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IMMUNOHISTOCHEMICAL STUDY OF DIFFERENTIATION OF THE CEPHALIC LOBE OF THE CHICK EMBRYONIC ADENOHYPOPHYSIS

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It was shown by the method of indirect immunofluorescence that ACTH appears in the cephalic lobe of the adenohypophysis of chick embryos after the 8th day of development. A new tissue-specific antigen (A3) was found in the adenohypophysis: It is located in the cephalic lobe and appears on the 7th day of embryonic development. It is concluded from the results of quantitative analysis of the distribution of ACTH and antigen A3 in cells of the adenohypophysis of 11-day chick embryos that antigen A3 is present in the corticotropic cells.

KEY WORDS: chick embryonic adenohypophysis; ACTH; tissue-specific antigen; immunofluorescence.

It has been shown by immunologic methods that ACTH in birds is contained in the cephalic lobe of the adenohypophysis [6, 7, 9]. The period of specific differentiation of the adenohypophysis in chick embryos begins with the 6th day of development. ACTH is found immunohistochemically after the 9th day [8] although, as the results of biological tests have shown, corticotropic activity appears on the 8th day [10, 12] and melanocyte-stimulating activity, also characteristic of ACTH, appears on the 5th day [5, 11] or at 6-6.5 days of development [4]. To discover the principles governing differentiation of the embryonic adenohypophysis it is interesting to study the dynamics of appearance not only of hormones, but also of tissue-specific antigens [1, 2]. The object of the present investigation was to make a comparative study of the appearance of ACTH and the tissue-specific adenohypophyseal antigen A3 and their localization in the adenohypophysis of chick embryos.

EXPERIMENTAL METHOD

Adenohypophyses of chick embryos (from Russian White hens) at 6-11, 13, 15, and 18 days of development and 6-day-old chicks were investigated. In some experiments the hypophyses of 8-day quail embryos, rabbits, a 20-week human fetus, and 11-, 15-, and 18-day CBWA mouse embryos also were used. Tissues were fixed in Bouin's mixture and embedded in paraffin wax; serial sections were cut to a thickness of 5 μ and treated by the indirect immunofluorescence method [3].

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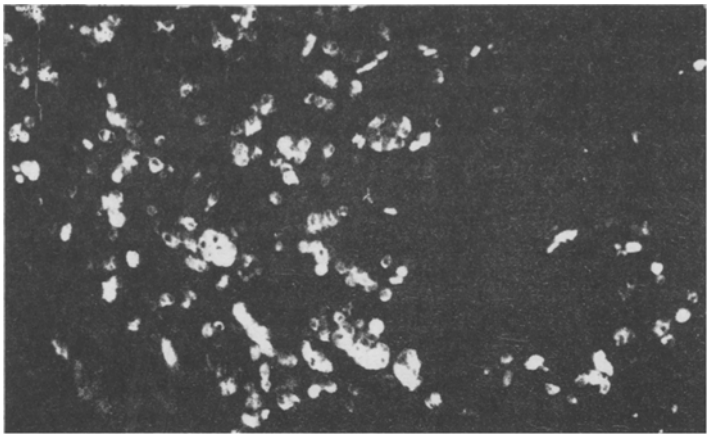

Fig. 1. ACTH-cells in cephalic lobe of adenohypophysis of 15-day chick embryo. Indirect immunofluorescence, ML-2B microscope, objective 10, homal 3.

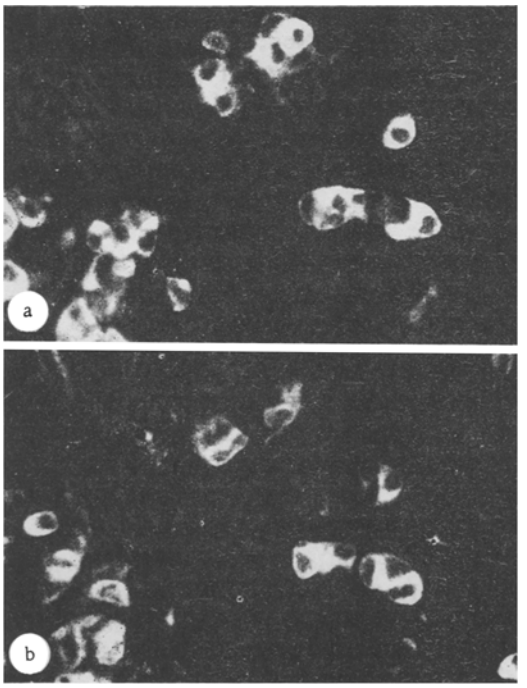

Fig. 2. Adenohypophysis of 11-day chick embryo, with location of ACTH-containing (a) and A 3-containing (b) cells in neighboring 5-μ sections. Indirect immunofluorescence, ML-2B microscope, objective 40, homal 5.

A sample of chromatographically pure hog ACTH was generously provided by Corresponding Member of the Academy of Medical Sciences of the USSR Professor Yu. A. Pankov (Institute of Experimental Endocrinology and Hormone Chemistry, Academy of Medical Sciences of the USSR, Moscow). Rabbits were immunized to a scheme of three injections with 10-day intervals into the footpads and into the region of the popliteal and inguinal lymph nodes, and reimmunized 1.5 months later. Single injections were given of 0.3 mg ACTH in 0.5 ml physiological saline and 0.5 ml of Freund's complete adjuvant.

Antisera against tissue-specific adenohypophyseal antigen were obtained by prolonged immunization of a rabbit with the cathodal electrophoretic fraction of an aqueous extract from the cephalic lobe of hen adeno-

TABLE 1. Detection of ACTH and Antigen A3 in Chick Embryonic Adenohypophysis Based on Results of Immunofluorescent Tests with Antisera against Hog ACTH and against Extract of the Cephalic Lobe of the Hen Adenohypophysis

Age of embryos, days	No. of pituitary glands studied	Antisera against ACTH				No. of pituitary glands studied	Antisera against A3	
		№ 2416	№ 2416a	№ 2426	№ 2426a		2429a	24296
6	2		—			2	—	
7	5		—		—	6	±	+
8	3	—	+	—		3	+	
9	3		+		+	3	+	+
10	3		+		+	3	+	+
11	6	—		±	+	5		+
13	2	±	+	+	+	1		+
15	3	+		+	+	2	+	+
18	4	+	+	+	+	3		+
6-Day chick	1		+			1		+

Legend. Antisera Nos. 2416 and 2426 were obtained after immunization of rabbits for 3 weeks, Nos. 2416a and 2426b after 1.5 months of immunization, and antisera Nos. 2429a and 2429b after immunization for 4 and 10 months respectively.

hypophyses. The methods of preparing the extracts and of fractionation and immunization were described previously [2]. The antisera were exhausted with lyophilized extracts of hen tissues (10 mg to 1 ml antiserum) and ACTH (2 mg to 1 ml antiserum).

EXPERIMENTAL RESULTS

Antisera against hog ACTH gave a positive immunofluorescence test with the anterior lobes of the pituitary gland of a 20-week human fetus, of adult rabbits, and of 15- and 18-day mouse embryos. Massive bright fluorescence of the cells was found in the cephalic lobe of the adenohypophysis (Fig. 1) but was absent in the caudal lobe in chicks and chick embryos, especially in the late stages of development, in reactions for ACTH. The fluorescence still remained after exhaustion of the antisera with chicken liver extract and it was completely blocked after exhaustion with the ACTH preparation.

The first cells to show weak specific fluorescence in reactions for ACTH were found in 8-day embryos (Table 1) in the anterior superior bands of the adenohypophysis. By the 11th day of development they were found over the whole area of sections of the cephalic lobe and consisted of single cells in the neighborhood of nonfluorescent cells or small groups of cells (Fig. 2). In 13 to 18-day embryos numerous bright cells were located in the parenchyma of the cephalic lobe and were absent in the epithelium of the residual cavity of the adenohypophysis (Fig. 1). Meanwhile, in some embryos solitary ACTH-cells also were seen in the caudal lobe from the ventral aspect or in the central cell bands; this indicates that the histological boundaries of these lobes overlap in the middle part of the adenohypophysis.

Antisera against the cathodal fraction of extract of the cephalic lobe of the chicken adenohypophysis, exhausted with lyophilized liver extracts, and similar antisera against the caudal lobe of the chicken adenohypophysis gave a positive immunofluorescence test with the cephalic lobe of the adenohypophysis of the chick and chick embryos, and also of the 8-day quail embryo, but did not react with the pituitary glands of mouse embryos. Fluorescence of the cells in the chick embryonic adenohypophysis also was found if the antisera were further exhausted with the preparation of hog ACTH or with extracts of hen kidney, spleen, lung, brain, retina, heart, muscles, and blood serum. The results thus show that an independent tissue-specific antigen (A3), different from ACTH, is present in the cephalic lobe of the hen adenohyophysis. Other evidence that antigen A3 is independent of ACTH is given by the fact that when chick embryonic pituitary glands are kept in paraffin blocks the intensity of fluorescence of the cells in tests for antigenic A3 fell sharply after 8-10 months, but was virtually unchanged in tests for ACTH.

Antigen A3 was found in the adenohypophysis after the 7th day of embryonic development (Table 1). Like ACTH, antigen A3 appeared in the anterior superior sector of the adenohypophysis. In the later stages of development the localization of antigen A3 was similar to the distribution of ACTH in the developing adenohypophysis.

TABLE 2. Number of Fluorescent Adenohypophyseal Cells of 11-Day Embryos in Reactions with Antisera against ACTH and Antigen A3 and with a Mixture of These Antisera (in an area of 0.02 mm^2)

No.	Antiserum	Embryo No. 1			Embryo No. 2		
		number of sections studied (n)	number of fluorescent cells (M ± m)	P	number of sections studied (n)	number of fluorescent cells (M ± m)	P
1	Anti-ACTH, No. 2426a, dilution 1:3	12	108±6	>0,05	13	78±6	>0,05
2	Anti-A3, No. 2429b, dilution 1:3	12	117±5	>0,05	13	93±6	>0.05
3	Total number of cells in neighboring sections	12	225±10	<0,001	13	171±9	<0,001
4	Mixture of antisera (No. 2426a 1:3+No. 2429b, 1:3)	12	119±5	—	13	91±7	—
5	Anti-ACTH, No. 2426a, dilution 1:6	11	105±6	>0,05			
6	Anti-A3, No. 2429b, dilution 1:6	11	119±5	>0,05			

Legend. P denotes significance of differences from number of fluorescent cells in tests with mixture of antisera.

The similarity of the localization of ACTH and antigen A3 in the chick embryonic adenohypophysis, which was particularly noticeable when fluorescent cells were compared in neighboring sections (Fig. 2), suggested that ACTH and antigen A3 may be present in the same cells. To study this problem, correlation between the fluorescent cells in the adenohypophysis of 11-day embryos was studied in serial sections treated with antisera against ACTH, against antigen A3, and with a mixture of these two antisera. Specifically fluorescent cells were counted in an area of 0.02 mm^2 near the anterior border of the cephalic lobe of the adenohypophysis (Table 2).

On the average the same number of fluorescent cells was found in each of the two embryos tested in reactions with the separate antisera. Further double dilution of the antisera did not lead to a reduction in the number of fluorescent cells by half. Consequently, the dilution of each antiserum when they were mixed likewise should not have affected the results of the tests. The number of cells detectable by a mixture of antisera was shown to correspond with the number of fluorescent cells in reactions with the separate antisera and was statistically significantly less than the total number of cells in these tests. In chick embryos ACTH and antigen A3 are evidently present in the same adenohypophyseal cells.

The results confirm published data on the localization of ACTH in chick embryos in the cephalic lobe of the adenohypophysis [7, 8]. ACTH could be detected immunohistochemically after the 8th day of development, which corresponds to the beginning of manifestation of corticotropic activity in the adenohypophysis [10, 12]. Differentiation of the cephalic lobe of the adenohypophysis in chick embryos is also connected with the appearance of tissue-specific antigen A3, which is characteristic of corticotropic cells and precedes the appearance of ACTH by 24 h. Detection of the tissue-specific antigen of the corticotropic cells suggests the existence of similar antigens characteristic of other types of adenohypophyseal cells.

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