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[8] Sequential numbers refer to the location of peptides, starting with the N-acetyl terminal peptide, #1.

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[10] Many of the peptides isolated by column chromatography were subsequently subjected to 2-dimensional electrophoresis-chromatography (mapping, according to B. Woody and C. A. Knight, *Arch. Biochem. Biophys.*, **78**, 460 (1958)) to further verify their identity and their intrinsic radioactivity. The quantitation of small amounts of C^{14}-labeled peptides by counting either segments of the paper maps (3MM) or plated eluates was poor for technical reasons which remain to be resolved. Attempts to use the mapping procedure directly on the digests were quite unsuccessful, probably because of above-mentioned quantitative aspects.

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*SOME OBSERVATIONS ON DIFFERENCES IN COMPOSITION BETWEEN THE NUCLEUS AND CYTOPLASM OF THE FROG OOCYTE**

BY HIROTO NAORA,† HATSUKO NAORA,† M. IZAWA, V. G. ALLFREY, AND A. E. MIRSKY

THE ROCKEFELLER INSTITUTE

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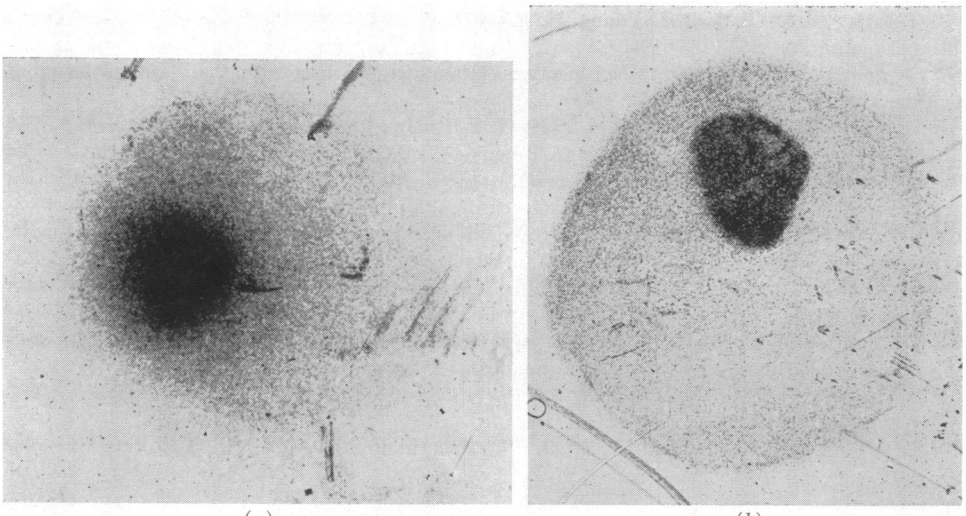
Much of what is known about the chemistry of the cell nucleus has come from investigations of isolated nuclei. In this work a difficulty that constantly must be borne in mind is that nuclei may be changed in the course of isolation; materials may be lost from the nuclei, and materials from the cytoplasm may contaminate the nuclei. We have found a way of isolating nuclei from frog oocytes in which we may be reasonably confident that nuclear composition remains unchanged. In this procedure the cells are frozen quickly and the nuclei are cleanly dissected out while they are still frozen. Nuclei can be removed only from those cells in which on freezing there appears a crack extending from the cell surface to the nucleus. Such a crack occurs in about one tenth of the frozen cells. With the finest-gauge cold hypodermic needles the frozen oocyte is opened up along the crack as far as the nucleus, which is then removed in one piece.

Using nuclei isolated by this procedure we have determined the concentration of sodium and potassium in the nucleus and cytoplasm and the penetration of radioactive sodium (Na^{22}) and potassium (K^{42}) ions into the nucleus and cytoplasm. We have also studied the penetration of C^{14}-labeled amino acids into both the nucleus and cytoplasm.

The sodium content of the nucleus was of special interest to us because several years ago we had found that the penetration of amino acids into isolated thymus nuclei is dependent upon the presence of sodium ions in the medium in which the nuclei are suspended (Allfrey *et al.*).[1] The maximum rate of penetration for



(a) (b)

FIGS. 1*a* and 1*b*.—Autoradiographs showing the intracellular distribution of Na^{22+} (1*a*) and C^{14}-leucine (1*b*) after exposure of frog oocytes to media containing $Na^{22}Cl$ (1*a*) and C^{14}-leucine (1*b*). The high concentrations of radioactive sodium and leucine in the nucleus are indicated by the high grain density over the nuclear areas in these cell sections. Very little of the leucine shown in the autoradiograph is incorporated into protein.

C^{14}-1-alanine, for example, is at a sodium ion concentration of 0.04 *M*. A sodium-dependent intracellular process is perhaps surprising, considering that the generality of cells are relatively rich in potassium but poor in sodium. The fact that amino acid metabolism in isolated thymus nuclei is sodium-dependent induces one to ask whether the sodium content of the nucleus is higher than it is in the surrounding cytoplasm. Metabolically active thymus nuclei are isolated in a sucrose solution so that the sodium ions which might originally have been present in them could very well have been extracted in the course of isolation; hence, the need for a sodium supplement in the medium to promote the penetration of amino acids.

Whatever sodium there might be in an oocyte nucleus would still be in it when removed while frozen from a frozen oocyte. The experiments to be described in this paper do in fact show that the sodium content per gram of water in the nucleus is several times higher than in the cytoplasm of the oocyte. (It should at this point be noted that, although the work on oocyte nuclei was done because of our previous experience with thymus nuclei, there is no more than a presumption that the results are significant for thymus nuclei or other nuclei.)

Autoradiographic Observations.—Our first observations on the sodium of the oocyte nucleus were made not on the isolated nucleus but on the nucleus *in situ*. Oocytes were placed in Ringer's solution containing $Na^{22}Cl$ at 16°C, and the penetration of the radioactive sodium was followed with autoradiographs. The energy of decay of Na^{22} is so high that nucleus and cytoplasm must be much larger than they are in most cells if the autoradiograph is to be something more than an indistinct blur. The frog ovarian oocyte is of a suitable size, the nucleus having a diameter of 400 μ and the cell of 1,600 μ.

Abelson and Duryee (1949)[2] were the first to make an autoradiographic study of

TABLE 1

SODIUM (Na^{23}) AND POTASSIUM (K^{39}) CONCENTRATIONS BY VOLUME AND WATER CONTENT IN CYTOPLASM AND NUCLEUS OF FROG OVARIAN EGGS*

Cell component	Na^{23} Concentration		K^{39} Concentration		Na:K ratio
	μeq/ml volume	μeq/ml H_2O	μeq/ml volume	μeq/ml H_2O	
Cytoplasm (C)	50 ± 4	88 ± 7	60 ± 5	106 ± 8	0.83
Nucleus (N)	253 ± 24	281 ± 28	227 ± 24	258 ± 26	1.11
N:C ratio	5.0	3.2	3.8	2.4	—

* These values represent average of ten measurements.

the penetration of sodium into the ovarian egg. In their experiments the oocyte remained for an hour in Ringer's solution containing radioactive sodium (Na^{24}) chloride and was then frozen. A 10 μ thick section containing the nucleus was cut by hand. The frozen section was attached to a glass slide. Over the section was placed a "plate" of photographic film, which was exposed for 4–10 days while the slide was on a cake of dry ice. We have repeated the experiments of Abelson and Duryee on sodium penetration and obtained results like theirs. The striking accumulation of Na^{22} is shown in Figure 1*a*.

Further experiments have shown that many substances accumulate in the nucleus. The autoradiograph given by K^{42} looks just like that shown in Figure 1*a* for Na^{22}. Such autographs are also given by $P^{32}O_4$, $S^{35}O_4$, C^{14}-leucine, and C^{14}-alanine (shown in Fig. 1*b*). The nuclear accumulation of these materials is not due to their incorporation into macromolecules. In the case of the amino acids, for example, there is only very slight incorporation into proteins. This can be shown by removing the photographic film overlying the section, treating the section with cold 5 per cent trichloracetic acid, then placing fresh film plate over the section and allowing exposure to proceed for several weeks. The developed film shows almost no incorporation in the nucleus and, indeed, practically no activity in any part of the oocyte.

Isolated Nuclei.—Many frozen oocytes were carefully examined while preparing sections for autoradiographs. In the course of this work the presence of superficial cracks on some oocytes were noticed. This observation led to the method of dissecting out frozen nuclei, as mentioned in the first paragraph of this paper. The frozen nucleus is taken out clean and whole.

Penetration of Na^{22}.—Ovarian eggs of Rana pipiens separated from each other were placed at 16°C in frog Ringer's solution containing $Na^{22}Cl$. After one hour the egg was quickly rinsed in nonradioactive Ringer's solution and frozen. The nucleus was removed. Radioactive counts were then made on both the nucleus and the remainder of the oocyte (cytoplasm). When the counts were calculated with reference to volume (and volumes of oocyte and nucleus can be measured readily because both are spherical) there were 3 to 4 times as many counts per unit of volume in the nucleus as in the cytoplasm (Table 1). Counts on nucleus and cytoplasm are in line with what one sees on an autoradiograph.

The time-course of Na^{22} penetration was measured because it seemed possible that there might be channels leading directly from the surface of the oocyte to the nucleus. Electron microscopy has revealed the presence of such channels in certain cells, those in the kidney tubule, for example.[3, 4] When the time of exposure to Ringer's solution containing $Na^{22}Cl$ was extended from $2^1/_2$ to 120 min the ratio of nuclear to cytoplasmic counts varied from 0.9 to 4.0 (calculated per unit of volume).

TABLE 2

RADIOACTIVE SODIUM (Na^{22}) CONCENTRATION IN CYTOPLASM AND NUCLEUS OF FROG OVARIAN EGGS*

Cell component	Na^{22} Concentration†	
	Cpm/ml volume	Cpm/gm H_2O
Cytoplasm (C)	5.7×10^5	1×10^6
Nucleus (N)	2.7×10^6	3×10^6
N:C ratio	4.7	3.0

* These eggs were loaded with $Na^{22}Cl$ (10 μc/ml Ringer's solution) for 60 min at 16°C without shaking.

† Radioactivity of Na^{22} in the medium (10 μc $Na^{22}Cl$/ml Ringer's solution) was 1.0×10^7 cpm/ml.

TABLE 3

SODIUM (Na^{23}), POTASSIUM (K^{39}), AND RADIOACTIVE SODIUM (Na^{22})* CONCENTRATIONS IN CENTRIFUGED FROG OVARIAN EGGS AFTER HOMOGENIZATION†

Fraction	Water content (gm H_2O/ml vol)	Na^{23} concentration		K^{39} concentration		$Na^{23}:K^{39}$ ratio	Na^{22} concentration	
		μeq/ml volume	μeq/gm H_2O	μeq/ml volume	μeq/gm H_2O		Cpm/ml vol	Cpm/gm H_2O
Top (yellow-lipid) layer	0.31	33	109	30	96	1.1	1.3×10^5	4.4×10^5
Middle (nearly transparent) layer	0.78	54	78	75	110	0.72	2.8×10^5	4.1×10^5
Bottom (black pigment and yolk platelet) layer	0.41	50	96	60	111	0.83	2.2×10^5	4.6×10^5

* Eggs for Na^{22} concentration measurement were first loaded with $Na^{22}Cl$ (10 μc/ml Ringer's solution) for 60 min at 16° without shaking.

† These eggs were homogenized with glass rod and then centrifuged at 15,000 g for 20 min using a Spinco SW-39 swinging basket.

This result indicates that Na^{22} penetrates into the nucleus after first traversing the cytoplasm, and this is consistent with electronmicrographs of oocytes which fail to show channels giving direct access to the nucleus from the surface of the cell.[5] Accumulation of Na^{22} in the nucleus is apparently not due to penetration from the external medium directly into the nucleus.

Sodium and Potassium Contents of Nucleus and Cytoplasm.—With a flame photometer 20 isolated nuclei were used for each determination. The results shown in Table 2 are given as microequivalents per milliliter of nucleus or cytoplasm and also as microequivalents per gram of nuclear or cytoplasmic water. The relative concentrations of sodium and potassium in the nucleus and cytoplasm are quite different depending on whether these concentrations are expressed with reference to volume or water content because for the oocyte the percentage of water in the nucleus is much higher than it is in the cytoplasm. The water content of the nucleus is 80% (gm H_2O per ml nucleus), while that of the cytoplasm is only 50%.

The low water content of oocyte cytoplasm is due to the presence of yolk platelets. This can be shown in the following manner: Some oocytes are broken by pressing on them with a fine glass rod. The broken cells are then centrifuged for 20 min at 15,000 g. Three distinct layers can be seen in the centrifuge tube. The top layer consists of lipid; the middle layer is almost transparent; and the bottom layer consists of black pigment and yolk platelets. The water contents as well as the contents of sodium and potassium of the middle and bottom layers are given in Table 3.

The results obtained on broken oocytes are confirmed by observations on intact cells. The intact oocyte can be separated into layers by suspending cells in a Ficoll

solution of the same specific gravity as the oocyte (1.14) and then centrifuging for 20 min at 15,000 *g*. After freezing, the two layers of the elongated oocyte can now be separated by a razor blade, an upper layer corresponding to the top and middle layers of the broken cell centrifugate and a lower layer containing black pigment and platelets. Sodium and potassium concentrations in these two layers were measured by flame photometry (Table 4).

TABLE 4

SODIUM (Na^{23}) AND POTASSIUM (K^{39}) CONCENTRATIONS IN CENTRIFUGED FROG OVARIAN EGGS WITHOUT HOMOGENIZATION*

Fraction	Water content gm H_2O/gm wet weight	Na^{23} concentration μeq/gm H_2O	K^{39} concentration μeq/gm H_2O	Na:K ratio
Upper (yellow and nearly transparent) part	0.61	94	114	0.82
Lower (black pigment and platelet) part	0.44	104	118	0.88
Whole egg	0.49	93	115	0.81

* These eggs were centrifuged at 15,000 *g* for 20 min using a Spinco SW-39 swinging basket.

Replaceable Sodium of the Nucleus and Cytoplasm.—In Tables 1 and 2 figures are given for the sodium content of both nucleus and cytoplasm These figures are of two kinds: one group consists of Na^{23} determinations, made by flame photometry; in the other groups are Na^{22} determinations, made by radioactive counts. In both types of determination the ratio of nuclear to cytoplasmic sodium is the same −3.2. (And this is in line with the Na^{22} autograph shown in Fig. 1*a*.) The reason for this agreement is that when Na^{22} penetrates into the oocyte at 16°C for 60 min it constitutes about 15% of the total sodium in *both* nucleus and cytoplasm (Fig. 2).

This is the percentage of sodium that is replaceable. A similar result was obtained by Abelson and Duryee (1949)[2] using the whole frog egg; after incubation for 60 min with radioactive sodium (Na^{24}) chloride in Ringer's solution, about 12% of the total sodium was replaced. When the oocytes are kept in a sucrose-tris

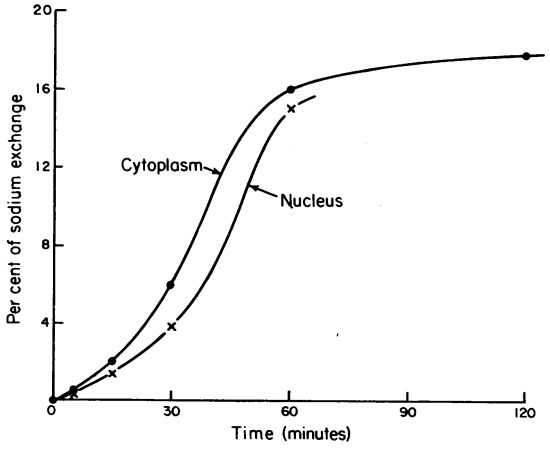


FIG. 2.—Exchange between Na^{22} in frog Ringer's solution and the cytoplasm and nucleus of the oocyte.

TABLE 5

CONCENTRATION OF L-ALANINE-1-C^{14} IN CYTOPLASM AND NUCLEUS OF FROG OVARIAN EGGS*

Cell component	L-alanine-1-C^{14} concentration†	
	Cpm/ml volume	Cpm/gm H_2O
Cytoplasm (C)	2.1×10^5	3.6×10^5
Nucleus (N)	7.3×10^5	8.4×10^5
N:C ratio	3.3	2.3

* These eggs were loaded with L-alanine-1-C^{14} (10 μc/ml Ringer's solution) for 60 min at 16°C without shaking.

† Radioactivity of L-alanine-1-C^{14} in the medium (10 μc L-alanine-1-C^{14}/ml Ringer's solution) was 3.8×10^6 cpm/ml.

buffer (0.22 *M* sucrose-0.01 *M* tris-HCl, pH 7.2) medium for 60 min at 16°C, sodium is released from both nucleus and cytoplasm, about 15 to 20% in each case; this sodium is replaced by returning the oocytes to Ringer's solution. These results show that about 15 to 20% of sodium in the cytoplasm and nucleus is easily exchangeable, but the remainder is not. The efflux of Na^{22} is more rapid than its influx; if oocytes into which Na^{22} has penetrated are then put into Ringer's solution containing $Na^{23}Cl$, the Na^{22} comes out more rapidly than it went in. It is of interest that although the external cell membrane and the nuclear membrane are both readily penetrated by sodium ions, the replaceable sodium (like the total sodium) is in much higher concentration in the nucleus than in the cytoplasm, both being lower than the sodium in the external medium (Ringer's solution).

Penetration of C^{14}-1-Alanine.—Autoradiographs described earlier in this paper (Fig. 1*b*) show that C^{14}-leucine and C^{14}-alanine accumulate in the nucleus of the frog ovarian oocyte. Isolation of nuclei after exposure of oocytes to labeled alanine containing Ringer's solution makes possible the quantitative assay of alanine in both nucleus and cytoplasm. For a radioactive count 10 isolated nuclei were used. Counts show that per gram of water there are 2.46 times as much C^{14}-1-alanine in the nucleus as in the cytoplasm (Table 5). In this experiment the oocytes remained at 16°C for 60 min in Ringer's solution containing C^{14}-1-alanine. At the end of this time the labeled alanine was still much more concentrated in the Ringer's solution than inside the oocyte, the outer concentrations (cpm per gram of cell water) being 11 times that of the cytoplasm and 4.5 times that of the nucleus.

In the experiments on penetration of labeled 1-alanine into the oocyte it was found that penetration is dependent upon the presence of sodium ions in the surrounding medium. The sodium-dependence of penetration of amino acids into the oocyte and also into other cells was subsequently studied in some detail. The results of this work will be presented in another paper.

Summary.—The nucleus can be dissected out clean and whole from the frozen frog oocyte. Sodium and potassium determinations on isolated nucleus and cytoplasm show that the Na content (microequivalents per gram water) of the nucleus is 3.2 times greater than that of the cytoplasm (excluding yolk platelets) and the K content of the nucleus 2.4 times that of the cytoplasm. The Na:K ratio of the nucleus is 1.1; that of the cytoplasm (excluding yolk platelets) is 0.72.

Penetration experiments using Na^{22}, K^{42}, C^{14}-leucine and C^{14}-alanine show in each case a marked accumulation within the nucleus of the oocyte. In these experiments the incorporation of amino acids into protein is very small.

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† Dr. and Mrs. Naora's present address is The Cancer Institute, Tokyo.

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*NUCLEOTIDE BASE CODING AND AMINO ACID REPLACEMENTS IN PROTEINS, II**

BY EMIL L. SMITH

COLLEGE OF MEDICINE, UNIVERSITY OF UTAH

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In a previous paper[1] it was shown that amino acid replacements in variants of human hemoglobin involve a single base change in the triplet codes, of messenger ribonucleic acid obtained from studies of amino acid incorporation.[2, 3] It was also possible to deduce certain interrelationships among the amino acid codes on the basis of these and other mutants. Additional information has now become available which permits assignment of base sequences for 18 of the amino acid codes when a sequence is assumed for a single code.

As before,[1] we shall use the following postulates: (1) The code is universal, (2) the code is of the nonoverlapping type, (3) a simple point mutation involves a single base substitution without alteration of sequence, and (4) each code is a triplet.

Base Code Interrelationships.—In this discussion the triplet code compositions previously reported by Speyer *et al.*[3] will be used. Let us assume that the base sequence for glutamic acid is UAG.[4] This code has been chosen as a starting point since there are more known interchanges for glutamic acid than for any other amino acid. Utilizing the amino acid substitutions known for human hemoglobin, previously summarized, [1, 5] we can deduce the following sequences in accord with Postulate 3: UCG for glutamine, UUG for valine, UGG for glycine, and UAA for lysine since all of these involve interchanges with glutamic acid.

The following known interchanges in hemoglobin also lead to code sequences: lysine (UAA)—aspartic acid (UGA), lysine (UAA)—asparagine (UCA), and glycine (UGG)—aspartic acid (UGA). It should be noted that since glutamic acid has been assigned UAG, aspartic acid must be UGA. Furthermore, it will be shown below that the histidine code is UAC; hence, the asparagine code has to be assigned UCA for its derivation by a single base change from the code for lysine.

Henning and Yanofsky[6] have recently reported that recombination of two mutants of *E. coli* restores the normal, wild-type A protein of tryptophan synthetase. A glycine residue (UGG) in the amino acid sequence of the normal protein is restored by a recombination involving one mutant which contains glutamic acid (UAG) in place of this glycine residue and another mutant which contains arginine (UGC) in place of this same glycine residue. Clearly, a crossover recombination can accomplish restoration of the glycine code (UGG) only if in the codes for glutamic acid (UAG) and arginine (UGC), the G of each code occupies a