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Relative Oöporphyrin Content and Porphyrin Forming Capacity of Wild-Type and White-Egg Japanese Quail Uterine Tissue. (31200)

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A previous study of eggshell pigmentation in wild-type and mutant white-egg strains of Japanese quail (*C. coturnix japonica*) showed that oöporphyrin is the principal pigment found on the eggshells and in the uterus of both quail types(1). It also was found that the oöporphyrin content of the wild-type uterus is reduced immediately following deposition of superficial eggshell pigment, *i.e.,* 2-3 hours before oviposition. These findings led to two questions relating to functional differences between the 2 quail phenotypes. 1) Does oöporphyrin occur in the mutant uterus in amounts equal to those previously reported in the wild-type uterus? 2) If not, might there be a difference between the 2 quail types in their capacity to produce oöporphyrin in the uterus?

Homogenates of chicken uterine tissue are able to synthesize oöporphyrin *in vitro* when incubated with the porphyrin precursor, delta-aminolevulinic acid (ALA). Furthermore, homogenates of white-egg layers synthesize as much oöporphyrin as do homogenates of brown-egg layers(2). By using similar methods I hoped to gain an insight into the relative porphyrin synthesizing capacities of wild-type and mutant quail uteri.

Materials and methods. The environmental regime and the method for recording the time of ovipositions in our quail colony have been described(1). The procedure for preparation and analysis of quail uterine pigment extracts—an adaptation of earlier work by Polin(2)—is described in the same publication(1). These were 3N HCl extracts; the amount of pigment present was expressed in optical density units at wavelength 410 mμ, determined with a Beckman DU spectrophotometer.

The method for estimating porphyrin synthesis in quail uteri was also a modification of Polin's techniques(2). All manipulations, up to incubation of homogenates, were carried out in an ice bath. Quail hens were decapitated and allowed to bleed freely. From each, 1 g of uterine tissue (almost the entire uterus) was homogenized in a Ten-Broeck tissue grinder with 20 ml of cold phosphate buffered saline adjusted to pH 7.2 with NaOH. This homogenate, plus a 5 ml aliquot of buffered saline used to rinse out the grinder, was poured into a 30 ml tube, mixed, and allowed to stand for 15 minutes. The upper 10 ml of homogenate was pipetted into another tube and thoroughly mixed. From here 1.6 ml aliquots of homogenate were placed in each of 4 screw cap tubes containing 0.08, 0.175, 0.35, and 0.7 ml of 0.008 M ALA* in buffered

* δ-aminolevulinic acid • HCl, Calbiochem, Los Angeles.

saline. A fifth tube, a control, contained 1.6 ml homogenate but no ALA. The volume of each tube was made up to 4.4 ml with buffered saline, they were tightly capped, and incubated in a water bath at 41°C for 20 hours. After incubation, 5 ml of concentrated HCl were added to each tube and they were allowed to stand for 2 hours in the dark. The contents of each tube were transferred to a graduated centrifuge tube with washings of buffered saline, and the volume of each was brought up to 12 ml with buffered saline. All were centrifuged at 3440 $\times$ g for 15 minutes. A 1 ml aliquot of the supernate was removed from each centrifuge tube, diluted with 4 ml 3 N HCl and the optical density curve was plotted between 360-480 mμ in a Beckman DU spectrophotometer. In this method oöporphyrin is specifically indicated by an absorption peak at 407-408 mμ. Readings were made against a control blank mixture of 1 ml buffered saline and 4 ml 3 N HCl. The amount of oöporphyrin synthesized in tubes containing homogenate plus ALA was estimated by subtracting from their optical density at 407-408 mμ, the optical density of tubes which contained homogenate but no ALA.

1. *Pigment in mutant uterus.* Uterine extracts were prepared from the following: (A) Six hens with hard-shelled eggs in the uterus, killed 3-6 hours before expected time of oviposition. There was no trace of superficial pigment on the eggs. (B) Four hens with hard-shelled eggs in the uterus, killed 2 hours or less before expected time of oviposition. Two of the 4 eggs had faint superficial pigment stipplings; the other 2 showed no trace of pigmentation.

2. In vitro *porphyrin synthesis.* Four pairs of hens, 1 wild-type and 1 mutant per pair, were selected so that within pairs both hens were expected to lay within the same hour. Both hens of a pair were killed, and their uterine homogenates prepared, simultaneously. All pairs were killed 3-6 hours before expected oviposition. There was a hard-shelled egg, with no superficial pigment, in the uterus of each hen.

Results. 1. *Pigment in mutant uterus.* The only absorption peak detected in mutant uterine extracts was at 410 mμ. The mean 410 mμ optical density of extracts from (A) hens killed 3-6 hours before lay was 0.420, while for (B) hens killed 2 hours or less before lay the mean was 0.365 (Table I). The means were significantly different at t = 0.05.

TABLE I. Optical Density (O.D.) at 410 mμ of Mutant White-Egg Quail Uterine Tissue Extracts Before and After the Time of Expected Superficial Eggshell Pigment Deposition.

Bird No.	Killed 3-6 hr before lay	Killed 2 hr or less before lay
1	.395	.325
2	.370	.390
3	.405	.370
4	.445	.375
5	.470	
6	.435	
Mean O.D.	.420	.365

2. In vitro *porphyrin synthesis.* The only strong absorption peak in all uterine tissue homogenates occurred at 407-408 mμ. The mean 408 mμ optical densities of wild-type and mutant homogenates at the 4 ALA levels concerned are illustrated in Fig. 1. They show a positive correlation between optical

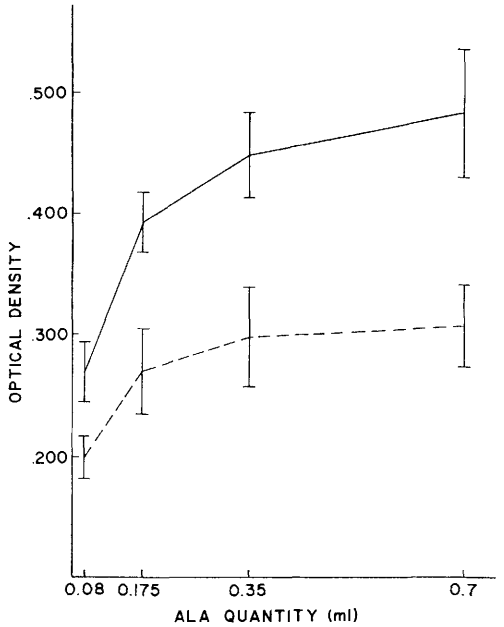

FIG. 1. Mean optical densities at 408 mμ of mutant white-egg and wild-type Japanese quail uterine tissue homogenates after 20 hr incubation with 4 different quantities of delta-aminolevulinic acid. Wild-type values are indicated by solid line, mutant values by dashed line. N = 4 birds per curve. Vertical bars indicate ± 1 standard deviation of means.

density and the amount of ALA available to the homogenates from both quail types. More importantly, they show that at each ALA level the mean optical density of mutant homogenates is much lower than that of wild-type homogenates.

Discussion. The 410 mμ absorption peak in these mutant uterine extracts confirms previous findings in wild-type and mutant, white-egg quail(1). The same peak is found in chicken uterine extracts and is attributed to the presence of oöporphyrin, 97.1% of which is protoporphyrin(2).

Comparison of the mean optical density values in Table I with published values for wild-type extracts(1) (obtained in the same laboratory with identical methods) clearly shows the oöporphyrin content of mutant uteri is much lower. For example, the mean optical density values of wild-type extracts at 4 and 2 hours before oviposition were 1.120 and 0.506 respectively(1). Compare with those the mean mutant values, 0.420 and 0.365, obtained respectively at 3-6 and 2 or less hours before oviposition. Note that there was a greater difference between wild-type and mutant extracts when donor hens were killed 3 or more hours prior to oviposition, *i.e.*, just before superficial pigment deposition(1,3) when uterine pigment concentration is at a high level(1).

The data also confirm previous findings in wild-type quail that superficial eggshell pigment deposition begins between the second and third hour before oviposition(1).

At one time I thought the white-egg phenotype might be due to a simple inability of the mutant uterus to release pigment for deposition. This idea is invalidated by the observation that oöporphyrin is significantly lower in the mutant uterus after the time of expected superficial pigment deposition.

Polin's finding(2) that uterine tissues from brown-egg and white-egg chickens were equal in their capacity for oöporphyrin synthesis suggested to him that "There is a possibility that ALA, or a precursor to ALA, is limiting in uterine tissue of the hen that lays a white egg. . . ." The quail data do not entirely conform to this idea even though the white-egg quail homogenates were capable of limited oöporphyrin synthesis (Fig. 1). At lower ALA levels, 0.08 and 0.175 ml, the ALA content did have a limiting effect on the amount of oöporphyrin synthesized by wild-type and mutant homogenates. However, at the highest ALA level, 0.7 ml, its limiting effect was greatly diminished. It is hard to see how additional ALA supplied to mutant white-egg homogenates could have caused them to synthesize an amount of oöporphyrin equal to wild-type homogenates.

Since the quail data indicate that mutant uterine homogenates were not capable of synthesizing as much oöporphyrin as wild-type homogenates, regardless of the amount of ALA available, it may be that a functional difference in the uterus determines the mutant phenotype. In spite of the valid reservations one must consider when interpreting data obtained *in vitro*, these findings strongly suggest further experiments designed to test critically a hypothesis that the basis of the mutant phenotype is a reduced ability in the uterus to synthesize oöporphyrin from its precursors.

Summary. Spectrophotometric analysis of wild-type and mutant quail uterine extracts has shown there is much less oöporphyrin in the mutant uterus than in the wild-type uterus, both before and after the expected time of superficial pigment deposition. The data also show that after the time of expected superficial pigment deposition there is significantly less oöporphyrin in the mutant uterus than there was before. *In vitro* incubation of wild-type and mutant quail uterine homogenates with delta-aminolevulinic acid (ALA) produced data indicating that mutant homogenates have less capacity for oöporphyrin synthesis than do wild-type homogenates. The evidence suggests that some factor other than ALA availability prevents mutant homogenates from synthesizing as much oöporphyrin as wild-type homogenates.

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