of cocks[5] 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 Wildhuhngefässe. Es scheint, dass das gesamte Gefäßsystem bei den Wildhühnern, in Anpassung an die erhöhte Herzfrequenz und den gesteigerten Blutdruck bei besondern Erregungszuständen, über Schutzmechanismen in der Gefässwand verfügt.

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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 amphibians[1], rabbits[2], rats[3] and man[4].

Evidence that this also happens in bird embryos is somewhat conflicting. The early works of WOLFF and STOLL[5] and FUGO[6] indicate that chick adrenal glands are independent of pituitary stimulation until the twelfth incubation day. CASE[7] 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 paper[10] 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 HAFFEN's[11] 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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been much higher than the corresponding physiological levels. In agreement with this, our results, as well as those of other authors (GYÉVAI, STARK and SZALAY[4]) show that, regardless of the fact that embryo extract may contain minute amounts of adrenotrophin, media containing it behave from the practical standpoint as nonhormonal.

Results obtained after embryonic hypophysectomy by means of irradiation[5] or surgical decapitation[6,7], indicate that in chicks adrenal gland function is dependent on pituitary stimulation during the last part of the embryonic life. MAZINA[8,9] using injections of ACTH, and STOLL, FAUCOUNAU and MARAUD[12] using injections of metopirone, have confirmed this conclusion. Our results, showing that the accumulation of lipids in chick embryonic adrenal glands maintained in vitro is dependent on the presence of ACTH in the medium, furnishes additional confirmation.

The fact that adrenals of 6 embryos cultured for 6 days accumulate lipids and cholesterol only if ACTH has been added to the cultured medium, suggests that pituitary action on adrenals is not restricted to the last part of the embryonic life, but is also necessary for initial differentiation. This assumption agrees with the results obtained in vivo by CASTAÑÉ DECOUD, PEDERNERA and NARBAITZ[10,13].

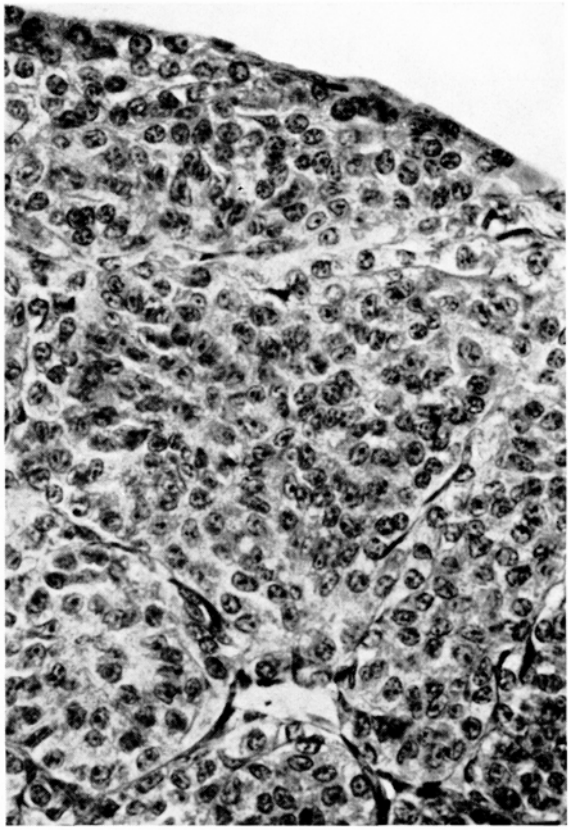

Fig. 1. Adrenal gland explant of 10-day-old embryos cultured for 12 days. Hematoxylin-eosin stained. × 490.

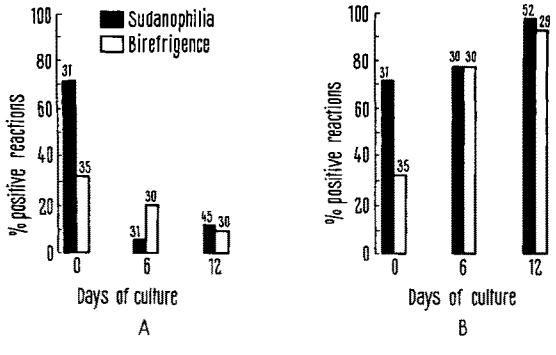

Fig. 2. Results obtained with explants of 6-day-old embryos cultured in media without (A) and with (B) the addition of ACTH (1 IU/ml). The numbers on each column indicate the total number of explants studied. O, controls.

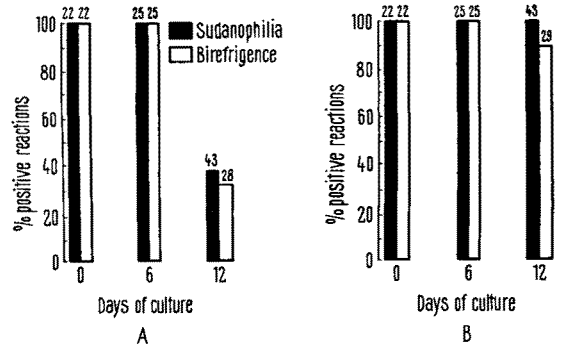

Fig. 3. Results obtained with explants of 10-day-old embryos cultured in media without (A) and with (B) the addition of ACTH (1 IU/ml). The numbers on each column indicate the total number of explants studied. O, controls.

Resumen. Suprarrenales de embriones de pollo de 6 y 10 días de incubación, fueron cultivadas en medios con y sin el agregado de ACTH. Los cultivos fueron mantenidos por períodos de 6–12 días. Los explantos fueron estudiados con técnicas histoquímicas para lípidos y ésteres de colesterol. Las suprarrenales de embriones de 6 días acumulan lípidos y colesterol solamente cuando son cultivadas en medios con ACTH. Luego de 12 días de cultivo la mayoría de las suprarrenales de los embriones de 10 días pierden su contenido de lípidos si han sido cultivadas en un medio privado de ACTH. El agregado de la hormona previene esa pérdida. Se discuten las implicancias fisiológicas de estos hallazgos.

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