Incorporation of Radioactive Glycine into Proteins of Frog Oocytes

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Recent work on the cytology of yolk synthesis in amphibians has been reported by Osawa (*1*), Wittek (*2*), Kemp (*3*), and Osawa and Hayashi (*4*). The last-named authors (*4*) have also presented biochemical evidence to show that protein continues to accumulate in oocytes of *Triturus pyrrhogaster* throughout the growth period in the ovary. Since the yolk platelets, according to Brachet (*5*), are composed chiefly of proteins, they provide a visible cellular inclusion convenient for studying protein synthesis. Friedberg and Eakin (*6*) and Eakin, Kutsky, and Berg (*7*) have published reports on the uptake of C^{14}-labeled glycine and S^{35}-labeled methionine into amphibian embryos; but it was noted (*6*) that the heavier fraction, containing the yolk of centrifuged embryos, took up comparatively little radioactive glycine from solution. One might expect, however, that amino acids would readily be incorporated into the yolk platelets during their synthesis in oocytes.

To test (*8*) this expectation 12 adult female frogs (*Rana pipiens*) obtained through a dealer in Wisconsin during the last week in July 1953 were injected intrapleuroperitoneally with C-2-C^{14}-labeled glycine purchased from Tracerlab, Inc. Six animals received 10 μc of the radioactive glycine dissolved in distilled water, and six received 20 μc. Two animals in each group of six were sacrificed at intervals of 1, 3, and 5 days after injection. Samples of stomach, liver, intestine, kidney, lung, brain, hand, and heart, as well as ovary, were fixed in Bouin's fluid for sectioning. Duplicate samples of each organ (brain omitted) and samples of blood collected from the ventricle were homogenized with 10-percent trichloroacetic acid in a Ten Broeck grinder. The fixed tissue was imbedded in paraffin (mp 56°–58°C) and sectioned at 10 μ. Sample slides were stained wtih hematoxylin and eosin. Slides of neighboring sections were used for making autoradiograms on Eastman medium-contrast lantern-slide plates after the technique of Dent and Hunt (*9*). Sections of tissues from a normal, uninjected animal served as controls for the radioactive tissues. Proteins precipitated with trichloroacetic acid were purified and isolated according to the technique of Levine and Tarver (*10*). Samples were plated on aluminum disks and were counted with a thin, end-window Geiger-Müller tube (TGC-2) obtained from Tracerlab, Inc. Counts were corrected for background, coincidence, and self-absorption.

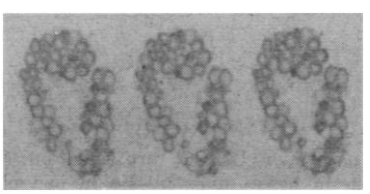

Fig. 1. Autoradiogram showing the peripheral localization of radioactivity in oocytes of a section of ovary from frog No. 4, injected with 10 μc of radioactive glycine and sacrificed 3 days later. (×4)

Table 1. Specific activity (counts/min mg) of samples of isolated proteins from tissues of six animals injected with radioactive glycine.

Samples	Animals injected with 10 μc			Animals injected with 20 μc		
	1	3	5	7	9	11
Kidney	151	128	183		497	618
Ovary	160	60	149	211	373	602
Liver	65	87	80	114	153	557
Intestine	61		17	147	157	477
Heart	69	65	54		232	361
Stomach	37	55	29	119		454
Hand	53	75	27	182	231	320
Lung	40	58	33	149	232	418
Blood		46	149	326	125	281

Figure 1 is an enlargement of an autoradiogram of a section of ovary from an animal injected with 10 μc of radioactive glycine and sacrificed 3 days later. The larger oocytes in this ovary were in stage Y_2 (*3*) with respect to deposition of yolk, and it is clear that radioactivity is highest at the periphery of these oocytes. None of the small oocytes in preyolk stages and interspersed among those containing yolk had sufficient radioactivity to sensitize the photographic film appreciably. Similar autoradiograms were obtained from other animals. It seems justifiable to conclude from these observations that radioactive glycine is selectively incorporated into the peripheral cytoplasm of cells that are actively synthesizing yolk. Unfortunately, the resolution of the autoradiographic technique used is not good enough to disclose at present what particular cytoplasmic components possess the highest radioactivity.

The specific activity of the isolated protein samples from single animals of each postinjection age, in both dosage groups, is shown in Table 1. Animals 1 and 7 were sacrificed 1 day after injection, animals 3 and 9 on the third day, and animals 5 and 11 on the fifth day. Data for the other six animals injected are similar. It can be seen that in general the figures for animals receiving injections of 20 μc run appreciably higher than those for animals injected with 10 μc. Because of individual variations, comparisons among animals in this limited series are not particularly significant; but counts on the different tissues for a given animal do reflect differences in level of incorporation. The various organs are arranged in Table 1 in order of decreasing relative activity. The ovary was among the three highest organs with respect to specific activity of incorporated glycine in nine of 12 animals

studied; and oocytes in the ovaries of the three animals exhibiting a relatively lower activity (for example, animal 3, Table 1) were either in preyolk stages or in early stages of the deposition of yolk. Further experiments with animals at graded stages of vitellogenesis are in progress to furnish more precise information on the correlation between the uptake of glycine and the synthesis of yolk.

References and Notes

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Crystal Structure of Rutherfordine, UO_2CO_3

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Crystals of the mineral rutherfordine, UO_2CO_3, used in this study (*1*) are pale yellow to brown and are lathlike elongated along [001], with large (100) and somewhat less dominant (010). Cleavage parallel to (010) is perfect. Precession and Weissenberg patterns yield the following data: orthorhombic, space group—$Pm2_1n$ (C_{2v}^7) or $Pmmn$ (D_{2h}^{13}); cell contents, $2UO_2CO_3$; $a = 4.84_5 \pm 0.010$ A; $b = 9.20_5 \pm 0.008$; $c = 4.29_6 \pm 0.006$; density (calc.) $= 5.72_4$ g cm^{-3}. (Moλ: $K_\alpha = 0.71069$ A, $K_{\alpha_1} = 0.70926$ A). Miller *et al.* (*2*) found the density of synthetic UO_2CO_3 to be 5.7 g cm.$^{-3}$

All of the observed reflections obey the criterion $h + k + l = 2n$, except a very weak set that appears only on strongly exposed photographs; for this set when $h + k = 2n$, $l = 2n + 1$, and when $h + k = 2n + 1$, $l = 2n$. The intensities of the strong reflections follow an essentially normal atomic form-factor decline with increasing $\sin\theta/\lambda$.

Through consideration of these experimental results and a knowledge of the nature of the uranyl ion, $(O\text{-}U\text{-}O)^{++}$—namely, that it is collinear (*3*) and has U-O distances of 1.93 A (*4*)—it was possible to derive the structure of UO_2CO_3 without recourse to quantitative intensity measurements. This structure is illustrated in Figs. 1 and 2 (top). It is isomorphic with the space group Pmmn and has the following atomic parameters (assuming C-O distances of 1.28 A).

2 U at (b): $\pm(\frac{1}{4}, \frac{3}{4}, z)$; $z = 0.750$
2 C at (a): $\pm(\frac{1}{4}, \frac{1}{4}, z)$; $z = 0.601$
2 O_I at (a): $\pm(\frac{1}{4}, \frac{1}{4}, z)$; $z = 0.303$
4 O_{II} at (e): $\pm(\frac{1}{4}, y, z; \frac{1}{4}, \frac{1}{2} - y, z)$; $y = 0.960$, $z = 0.750$
4 O_{III} at (f): $\pm(x, \frac{1}{4}, z; \frac{1}{2} - x, \frac{1}{4}, z)$; $x = 0.021$, $z = 0.750$
(origin at $\bar{1}$)

The structure described is the ideal one for rutherfordine. Actually, the x-ray patterns of the crystals examined exhibit diffuse streaking, in the direction of the b^*-axis, of the weak reflections. This observation is explained in the following way. The carbonate groups may in alternate layers point in opposite di-

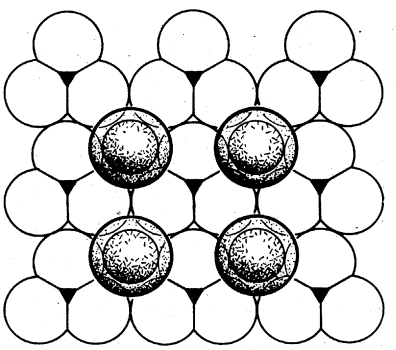

Fig. 1. Structure of a layer in UO_2CO_3, parallel to (010). The UO_2^{++} groups lie normal to the plane with the uranium atoms lying in the hexagonal holes formed by the carbonate groups. Each uranium atom has six bonds lying in the plane, with distances U-O_{III} (4) = 2.52A, U-O_I (2) = 2.43 A, and two bonds normal to the plane with U-O_{II} = 1.93 A (assumed).

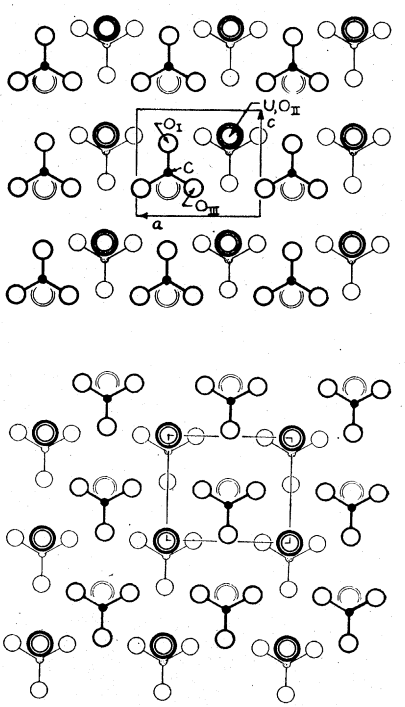

Fig. 2. Structure of UO_2CO_3 projected on (010). The atoms drawn in heavy lines all lie in one plane, those drawn in light lines in another plane, with the two planes separated by $b/2 = 4.60$ A. Structure A (ideal) (top) and structure B (bottom) differ in the way that the CO_3^{--} groups point in successive layers.