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TECHNICAL NOTE

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Pig-mentation: Postmortem Iris Color Change in the Eyes of *Sus scrofa**

ABSTRACT: Experienced forensic pathologists and examiners may be familiar with the phenomenon of postmortem iris color change; however, only Knight, *Simpson's forensic medicine*, Arnold, London, 1997; Ref. 1 and Saukko and Knight, *Knight's forensic pathology*, 3rd ed., Arnold, London, 2004; Ref. 2 have referred to it in the literature, and to date, there have been no published scientific research studies on this taphonomic artifact. A controlled experiment was conducted of postmortem changes to isolated *Sus scrofa* eyes. The eyes (n = 137) were separated into three groups and each sample was observed for 3-day postmortem at a different temperature. In addition, a *Sus scrofa* head was obtained to observe postmortem changes of eyes *in situ*. All isolated blue eyes in the experiment, at room temperature and higher, changed to brown/black within 48 h. The *in situ* blue eye, at room temperature, turned brown/black within 72 h. If iris color consistently changes postmortem in humans, then this taphonomic artifact must be incorporated into victim identification protocol, including disaster victim identification software, and autopsy reports to prevent inaccurate victim identification and inappropriate exclusion from the identification process.

KEYWORDS: forensic science, postmortem, iris color change, eye color change, victim identification, forensic taphonomy

Postmortem iris color change in light-eyed individuals has been reported in the literature, albeit without supporting studies (1,2). Saukko and Knight (2) conclude that eye color is unreliable for identification. The implication is that the eye color changes before the globe decomposes, hence the possibility of incorrect eye color assessment.

This phenomenon has been confirmed by forensic practitioners (J. Arnold and C. Barker, pers. comm.) and by forensic pathologists who describe the reaction of families of the deceased to erroneous eye color as stated in autopsy reports (P. Ellis and J. Fernandes, pers. comm.).

The eye has been studied for postmortem changes for potential indicators of time of death estimates, for example, potassium ion concentration (3–10), for the detection of alcohol or narcotics (11,12), and for the presence of individuating pathology such as hypoglycemia (13–15). However, postmortem iris color change is not found in the literature aside from the two references cited above. While experienced pathologists may be familiar with this artifact, not all death investigators or coroners are, and the latter may have jurisdiction over human remains.

Eye color is routinely described in autopsy and victim identification reports (2,11,16–18). While eye color is not heavily relied upon for identification (sex, height, and estimated age carry more weight) (J. Arnold, pers. comm.), victim identification software currently available would exclude a potential match due to discrepancies, including eye color, unless parameters are preset to limit the required number of fields for a potential match (R. Venables and D. Horgaard, pers. comm.). If Knight is correct that postmortem iris color change is an inevitable taphonomic artifact, then victim identification protocols, from autopsy reports to disaster victim identification software, would have to accommodate this phenomenon.

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Methods

To test Knight's assertion that eye color changes before the eyeball is fully decomposed, a series of pilot experiments were undertaken using enucleated (excised) *Sus scrofa* (domestic pig) eyes supplied by a local butcher 1 day after slaughter. *Sus scrofa* eyes are anatomically similar to those of humans (19). Isolated eyes of the pilot experiments were kept at room temperature in an isothermal fume cupboard, and all of the blue eyes turned brown/black within 2–3 days postmortem and prior to decomposition of the eye itself. In addition, dissection of an eye 3-day postmortem showed the vitreous humor, which is normally transparent and gel-like in life, to be black and watery.

The main experiment consisted of a controlled observational study of a total of 137 isolated *Sus scrofa* eyes collected from a local abattoir on the day of slaughter. The eyes were observed over several days in three different environments, each at a different temperature, and monitored every 12 h, starting from their placement in one of the three environments:

Environment A: *LMS Series Four Cooled Incubator* (*4–8°C*).
Environment B*: Isothermal fume cupboard* (*room temperature, 21–26°C*).
Environment C: *Gallenkamp Plus II Oven* (*30–36°C*).

In addition, one *Sus scrofa* head was obtained to study postmortem changes *in situ*. As in humans, blue eyes in pigs are less common than brown; in total there were 13 pure blue eyes for the main experiment.

The eyes used in this experiment were obtained from intensively reared (nonorganic) domestic pigs bred in Southwest England and

killed for consumption. As such, there were no ethical issues raised by this project.

At the time of slaughter the animals were *c.* 4–5 months old. The method of slaughter was an electric shock to the ear, which stuns the animal, followed by exsanguination by severance of one of the carotid arteries, performed manually. All of the animals had blue, brown, or bi-colored (mixed blue and brown) eyes; these were enucleated by abattoir staff.

Eyes were collected on two occasions. In Part 1 of the experiment, 119 eyes were collected, bagged, and stored on ice *c.* 4–5 h after slaughter. There was a total of three pure blue eyes in Part 1.

On the second occasion (Part 2), collection took place within an hour postmortem. Including the *Sus scrofa* head, there was a total of 10 pure blue eyes in Part 2. Including the two *in situ* eyes, there were a total of 18 eyes in Part 2 of the experiment.

In both parts of the main experiment, the eyes were transported immediately to the laboratory, where they were catalogued and placed on hard plastic trays in one of three environments. Details of the temperature and relative humidity of each environment from both experiments at each 12-h interval are shown in Tables 1 and 2.

The *Sus scrofa* head obtained in Part 2 of the experiment was stored in the isothermal fume cupboard (Environment B, 22°C). This animal had *heterchromia iridium*, or two different colored eyes: one brown and one blue.

Over the course of Parts 1 and 2 of the experiment, the following activities took place every 12 h:

- Recording of temperature and humidity in each environment (see Tables 1 and 2).
- Photography of entire trays and close-ups of selected eyes in a darkroom within the laboratory facilities using a Ricoh Caplio RR30 digital camera and two 60-watt Craftlight High Quality Daylight light bulbs.
- One milliliter of vitreous humor was withdrawn from one eye from each environment, using calibrated micropipettes following incision of the sclera, and stored in the cooled incubator (the eye was subsequently disposed of, i.e., vitreous humor was withdrawn from a separate eye on each occasion).
- Notations were made on the decomposition process and iris color change (if any).

Finally, the vitreous humor samples were examined under a light microscope, and photomicrographs were taken of several of the samples using a Canon EOS 20D digital camera (Fukushima, Japan).

All biological material was disposed of through autoclaving following the pilot experiments and the main experiment.

Results

The results are presented for each of the two parts of the main experiment and for the changes observed in vitreous humor samples. For the sake of simplicity, the mean temperatures of each environment are expressed.

Part 1

One of the early postmortem changes seen in the sclera (the white area of the globe), is referred to as *tache noire* (literally, "black spot"), a result of desiccation of the sclerae when the eyelids of the deceased are open. A striking difference among the eyes collected for Part 1 of the experiment was the proportion of the sclerae that exhibited *tache noire*, from none or very little, to over 75% coverage of the surface of the globe; an example of what Dolinak et al. (20) refer to as "global *tache noire.*"

Of 119 eyes collected in Part 1, only three were pure blue; four of the eyes had bi-colored irises. One blue eye was placed in each of the three environments.

A change in iris color in the blue eye of Environment C (Gallenkamp oven, 32°C) began within 12-h postmortem. This commenced with a distinct darkening around the pupil, and was

TABLE 1—*Temperature and humidity readings for Environments A, B, and C for Part 1 of the main experiment.*

	A		B		C	
Environment PMI (hours)	Temp (°C)	Humidity (%)	Temp (°C)	Humidity (%)	Temp (°C)	Humidity (%)
12	7	42	26	40	30	70
24	4	45	23	57	31	50
36	7	43	26	38	35	40
48	8	46	24	40	n/a	n/a
60	6	43	n/a	n/a	n/a	n/a
Mean	6.4	44	24.8	44	32.0	53

PMI, postmortem interval.

TABLE 2—*Temperature and humidity readings for Environments A, B, and C for Part 2 of the main experiment.*

	A		B		C	
Environment PMI (hours)	Temp (°C)	Humidity (%)	Temp (°C)	Humidity (%)	Temp (°C)	Humidity (%)
12	7	55	23	64	36	43
24	6	52	22	63	35	43
36	6	53	22	58	35	39
48	5	53	21	50	n/a	n/a
60	5	55	23	50	n/a	n/a
Mean	5.8	54	22.2	57	35.3	42

PMI, postmortem interval.

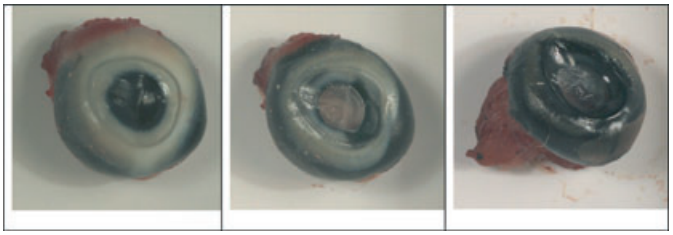

FIG. 1—*Blue eye in Part 1 at 32°C (Environment C) at 12-, 24-, and 36-h postmortem. Photo: Bonita Dainowski.*

complete within 36 h, by which time the iris color was unrecognizable as blue. It was difficult to assess iris color in isolation because the rest of eye (the sclera) was almost entirely black (global *tache noire*) and desiccated (Fig. 1). This is also true of the brown eyes in the same environment. At 36 h, all of the eyes were desiccated and hard, and the iris color was undeterminable.

For the blue eye in the isothermal fume cupboard (Environment B, 25°C), the change of iris color was underway by 24-h postmortem. By 36-h postmortem, there was evidence of darkening at the periphery of the iris. Iris color was completely altered by 48-h postmortem, by which time all of the eyes were decomposed, desiccated, and exhibited global *tache noire* (Fig. 2).

The blue eye in the cooled incubator, 6°C (Environment A) retained its color for 60-h postmortem, although there was evidence of darkening around the pupil at that time, marking the onset of iris color change (Fig. 3).

At 60-h postmortem, most of the sclera was black with *tache noire* and the anterior chamber had collapsed, an artifact that appeared much earlier in the eyes in moderate and high temperatures (21°C and higher). The color of the brown eyes also persisted in the colder environment. After 60 h the original iris color was still recognizable, despite the fact the sclera of the eye was completely black or almost completely black (global *tache noire*).

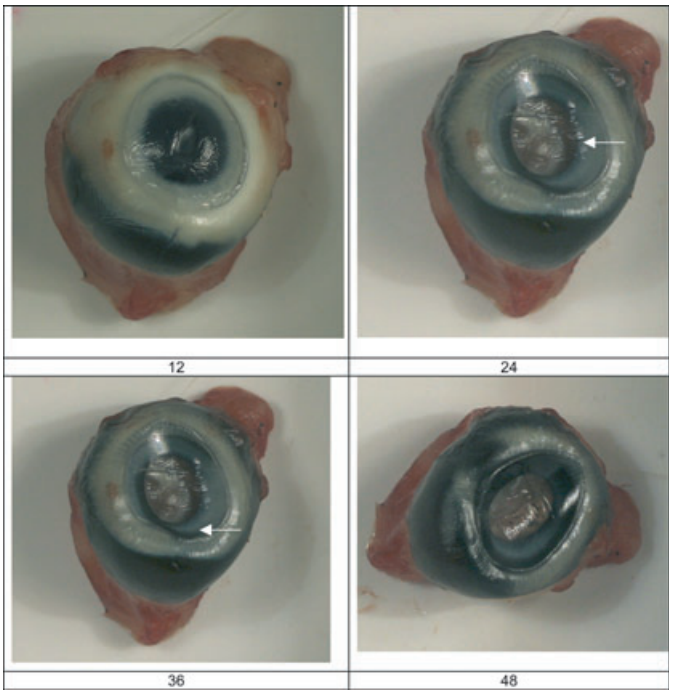

FIG. 2—*Blue eye in Part 1 at 25°C (Environment B) at 12-, 24-, 36-, and 48-h postmortem. Arrows indicate areas of iridial darkening. Photo: Bonita Dainowski.*

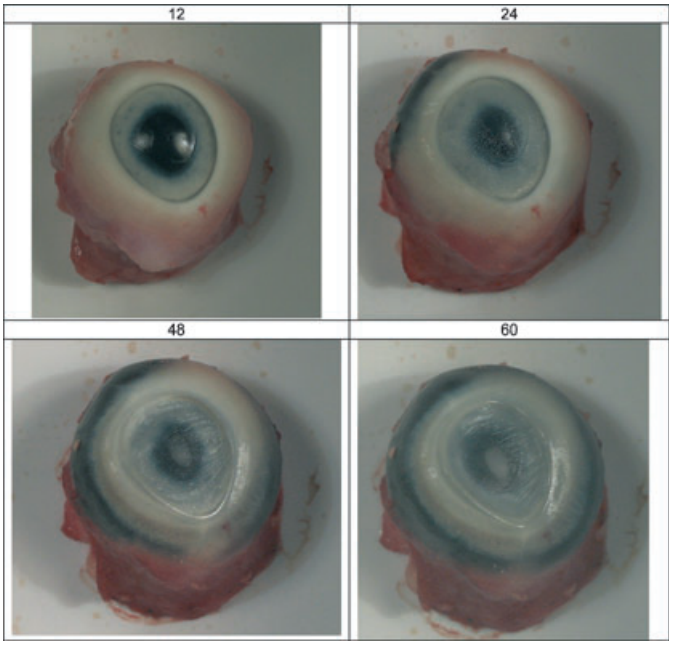

FIG. 3—*Blue eye in Part 1 at 6°C (Environment A): 12-, 24-, 48-, and 60-h postmortem. Photo: Bonita Dainowski.*

Part 2

The postmortem interval at collection and distribution of Part 2 was 4–5 h shorter than that of Part 1. Of note, there was noticeably less *tache noire* on the sclera observed in this set of eyes at the start of Part 2 of the experiment than that of Part 1. Of the 18 eyes collected, nine of these were pure blue. In addition, one of the eyes of the *Sus scrofa* with *heterochromia iridium* was blue.

The eyes in Part 2 of the experiment decomposed in a similar sequence to those of the first experiment. There were four blue eyes in the cooled incubator (Environment A, 6°C), and five in the Gallenkamp oven (Environment C, 35°C). Only the *Sus scrofa* head was placed in the isothermal fume cupboard (Environment B, 22°C) during Part 2 of the main experiment. The latter group, in the Gallenkamp oven, desiccated very quickly, and most of the sclerae exhibited global *tache noire* by 24-h postmortem, by which time the irises changed from blue to brown/black.

By 72-h postmortem, 100% of the eyes in Environment A, the cooled incubator, showed blackening around the pupillary margin, the initial stage of iris color change. In addition, by 72 h, the sclerae were almost completely affected by *tache noire*.

The *Sus scrofa* head was kept at room temperature (Environment B, 22°C). No changes in color were observed for the first 36 h. At 48 h, the characteristic darkening around the pupil that precedes color transformation was observed in the blue iris. At 60 h, color change was well underway, and by 72-h postmortem, the blue iris had turned entirely brown/black, except for a slip of blue on the medial periphery (Fig. 4). Unlike the isolated eyes, which were affected by *tache noire* increasingly as the blue irides changed color, the sclera of the *in situ* eyes remained white and fresh looking, despite the fact that the eyes opened spontaneously between 12- and 24-h postmortem. No obvious color change occurred in the brown iris.

In both Parts 1 and 2 of the experiment, iris color change occurred within 48 h in 100% of the blue irides of isolated eyes in warm environments (21°C or higher), and in a similar pattern. Color change began at the pupillary margin and moved outward

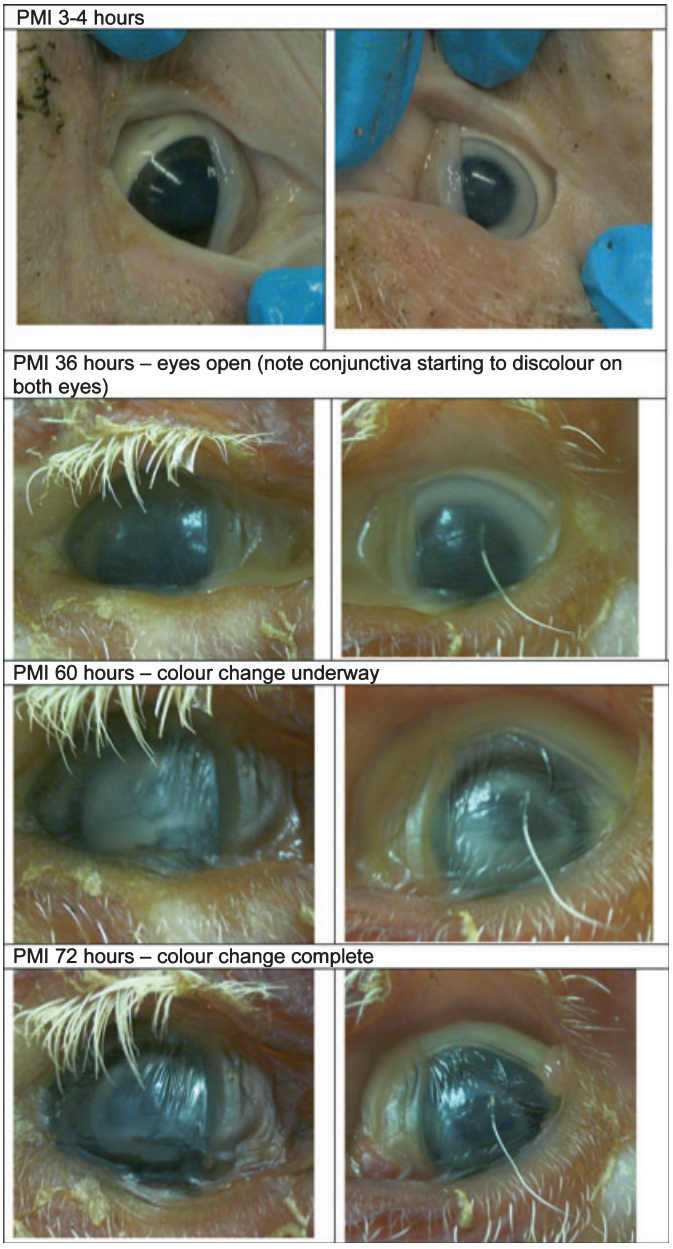

FIG. 4—*Part 2, 22°C (Environment B), in situ eyes: right (brown), left (blue). Photo: Bonita Dainowski.*

toward the periphery of the iris. In addition, some of the eyes exhibited darkening at the periphery of the iris subsequent to the initial darkening around the pupil (Fig. 5).

Irides of isolated eyes kept in a cool environment (4–8°C) retained their color for at least 72-h postmortem.

Brown irides tended to retain their color, regardless of the postmortem temperature, until the eyes were fully decomposed, although the pupillary margins (and in some cases, the periphery of the iris) exhibited the darkening that preceded color change in blue eyes.

Changes to Vitreous Humor

In Part 1 of the experiment, vitreous humor extracted at 12- and 24-h postmortem was transparent and gel-like, as expected, but by 36-h postmortem the vitreous was watery and dark, particularly so

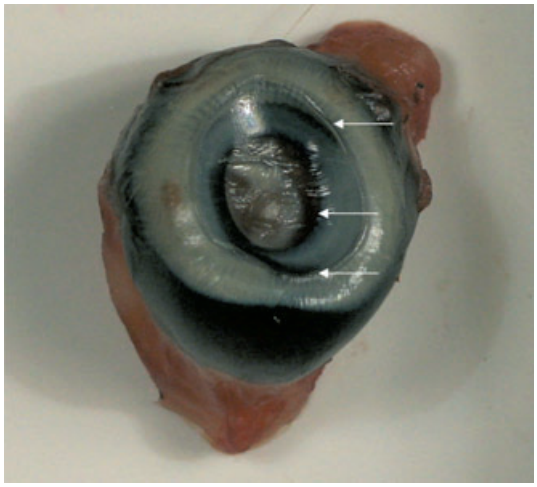

FIG. 5—*Darkening of blue iris in pupillary margin and periphery of iris (Part 1, 25°C, Environment B, 36 h). Photo: Bonita Dainowski.*

at temperatures of 21°C or higher, and became increasingly dark with postmortem interval. In Part 2, the vitreous of the eyes in the Gallenkamp oven (Environment C, 35°C) was dark by 24-h postmortem.

Within the test tubes themselves, free melanin granules precipitated to the bottom of the tubes. An increase in the volume of precipitated granules would appear to be correlated to postmortem interval (Fig. 6). Although quantitative measurements were not taken, the amount of melanin was visibly greater in the test tubes. This was most evident in vitreous collected from eyes in the warmest environment (Environment C, 30–36°C).

Under a light microscope, it was possible to observe the foci of melanin-containing tissue and freed melanin granules from vitreous humor removed from an eye in Environment C, 36-h postmortem (Fig. 7).

Discussion

This experiment demonstrated that, in *Sus scrofa* eyes, blue irises change color postmortem in warm or hot environments with moderate humidity: 100% of the blue irides at room temperature or

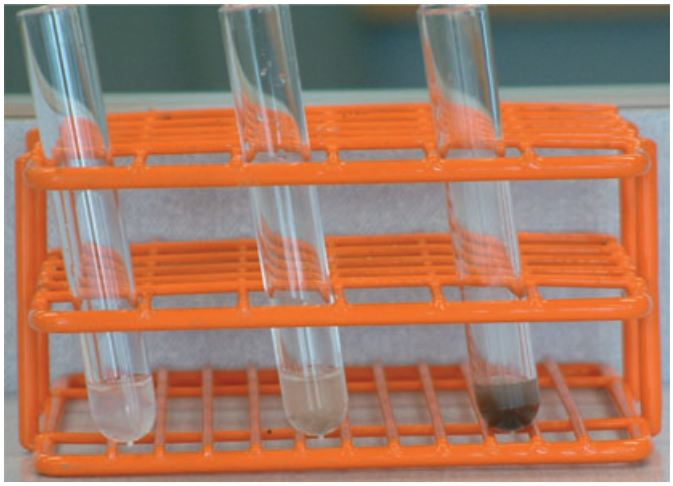

FIG. 6—*Vitreous humor collected from three eyes in Part 2, 35°C (Environment C) at 12-, 24-, and 36-h postmortem. Photo: David Quincey.*

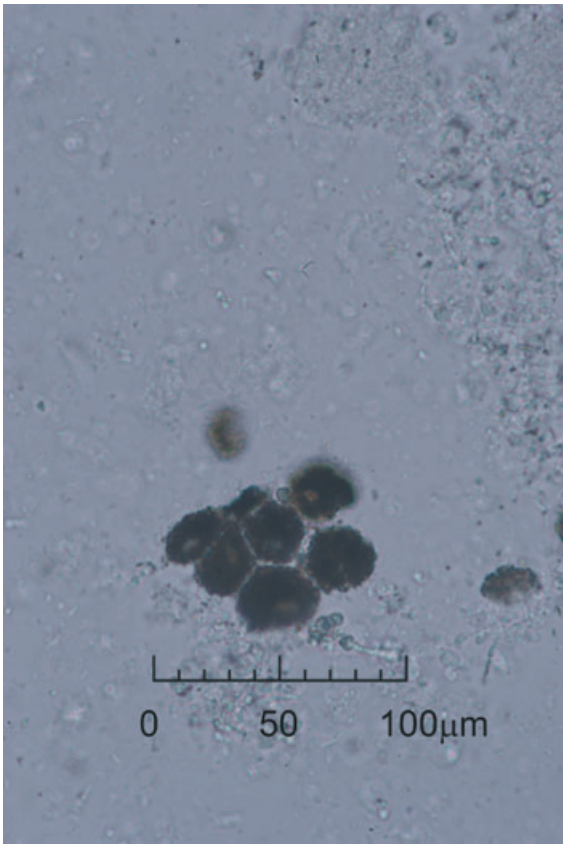

FIG. 7—*Vitreous humor from eye in Part 2, 35°C (Environment C) at 36-h postmortem. Photo: David Quincey.*

higher turned dark brown or black prior to the complete decomposition in the eye. The blue portion of the bi-colored irides underwent similar changes; areas of the iris that were originally blue became indistinguishable from the brown areas. If this postmortem artifact occurs in humans, it may lead to confusion of identity and possibly prevent a potential match from being made between unidentified remains and a missing person report, particularly when relying on electronic victim identification software in the context of mass fatality incidents.

While there is a predictable sequence of events in the decomposition of *Sus scrofa* eyes, iris color change cannot be used to determine or estimate time of death. The variable of temperature alone, at least in isolated eyes, precludes postmortem iris color change as a reliable indicator of the postmortem interval.

Another important variable is humidity. While eyes may spontaneously open some hours after death (21), some will remain closed, particularly if the death is caused by drowning or carbon monoxide poisoning (*ibid.*). The position of the eyelid affects the rate at which the eye desiccates and, by extension, the rate of iris color change. Similarly, an arid or particularly humid environment will respectively increase or decrease the rate of eye decomposition.

Apart from the artificial laboratory environment, limitations of this experiment include the low number of blue irides ($n = 13$), and the rapid desiccation of the isolated eyes. To this end, more regular monitoring of changes (e.g., every 6 h) may be useful. An inherent drawback to any monitoring scheme, however, is the removal of the eyes from their respective environments, and exposure to hot lights during photography, both of which are likely to affect desiccation rates. Future studies should use *in situ* eyes and record changes in relation to time of death. The fact that the one *in situ* blue eye in this experiment ($n = 1$) turned brown may or may not be a meaningful result. Furthermore, organic animals may produce different results than intensively reared animals, given that hormones, antibiotics, and other additives to feed would introduce variables which will not apply to most humans, and this needs to be examined. Finally, intrinsic factors such as sex, diet, and exact age of the animal at death may have an effect on the rate of decomposition, and this information was not available for the experiment.

The mechanism behind iris color change may be related to postmortem changes in the vitreous humor, which changes in composition, color, and viscosity after death. Free melanin granules are found in vitreous humor as early as 24-h postmortem. The melanin is released either from the iris itself (iris pigment epithelium) or, more likely, from the retinal pigment epithelium or choroids, both of which are heavily pigmented.

Degrading iris pigment epithelium is unlikely to contribute to postmortem iris color change for two reasons. First, melanin granules released from the iris pigment epithelium would result in an initial lightening of the iris. It is not likely that the melanin is moving away from the iris; clearly, melanin content of the iris is increasing with the postmortem interval. Second, there is very little iris pigment epithelium as compared with retinal pigment epithelium and choroids, which line the entire posterior chamber.

Postmortem breakdown of melanocytes within the posterior lining of the globe would result in the release of melanin granules directly into the vitreous, in a process driven by autolysis, commencing within minutes after death (22).

While it is likely that melanin granules freed from degenerated melanocytes of the retinal pigment epithelium and/or the choroid layer underlie postmortem iris color change, this hypothesis does not inform as to why the irides of brown eyes did not turn significantly darker brown or black in this experiment. In future studies, scanning electron microscopic examination of vitreous and iridial tissue may be useful for identifying the mechanism of the phenomenon.

Further investigation is required into the interval between death and iris color change. Research on human remains is necessary to determine the frequency and consistency of postmortem iris color change. If iridial color change occurs in humans as well as *Sus scrofa*, then this postmortem artifact must be taken into consideration when comparing data sets of unidentified remains and antemortem data of missing individuals.

In mass fatality incidents, eye color discrepancies between antemortem and postmortem data can cause delays in the identification of victims. Furthermore, in the interest of the families of deceased who are given access to autopsy reports, erroneous eye color data unnecessarily upsets them.

Perhaps the protocol for victim identification should be modified to record only irides that fall into one of two categories: blue/grey or green/hazel. Otherwise, eye color should not be recorded at all. Brown iris color, at least in the eyes of *Sus scrofa*, is not a reliable indicator for identification, as its original color could have been different.

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