuged. The supernatant contained the desoxyribonucleotides which were purified by the procedure described for the nucleotides of liver and pancreas nuclei.

When rates of incorporation of N^{15} into the chromosomal components of liver, pancreas, and kidney are compared certain significant differences in the kidney are noted. There have been many investigations showing that uptake of N^{15} by different tissues of an organism is highly variable, but in most instances the tissues studied, such as intestinal epithelium or regenerating liver, contain considerable numbers of dividing cells, so that variations in uptake are, at least in part, due simply to differences in rate of synthesis of new cell substance. In the liver, pancreas, and kidney of adult mice, however, cells may be considered to be non-dividing during the short period of our experiments, so that the complication of growth does not arise. Incorporation of N^{15} into the DNA of kidney cells is less than one-third of that found in liver and pancreas cells. This difference is not due to a low rate of penetration of the N^{15}-containing glycine into the nuclei of kidney cells for the uptake of N^{15} by histone of kidney nuclei is the same as that observed in the histone of pancreas nuclei. Histone and DNA are closely associated in chromosomes but although the N^{15} uptakes by histone in pancreas and kidney are about the same, the uptakes by DNA are quite different. It should also be noted that in these tissues the quantity of DNA per nucleus is the same; what varies is the "activity" of the DNA. Comparison of N^{15} uptake by histone and DNA of three different tissues shows, therefore, that chromosomal "activity" varies in different cells, and that activity of the chromosomal components in one type of cell may vary independently of each other.

When the pancreas of a fasted animal is compared with that of an animal which is continuously feeding it is possible to see whether differences in cellular activity are reflected in different rates of N^{15} incorporation by the chromosomes.

The results show that N^{15} uptake into DNA of the more active cells is some 50 per cent higher. The more active cells may be said to have more "active" chromosomes.

SUMMARY

1. The uptake of glycine-N^{15} by components of cell nuclei was studied. The nuclear components were derived both from tissues with high metabolic rates–mammalian liver, kidney, and pancreas–and from cells with relatively low rates of metabolism–avian erythrocytes and echinoderm sperm. N^{15} uptake by nuclear components of liver, kidney, and pancreas was far more rapid than by those of erythrocytes and sperm.

2. The nuclear components of liver, kidney, and pancreas for which measurements were made were DNA, histone, and residual protein of chromatin. Uptake into DNA was low, into histone higher, and into residual protein much higher still, being comparable with that into mixed cytoplasmic protein.

3. A comparison of the uptake of N^{15} by the chromosomal components, histone and DNA of liver, pancreas, and kidney showed that chromosomal "activity" varies in different cells and also in the same cell depending upon its over-all activity.

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