

Comparative Histology of the Vascular System in the Domestic and Game Cock

Game chickens have undergone selection for fighting ability for thousands of years¹. Concomitant to this selection were correlated responses which have survival value in the cockpit. Examples of such responses are the rapid blood prothrombin and coagulation times of game chickens in comparison to domestic fowl².

Preliminary studies on the comparative histological structure of veins and arteries of domestic and game cocks demonstrate a noticeable difference in these vessels. Studies to date have been limited to comparisons of iliac arteries and veins in 2 White Rocks and 2 game cocks of comparable ages (20 months) and weights (2.3 kg).

Vessels were dissected from the same region (axis of the thigh and body) and placed in Bouin's fluid for 48 h. They were then dehydrated in a graded series of alcohols, cleared in xylene, and embedded in paraffin. Sections cut at 6 μ were stained by (1) Delafield's hematoxylin and counterstained in 1% eosin Y in 95% alcohol and (2) Verhoeff's method for elastic fibers³ followed by Delafield's hematoxylin and eosin.

A gross structural difference was noted during dissection of the arteries and veins. These vessels appeared much stronger, smaller in diameter, and were quite tough in the game cocks. In comparison, the vessels of the domestic cocks appeared quite flacid (particularly the vein), larger in diameter, and gave the impression of being weaker structures.

Histological examination revealed that these gross observations were correct. The histological results are summarized in the Table and represent measurements taken from sections of the iliac artery and vein.

Although probability levels have not been set because there was no statistical analysis, large differences between genetic stocks are evident. The overall size of the iliac vein (Figures 1 and 2) and artery was much greater for the domestic than for the game cock. Contrariwise the arterial tunica adventitia and tunica media (Figures 3 and 4) were much thicker for the gamecock than for the domestic cock. No differences were noted between stocks for either the number or arrangement of elastic fibers in the iliac vessels. The greater thickness of the arterial tunica media

in the game cock would, however, indicate a proportionally greater number of elastic fibers in this vessel.

The structural differences in the veins and arteries of game and White Rock chickens must have evolved from artificial selection as stocks were developed for fighting purposes and food production. The increased heart rates that a fighting cock is exposed to for long periods during encounters⁴, coupled with the normal high blood pressures

¹ A. RUPORT, *The Art of Cockfighting* (Devin-Adair Co., New York 1949).
² S. C. MOHAPATRA and P. B. SIEGEL, *Poultry Sci.* 46, 1294 (1967).
³ R. D. LILLIE, *Histopathologic Technic and Practical Histochemistry* (McGraw-Hill, New York 1954).
⁴ C. L. HARRIS and P. B. SIEGEL, *J. appl. Physiol.* 22, 846 (1967).

Measurements (μ) of parts of the iliac artery and vein^a

	Artery		Vein					
	Game	White Rock	Game	White Rock	max.	min.	max.	min.
Thickness of tunica media	182	135	135	135	27	18	27	18
Thickness of tunica adventitia	630	450	455	450	540	455	450	410
Long axis of the elipsoidal vessel	1683	1620	1995	1820	2366	2295	3285	2821
Short axis of the elipsoidal vessel	1628	1620	1305	1092	1137	900	990	910

^a These represent maximum and minimum values observed for the samples measured.

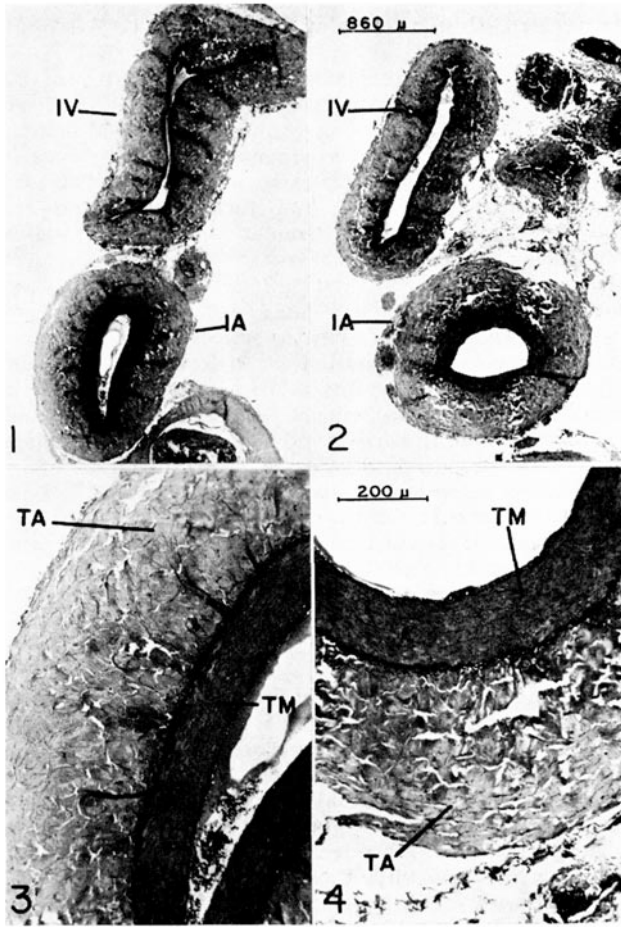


Fig. 1. Transverse section of the iliac artery and vein from a domestic White Rock cock. Verhoeff's stain for elastic fibers, Delafield's hematoxylin and eosin Y. IV, iliac vein; IA, iliac artery. Scale same as Figure 2.

Fig. 2. Transverse section of the iliac artery and vein from a game cock. Verhoeff's stain for elastic fibers, Delafield's hematoxylin and eosin Y. IV, iliac vein; IA iliac artery.

Fig. 3. Enlargement of a portion of the iliac artery shown in Figure 1. TA, tunica adventitia; TM, tunica media. Scale same as Figure 4.

Fig. 4. Enlargement of a portion of the iliac artery shown in Figure 2. TA, tunica adventitia; TM, tunica media.

of cocks⁶ could cause rupture of blood vessels lacking certain protective mechanisms. The need for such a mechanism in domestic chickens is not necessary. It is of further interest that in our laboratory, where all chickens that die receive post-mortem examinations, we have never observed any mortality from aortic rupture in game cocks, whereas mortality from this condition is not unusual in White Rock cocks.

Zusammenfassung. Vergleichend-histologische Untersuchung der Darmbeingefäße bei Wildhühnern und der White-Rock-Rasse zeigen eine stärkere Entwicklung der Wildhühnergefäße. Es scheint, dass das gesamte Gefäßsystem bei den Wildhühnern, in Anpassung an die er-

höhte Herzfrequenz und den gesteigerten Blutdruck bei besonders Erregungszuständen, über Schutzmechanismen in der Gefäßwand verfügt.

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⁶ B. W. HAWKES and P. B. SIEGEL, *Va J. Sci.* 15, 264 (1964).

Differentiation in vitro of Chick Embryo Adrenal Glands

Adrenal gland function appears to be controlled by pituitary secretion during the fetal life. This has been demonstrated in amphibians¹, rabbits², rats³ and man⁴.

Evidence that this also happens in bird embryos is somewhat conflicting. The early works of WOLFF and STOLL⁵ and FUGO⁶ indicate that chick adrenal glands are independent of pituitary stimulation until the twelfth incubation day. CASE⁷ and MAZINA^{8,9}, using histochemical techniques, confirmed that adrenotrophic stimulus is necessary after the twelfth day of incubation, but there is no confirmation up to now to support the assumption that adrenal differentiation is independent of that stimulus in younger embryos. In a previous paper¹⁰ it was shown that histochemical techniques for lipids and cholesterol appear earlier and are more intense when embryos are injected with ACTH. The present work was undertaken in order to study the action of ACTH on embryonic adrenal glands grown in organ culture.

Material and methods. Over 500 embryos of the Hyline breed were distributed in experimental groups as indicated in Figures 2 and 3. Whole adrenals were dissected and explanted using WOLFF and HAFEN's¹¹ technique. Culture medium contained equal parts (v/v) of embryo extract (from 9-day-old chick embryo) and 1% agar in Hank's saline solution. As indicated in Figures 2 and 3, in several experimental groups 1 IU of ACTH (Actonar, Laboratorios Acton, Argentina) per ml was added to the medium. Media were changed every 4 days.

Non-cultured controls and cultured explants were fixed in 10% formalin with 1% CaCl. Tissues used for histological study were embedded in paraffin and stained with hematoxylin-eosin while those reserved for histochemical purposes were embedded in gelatin and sectioned with a freezing microtome. Sections were observed with a polarizing microscope in order to detect birefringent crystals (generally cholesterol and its esters) or stained with Sudan black B as a general stain for lipids.

Results. Adrenal explants in all experimental groups grew and differentiated adequately as judged by their histological aspect (see Figure 1). No attempt was made, however, to make a quantitative study of growth differences; attention was focused on establishing the % of explants accumulating lipidic material during the culture period.

Figure 2A summarizes the results obtained with adrenal glands of 6-day-old embryo culture on media without

ACTH. After 6 days of culture only 6% of the explants showed sudanophilic material and 20% birefringent crystals; after 12 days of culture 13% showed sudanophilia and 10% birefringent material. When compared with controls, also shown in Figure 2A, these results appear to indicate that most of the explants which are demonstrable lipidic material at the moment of explantation lose it in the course of the first 6 days of culture and that no new accumulation occurs after that.

Figure 2B shows the result obtained with similar explants cultured in medium with ACTH. After 6 days of culture 76% of the explants contain sudanophilic material and 77% of them birefringent crystals. Tissue fixed on the twelfth day of culture showed sudanophilic material in 98% and birefringence in 93% of the cases. These results show, when compared with controls, that accumulation of lipidic material occurs during the first days of culture and continues thereafter.

The results obtained by culturing adrenal glands of 10-day-old embryos are expressed in Figure 3 A and B. After 6 days of culture, all the explants cultured in media with or without ACTH contain sudanophilic and birefringent material; after 12 days over 60% of the explants cultured in media without ACTH have lost their lipidic material. On the contrary, when cultured with ACTH few of the explants lose this material.

Discussion. In experiments by other authors in which adrenotrophic and other hormones have been used in vitro, the amounts of active substances added to the media in order to obtain noticeable effects have always

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⁶ N. M. FUGO, *J. exp. Zool.* 85, 271 (1940).

⁷ J. F. CASE, *Ann N.Y. Acad. Sci.* 55, 147 (1952).

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¹¹ E. T. WOLFF and K. HAFEN, *Tex. Rep. Biol. Med.* 10, 463 (1952).