Properties of Electroretinogram in Albino and Wild-Type Japanese Quail with Special Regard to the C-Wave

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Abstract. Electroretinograms (ERGs) of normally pigmented and albino quail were recorded under white flash light stimulation. In ERGs taken during first 3 days after hatching at a flash intensity of 10 joule (J), the c-wave was found only in the albino, while in the older birds it was found in ERGs of both phenotypes. The c-wave of the chick was much faster in the time course than that of the adult. In the albino chick (day-5), when the flash intensities were increased from 1 to 10 J, there was a marked decrease in the b-wave which was always associated with an increase in the c-wave. In some albino adult quail, low intensity of flash light (0.5–1.0 J) could bring about distinctive slow positive potential (c-wave) and oscillatory potential (wavelets), while in the pigmented adult quail, higher intensity of flash light (10–40 J) was required to elicit comparable responses. The a- and b-waves of albino were larger in amplitude than those of pigmented quail at any given flash intensity. These results indicate that the sensitivity of the mixed retina of the quail to a light stimulus is enhanced by the albinism.

In the trout, absence of melanin pigment in the retina lowers its threshold to photic stimuli [1]. In the rat, however, opposite results are known [7]. These contradictory observations suggest that enhanced electrical responses of the albino retina in the former have not been brought about simply by an increase in intra-ocular scattering of light.

In the eyes of lower vertebrates, recent reports have shown that pigment epithelium which is not photosensitive in itself, plays an important role as [K+] electrode during and after a light stimulation [14], and this might explain the origin of the c-wave as the result of interaction between the apical process of the pigment epithelium and adjacent photosensitive elements [8, 15, 20].

The present study was undertaken to investigate the influence of ocular pigmentation on the electrical responses of quail eyes from hatch to maturity by means of electroretinogram and visual evoked potential.

Materials and Methods
The experiments were performed using our stock of two lines of Japanese quail (Coturnix coturnix japonica); one was a normally pigmented line (Group 1) and the other was a hybrid albino (Group 2) which was lacking in the ocular pigmentation as the sex-linked albino [11].

Before the photic stimulation, the eyelid and nictitating membrane of the left eye of the bird were removed surgically. The eyelid of the remaining side was sutured and covered with a piece of black plastic tape. The bird was then fixed in a holder. A platinum electrode was placed on the cornea for recording electroretinogram (ERG), and a stainless steel electrode insulated with synthetic resin (Cashew #91) except the tip, was inserted into the contra-lateral optic lobe for recording visual evoked potential (VEP). A stainless insect pin was placed near the nostril as the reference electrode.

Each experiment was commenced immediately after putting the room light off. An ophthalmic
strobolight (3G 21P, San-Ei) was used for photic stimulation. Light flash, 1/sec in frequency, 100 
μsec in duration, 0.5–40 joule (J) in intensity, was directed towards the left eye located 50 cm apart 
from the light source.

In each bird, 20 sweeps of ERGs or VEPs were fed to the signal processor (7806, San-Ei) at a time 
constant of 0.3 sec while being monitored each sweep on the display panel. Averaged results were 
written out on an X-Y recorder (6400 A, Matsushita). At the end of each experiment, the brain 
tissues were examined macroscopically whether the electrode for VEP had been inserted correctly into 
the desired site.

During ERG recording of the quail chick, the body temperature was maintained at 39°C by a 
heat source placed under the body.

The age of the bird was expressed in day, designating the day of hatching as day-1. The bird 
older than day-30 was expressed as adult. The amplitude of each wave was measured by the height 
from the bottom of the a-wave as shown in Figure 1. When the b-wave did not appear in a typical

![Figure 1. Measurement of amplitude](image)

Remarks.
When the b-wave was partially hindered by the c-wave, ERG at low flash intensity was used for reference to measure the amplitude of the b-wave.

![Figure 2. Changes in ERGs with age in albino and pigmented quail at flash intensity of 10 J](image)

Remarks.
Two different wave forms were observed in albino quail at day-8.
form owing to associated c-wave, a wave having the peak and latency similar to those found in typical b-wave obtained at a low flash intensity was assigned for the b-wave (Fig. 1), since it was known in the chicken that the latency of the b-wave was substantially unchanged regardless of the increase in flash intensity [5] and this was confirmed in our preliminary experiments on normally pigmented quail.

In experiment 1, ERGs of normally pigmented and albino quail (day-1, 2, 3, 5, 8 and adult) were recorded at a flash intensity of 10 J. In the chick of Group 2 at day-5, ERGs at a flash intensity of 1 J were also examined. In experiment 2, ERGs were recorded at various flash intensities (0.5, 1, 10, 20 and 40 J) in both groups. In some cases VEPs were recorded.

Fig. 3. Changes in amplitude of the b- and c-waves of albino quail with advance in age at a flash intensity of 10 J

![Graph showing changes in amplitude of b- and c-waves with age](image)

Remarks.
- : b-wave.
■: c-wave.
Number of birds used is indicated in the parenthesis. Each point and vertical line represent the mean plus 1 SD.

Results

Experiment 1. Effect of age

Figure 2 shows the changes in the wave form of ERGs of two groups with advance in age. In Group 2 a slow positive potential (c-wave) having fast time course was evident at day-1. Age difference in the amplitude of the b- and c-waves of Group 2 at a flash intensity of 10 J is shown in Figure 3. The b- and c-waves were approximately equal in size when measured immediately after hatching. Amplitude of both waves, especially that of the c-wave, then increased between day-2 and day-3. In the amplitude of the b-wave, however, a transient decrease was observed during day-3 and day-5. At day-8 both the b- and c-waves were in adult level with regard to their amplitude, but there were variations in the wave forms which

Fig. 4. Typical wave forms and amplitudes of the b- and c-waves of albino quail chick at the flash intensities of 1 and 10 J

![Waveforms and amplitudes](image)

Remarks.
- : b-wave.
- : c-wave.
Each column and horizontal bar represent the mean plus 1 SD of 6 birds used.
might be classified into two types; one with fast c-wave as in the type of the immature and the other with slow c-wave as in the type of the adult.

ERGs of the albino chick at day-5 at different flash intensities are shown in Figure 4. The amplitude of the b-wave at 1 J was greater than that at 10 J, while in the c-wave the relation was inverse.

First sign of the c-wave appeared in some birds of Group 1 at day-5. From its beginning the c-wave was almost identical with that of the adult, both in the latency and the form. The amplitude of the b-wave of Group 1 never decreased with advance in age and attained to the adult level at day-5.

The wavelets on the a- and b-waves in both groups increased in amplitude with the growth of the birds.

Experiment 2. Effect of flash intensity

Figure 5 shows typical pattern of ERGs in adult quail of the two phenotypes taken at various flash intensities. The occurrence rate of the c-wave and the wavelets in ERGs of the adult quail examined under various flash intensities is summarized in Figure 6. Only the c-wave larger than 3/5 of the corresponding b-wave in amplitude was taken into account in preparing this figure. The wavelets were defined as a small sharp peak appeared on the branch of the a- and b-waves. They were classified into three groups according to the location in which they were found: (1) descending branch of the a-wave; (2) ascending branch of the b-wave; (3) near the peak of the b-wave.

The c-wave and wavelets were already evident in several birds of Groups 2 at a flash intensity of 1 J, but neither was observed in all birds of Group 1 at the same flash intensity. In the tests at 10 J, the c-wave appeared only in 2 out of 9 birds in Group 1, while in 9 out of 10 in Group 2. Finally the c-wave at 40 J was evident in all birds of two groups. It was a common trend that the number and amplitude of the wavelets increased at higher flash in-
Fig. 6. Frequencies of the c-wave and wavelets at various flash intensities

Remarks.
1: albino.
2: pigmented.
Numerals in the middle small rectangles represent flash intensities.

Fig. 7. Typical ERGs and VEPs of albino and pigmented quail at various flash intensities
tensions as seen in ERGs and VEPs shown in Figure 7, in which it was also exhibited that the wavelets of ERGs corresponded fairly well to those of VEPs in their latency.

The interrelation between the amplitude of the a- and b-waves, and the flash intensities are given in Figure 8, where it is clearly shown that the response of Group 2 was greater than that of Group 1, and attained to the plateau at a flash intensity of 10 J in both groups.

**Discussion**

The combined results of experiments 1 and 2 showed that there were two types of c-wave. One was characterized with very clear sharp peak and fast time course and was seen only in ERGs of the chick of Group 2 (Fig. 2). The other had a slow time course and was seen in ERGs of the adult of both groups (Fig. 5).

The c-wave has long been regarded as rod specific, because no sign of c-wave was demonstrated in ERGs of the pure cone retina [2,6]. Recent reports [14] have shown that there are cone specific and rod specific c-waves and they differ from each other in their time courses. The former is called as the fast c-wave which is swamped by the other components of ERG. Referring to these observations we may assume that the fast c-wave of quail chick seen in this experiment is cone specific and the slow one is rod specific. Indirect evidence supporting this assumption was reported in the chicken that during perinatal development, scotopic mechanisms are not operating, though the retina is equipped with cones and rods [9].

In the quail chick of Group 1, the flash intensity of 10 J might have been inadequate in eliciting the c-wave, because even in the adult, only limited number of birds responded with the c-wave at this flash intensity and unequivocal c-wave was ob-
served in all birds at 40 J.

There was disagreement about the age when the c-wave first appeared in the chicken. Blozovsky and Blozovsky [3] reported its appearance at day-6. On the other hand Ookawa [16] demonstrated its occurrence as early as day-1. This discrepancy might be attributable in part to the use of anesthetics or to the body temperature during the recording as suggested by Ookawa, but the effect of flash intensity may not be ignored in evaluating the results as demonstrated in our present experiments in quail.

Peculiar decrease in amplitude of the b-wave was found between day-3 and day-5 only in the albino quail chick (Fig. 3). Whenever the c-wave was lacking, we found large size of the b-wave at day-5 in the quail chicks of both phenotypes (Figs. 2, 4). It is likely that the condition which enhanced the c-wave had concomitantly suppressed the b-wave.

The wavelets we observed were comparable to the oscillatory potential recorded in other avian and mammalian species [4, 12, 13, 17]. The origin and significance of the wavelets have not been well established. In the pigeon and monkey, their wavelets showed maximum amplitude at the retinal inner plexiform layer when examined with micro-electrode [18, 19]. Amacrine membrane is another possible site of the origin, since reversible loss of the wavelets and reversible changes in the ultrastructure of amacrine membrane were observed after intraretinal glycine injection [10].

Results of present experiment (Fig. 7) suggest that the oscillatory potential are closely related with VEPs, and this is supported by the evidence that the oscillatory potential ceased 24 hr after the optic nerve section coincident with the loss of detectable ganglion discharge [19].

Lack of ocular pigmentation in quail facilitated the occurrence of the c-wave and wavelets, and enhanced the size of the a- and b-waves in our results. In the rod dominant retina of the rat, the c-wave was demonstrated only in the pigmented animals [7], while in the mixed retina of the trout large size of the a- and b-waves and small but evident c-wave were observed in the albino [1]. The effect of the ocular albinism on the electrical responses of the retina might be determined rather by the type of the retina than the species used.

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References
アルビノ及び野性型ウズラにおける ERG 特に c 波の特性に関する研究：小西秀彦・本間達隆（東京大学農学部家畜生理学教室）——野性型ならびに遺伝的アルビノウズラに白色光刺激を与え、両者の
網膜電図（ERG）を飼育後日齢を追って比較検討した。距離 50 cm、10 J の光刺激に対し、ERG の
主成分の一つである c 波は、飼育後 3 日間でアルビノ個体にのみ発現し、野性型個体では更に日齢が進
んで初めてその発現をみた。なお幼鶏の c 波は成鳥のそれより時間が経過が早いのが特徴であった。アル
ビノ幼鶏において、光強度を 1 J から 10 J に増加することにより b 波では振幅の減少が、c 波では
増加が認められた。成熟アルビノでは 0.5-1 J の光刺激で明瞭な c 波の光刺激を示す個体が
存在したが、野性型で同様の反応を得るには少なくとも 10 J の刺激を要した。また本実験で用いた刺激
強度の範囲では、同じ強度の光刺激に対しアルビノで観察される ERG の a, b 波の振幅は、常に野
性型のそれを上回る値を示した。以上の結果から、複合網膜をもつウズラにおける色素上皮のメラニン
欠損は、光刺激に対する感度の増加をもたらすことが明らかとなった。