Iodine Incorporated in Cell Constituents during Sensitization to Radiation by Iodoacetic Acid

Abstract. *Iodine atoms are incorporated in bacterial membrane proteins when cells are irradiated in the presence of iodoacetic acid labeled with iodine-131. Such atoms are produced on reaction of iodoacetic acid with the gamma ray–induced hydroxyl radicals in the surrounding medium.*

Iodoacetic acid and its derivatives such as iodoacetate and iodoacetamide have been shown to sensitize microorganisms to the lethal effects of x-rays and gamma rays (*1*). It was recently reported that the species responsible for such sensitization are the free iodine atoms (*2*) formed by reaction of the sensitizers with the hydroxyl radicals and hydrated electrons produced during radiolysis of water (*3*).

In order to obtain further insight into the mechanism of action of iodoacetic acid, and to locate the sites involved in the process of radiosensitization, we have investigated the incorporation of labeled iodine into constituents of bacterial tissue.

Labeled (I^{131}) iodoacetic acid was prepared by simple exchange reaction. *Escherichia coli* B/r (Hill) was grown in nutrient broth for 18 hours at 37°C. The cells were harvested after three washings with sterile 0.1*M* phosphate buffer and resuspended in sterile phosphate buffer. Labeled iodoacetic acid (20 μc) was added to the suspension, and irradiation was carried out in air with 100 krad of Co^{60} gamma rays. After irradiation, samples were washed three times with sterile phosphate buffer to remove extracellular radioisotope. Chemical fractionation was carried out by a modification of reported methods (*4*). Fractionation into cell wall, cell membrane, and cytoplasmic constituents was effected by treatment of irradiated bacterial cells with lysozyme, followed by sonication (*5*).

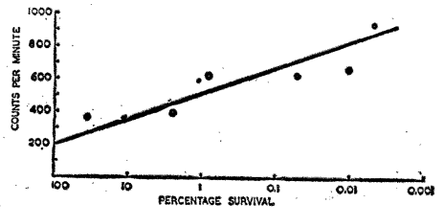

Fig. 1. Survival of cells of *Escherichia coli* relative to incorporation of iodine-131 under an atmosphere of nitrogen.

Table 1. Chemical fractionation of cells. Abbreviations: TCA, trichloroacetic acid; RNAse, ribonuclease; DNAse, deoxyribonuclease; IAA, iodoacetic acid labeled with iodine-131.

Sample irradiated	Insoluble fraction	
	Count per minute	Activity (%)
Treatment with cold 5-percent TCA		
No irradiation (control)	43	100
With IAA added later	17	100
With 0.001*M* KCNS and IAA	46	100
With IAA	35,228	100
Treatment with 75-percent ethanol		
With IAA		97.7
Treatment with RNAse (100 μg/ml)		
With IAA		92.5
Treatment with DNAse (100 μg/ml)		
With IAA		83.0

The chemical fractionation of the irradiated cells has demonstrated that the free iodine is incorporated not in the nucleic acids but in the proteins of the system (Table 1). The presence of a hydroxyl-radical scavenger (KCNS), which is known to reduce the sensitizing effect of iodoacetic acid (*6*), also reduces uptake of the isotope during irradiation; this fact further supports the hypothesis that the sensitization caused by iodoacetic acid is due to the transients produced mainly by the reaction of hydroxyl radicals. Since even after irradiation the bacterial cell wall is impermeable to iodoacetic acid, and radioactivity is not detected in cells treated with irradiated iodoacetic acid (Table 1), these results implicate the release of iodine atoms from the sensitizer in the presence of cells during irradiation. Figure 1 shows that the amount of radioactive material incorporated into the cells is directly related to radiation lethality.

In a radiation-resistant bacterial strain such as *E. coli* B/r there is an enzymic repair system that is postulated to be located in the cell membranes. Since the sensitizing effects of iodoacetamide and iodoacetic acid proved to be more marked in radiation-resistant strains, the inhibition of repair processes after irradiation was believed to be implicated (*7*). On the basis of this hypothesis, the incorporation of radioactive iodine into the proteins of cell membrane would be expected. Our results demonstrate that about 60 percent (36,613 count/min) of the total activity was associated with the cell-membrane fraction; the remainder was more or less equally distributed between the proteins in the cell-wall (12,400 count/min) and cytoplasmic (10,200 count/min) fractions. We cannot say whether these proteins were released from the membrane during the experimental procedure or originally belonged to the fractions concerned.

Thus from these observations one can infer that, during the process of radiosensitization by iodoacetic acid, iodine atoms are incorporated in membrane proteins which may constitute the repair system.

M. A. Shenoy
B. B. Singh
A. R. Gopal-Ayengar
Biology Division, Bhabha Atomic Research Centre, Modular Laboratories, Trombay, Bombay-74, India

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Yolk Protein: Structural Changes during Vitellogenesis in the Cockroach Leucophaea maderae

Abstract. *Most of the yolk protein in the mature egg of* Leucophaea maderae *consists of one large component, whereas a second smaller protein is present during the early stages of vitellogenesis. The large protein can be converted to the smaller one and to even smaller units by mild alkaline conditions in vitro. After injection of uniformly labeled leucine-C^{14} into females with developing eggs, the smaller yolk protein becomes labeled first, the label is then transferred to the large protein upon prolonged exposure.*

The most obvious manifestation in the maturation of the oocyte in *Leucophaea maderae* is the deposition of a large amount of yolk. Vitellogenesis begins soon after the imaginal molt and is initiated by secretions from the corpora allata (*1*). During maturation, the protein content of the ovaries increases

70-fold, largely as the result of yolk protein deposition. The immature oocyte is about 0.85 mm long and reaches a maximum of 5.5 to 6.0 mm in 15 to 20 days. At the time the terminal oocytes are mature, approximately 25 percent of the dry matter of the ovary is protein. The protein of the ovaries has been made soluble in solutions of high ionic strength and subjected to electrophoresis on polyacrylamide gels. The yolk proteins were resolved into five components. When salt solutions of ovarian proteins were diluted with water, three of these components, representing 90 percent of the soluble protein, precipitated. Despite this distribution, most of the total yolk protein was a single component as judged by its electrophoretic properties; by appropriate staining, it was identified as a lipoprotein (*2*).

The yolk protein fraction, which is insoluble at low ionic strength, has been further examined by sedimentation in the analytical ultracentrifuge and in sucrose density gradients. The incorporation of uniformly labeled leucine-C^{14} into ovarian proteins has been measured at intervals during periods of yolk deposition. Our results suggest that the primary yolk protein is a polymeric structure which is gradually assembled in the oocyte during yolk deposition.

Cockroaches were taken from a colony maintained as previously described (*1*). Ovaries were dissected at various times during vitellogenesis, homogenized in 0.4*M* NaCl at a ratio of 10 or 20 ml of saline to 1 g of ovary, and centrifuged at 12,000*g* in an SS-34 rotor in the Servall centrifuge at 0°C. The supernatant was diluted with one volume of ice-cold water and held at 0°C for at least 15 minutes. The precipitate was removed by centrifugation for 30 or 45 minutes at 12,000*g* and recovered in an appropriate salt or buffer solution. In some experiments the proteins were precipitated a second time. This fraction shall be described hereafter as yolk protein. Unbuffered salt solutions of yolk protein were found to be *p*H 5.8.

Sedimentation studies were performed at 20°C with a Spinco model E ultracentrifuge with standard and wedge cells and schlieren optics. Photographs were taken on Kodak metallographic plates, and the boundary positions were measured with a Nikon comparator. A plot of the migration of the protein as a function of time was used to obtain S_{20}, which was not corrected to standard conditions. The relative amounts represented by the peaks were determined by cutting out and weighing tracings of the peaks. The data were corrected for radial dilution but not for the Johnston-Ogston effect.

The deposition of yolk protein was measured as the incorporation of uniformly labeled leucine-C^{14} (250 mc/mmole, New England Nuclear) into yolk protein after injection of 50 μl (2.5 μc) into females with developing oocytes. Yolk protein was prepared as described and precipitated in 10 percent trichloroacetic acid (TCA). It was washed with 5 percent TCA to remove acid-soluble material and with water-saturated ether. The dried material was dissolved in 0.1*N* NaOH, and samples were counted in Bray's solution (*3*) in a Packard liquid scintillation counter. Density-gradient centrifugation was performed in a Spinco model L centrifuge with a SW 25.1 rotor. After centrifugation, the bottoms of the tubes were punctured, and fractions of 40 drops each (0.7 ml) were collected. These were diluted with one volume of 0.4*M* NaCl. After the optical density was read, a sample of unlabeled yolk protein was added to each fraction, and the total fraction was precipitated in 10 percent TCA. The precipitate was collected on membrane filters (B-6, Schleicher and Schuell Co.). The membranes were added to scintillation vials together with 1 ml of 0.1*N* NaOH and counted in Bray's solution.

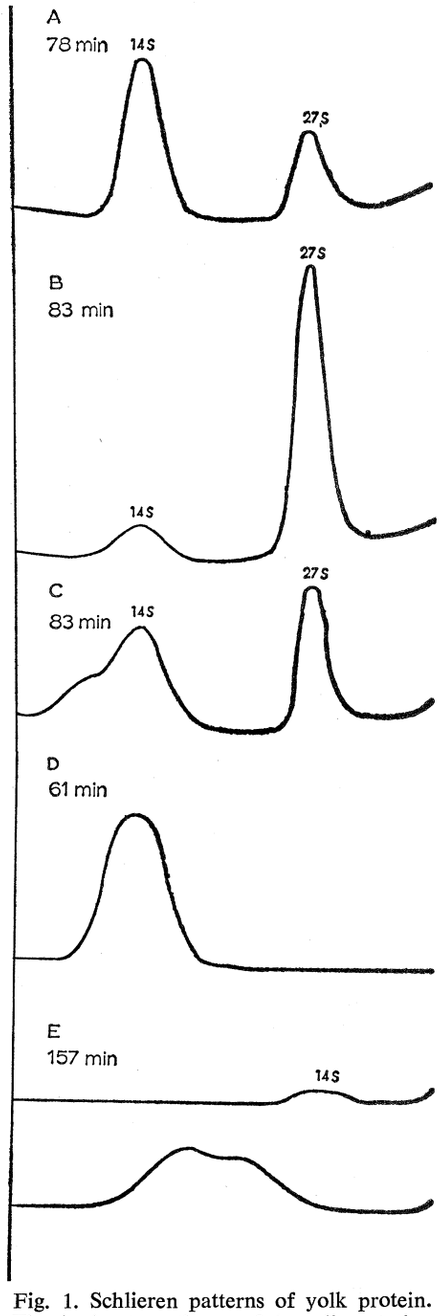

Fig. 1. Schlieren patterns of yolk protein. The ionic strength was 0.4; sedimentation was at 31,410 rev/min in all cases. (A) Yolk protein from eggs early in development. Egg size, 2.33 mm; protein concentration, 4.8 mg/ml; *p*H 5.6. (B) Egg size, 4.25 mm; protein concentration, 5.7 mg/ml; *p*H 5.6. (C) Egg size, 4.25 mm; protein concentration, 5.7 mg/ml; *p*H 8.1. (D and E) Egg size, 3.70 mm; protein concentration, 5.1 mg/ml; *p*H 9.0. The upper pattern of (E) shows the 14*S* component from a preparation at *p*H 5.6 for comparison.

The yolk protein fraction contained two components with sedimentation coefficients of 27*S* and 14*S*. In addition, a larger component with sedimentation values ranging between 39*S* and 44*S* was seen consistently at *p*H 7.4 and lower. This fraction, which will be designated 40*S*, constituted a very small proportion of the yolk protein fraction. The fact that for this fraction the ratio of the optical density at 260 nm to that at 280 nm was equal to or greater than 1 suggests the presence of both nucleic acids and protein. Three components were also found in density-gradient separations, but the 40*S* and 27*S* components were not completely resolved.

When yolk protein fractions from oocytes at different stages of development were examined, we found that the relative proportions of the 27*S* and 14*S* components varied with age. Early in vitellogenesis the 14*S* component is dominant, but in the mature oocyte most of the yolk protein exists as the 27*S* fraction (Fig. 1, A and B). The 40*S* component moved to the bottom of the cell during the first 60 minutes of centrifugation and therefore does not appear in Fig. 1.

The relative proportions of the 27*S* and 14*S* components can be altered in vitro by changing the *p*H of the yolk protein fraction. In Fig. 1, B and C, are shown the results of an experiment

in which yolk protein fraction was divided into two parts and each part was dissolved in phosphate buffer (ionic strength, 0.4) (*4*) so that one part was *p*H 5.6 and the other *p*H 8.1. These fractions were held at 4°C for at least 24 hours and then centrifuged as described in Fig. 1. At the higher *p*H, the amount of the 27*S* component was reduced; the 14*S* increased; and a new, lighter fraction appeared. Similar experiments were performed at *p*H 6.4, 7.4, 7.8, and 9.0. At *p*H 7.4 and below there was no change in relative proportions of the two peaks. The centrifugal pattern at *p*H 7.8 was similar to that shown in Fig. 1C, including the small shoulder which appeared on the 14*S* component. Adjusting the yolk protein fraction to *p*H 9 resulted in the further disaggregation of the yolk proteins. As shown in Fig. 1, D and E, the 27*S* and 14*S* components no longer existed, and in their place was a fraction of lighter weight, which on prolonged centrifugation proved to be heterogeneous. Part of this material may correspond to that which appeared as a shoulder on the 14*S* component (Fig. 1C), but this cannot be proved on the basis of the data available. At *p*H 7.8 and 8.1, an incubation period was necessary to disaggregate the protein. Centrifugation performed within 2 hours of *p*H adjustment revealed little change in the relative peak proportions. The changes observed at *p*H 9.0 took place within a 2-hour period, and the centrifugal patterns after 61 minutes and 157 minutes are shown in Fig. 1, D and E.

The incorporation of uniformly labeled leucine-C^{14} into yolk proteins was measured in intact females with developing oocytes ranging in size from 2.00 to 4.00 mm. At intervals after injection, the ovaries were removed, and yolk protein was prepared. Rapid incorporation began about 4 hours after injection of the labeled amino acid. The 27*S* component became labeled only after prolonged periods of exposure. During the first 8 hours, most of the radioactivity of the yolk protein was recovered in the 14*S* fraction. Yolk protein was separated by density-gradient centrifugation after exposure of the abdomens to labeled leucine for 8, 24, and 72 hours (Fig. 2). During the first 24 hours, the specific activity of the 14*S* component substantially increased with a much slower increase in the heavier material. In the interval between 24 and 72 hours, the specific activity of the 27*S* component continued to increase, whereas that of the lighter material decreased. These results suggest that the label in the 14*S* component gradually moved into the heavier fraction. The fact that the incorporation into the lighter fraction always preceded that of the heavier one excludes the possibility that the two fractions are independent of one another with one being made more rapidly during earlier phases and the other during later phases of vitellogenesis.

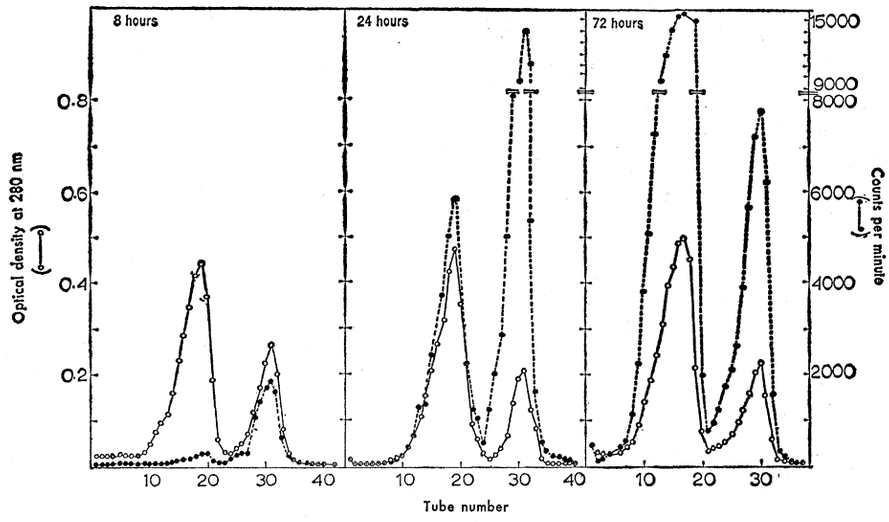

Fig. 2. Sucrose density-gradient pattern of yolk proteins labeled with C^{14}. Sixteen animals were each injected with 1.34 μg of uniformly labeled leucine-C^{14} (50 μl, 2.5 μc), and yolk protein was prepared from the ovaries as described in the text. One milliliter containing 6 mg protein was layered on a linear sucrose (5 to 20 percent) NaCl (0.4 to 0.8*M*) gradient and centrifuged for 16 hours at 25,000 rev/min. The patterns were obtained from ovaries of six animals that had been exposed to leucine for 8 and 24 hours. Four animals were used to obtain the pattern of the 72-hour pulse.

It is generally believed that in insects and other animals at least some vitellogenic proteins are synthesized at a site remote from the ovary, transported by the hemolymph, and encapsulated in vesicles in the oocyte by a process known as pinocytosis (*5*). The experiments described here and earlier (*2*) show that the yolk protein in *L. maderae* consists almost entirely of a single, large component which is made up of subunits. During deposition, the large protein, 27*S*, is gradually assembled from subunits of an intermediate size, the 14*S* component. It may be that the 14*S* component is taken up from the hemolymph during vitellogenesis. However, it is not known whether the smaller units found under mild alkaline conditions are involved in the assembling.

The possibility that changes in the properties of yolk proteins may occur during deposition of yolk has been suggested from examination of the structure of insect eggs with the electron microscope. The spaces surrounding the follicle cells of cecropia contain proteins, taken up from the hemolymph, that were flocculent. Inside the ovarian wall this material becomes granular (*6*). Roth and Porter (*7*) found a coarse granular material on the inside of the basement membrane and no resolvable material outside. This suggested to them that "once inside, the protein is complexed into relatively large aggregates which are too large to diffuse out through the membrane." This process seems to be the reverse of that in the chicken egg. The bulk of avian yolk is synthesized in the liver, and during deposition as granular yolk the serum proteins appear to be split in half (*8*).

VICTOR J. BROOKES
ROGER K. DEJMAL
Science Research Institute, and
Department of Entomology, Oregon State University, Corvallis 97331

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