The results are evidence of the similarity or identity of antigens specific for stable L-forms of group A streptococci and antigens composing the cytoplasmic, including sarcoplasmic, membrane of myocardial muscle fibers. This phenomenon may partly explain why the body does not recognize L-forms as foreign, and this may be one of the conditions responsible for their long persistence.

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TISSUE-SPECIFIC ANTIGEN IN THE CAUDAL LOBE OF THE CHICK ADENOHYPOPHYSIS

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A tissue-specific water-soluble antigen was discovered in the chick adenohypophysis by methods of immunochemical analysis. The content of this antigen was found to be highest in the caudal lobe of the adenohypophysis. During embryonic development the antigen was detected by immunoelectrophoresis and immunofluorescence after the 13th day. Two forms of the antigen were found in chick adenohypophysis – one with high and one with low electrophoretic mobility. It is concluded that cells of the adenohypophysis differ in their level of differentiation. KEY WORDS: chick adenohypophysis; caudal lobe; tissue-specific antigen.

The adenohypophysis of the chick embryo is widely used for the comparative study of the principles of morphogenesis and development of the functions of this organ in the individual development of vertebrates [3-7, 9-11]. Yet there have been few studies of the immunochemistry of development of the chick adenohypophysis [2, 8]. Together with the study of the dynamics of appearance and cellular localization of hormones for the analysis of differentiation of this adenohypophysis, the investigation of its tissue antigens may also yield important results.

The object of the present investigation was to study the tissue antigens of the chick adenohypophysis with the object of discovering specific marker antigens of differentiation.

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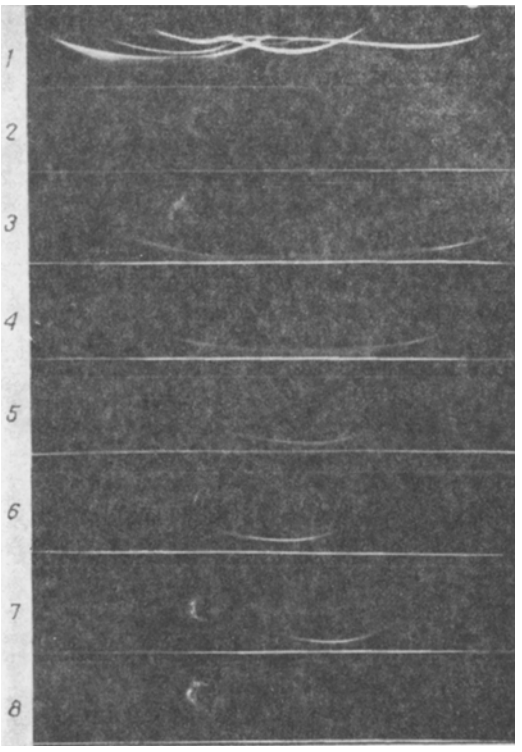

Fig. 1. Immunoelectrophoretic analysis of A1. 1) Control: reaction of normal human serum (NHS) with anti-NHS; 2-8) detection of A1 in hens' adenohypophysis and chick embryos by antiserum against cathodal fraction of adenohyophysis after exhaustion with hens' liver; 2) extract of cephalic lobe (40 mg/ml); 3) extract of caudal ions (40 mg/ml); 4) the same (10 mg/ml); 5) ditto (2.5 mg/ml); 6, 7, 8) pituitary gland homogenates from 19-, 15-, and 13-day embryos respectively.

EXPERIMENTAL METHOD

Experiments were carried out on hens of the Russian White breed. Adenohypophyses removed intact or divided into three parts (cephalic, middle, and caudal) were homogenized in 10 volumes of distilled water, pH 7.2. The extract was lyophilized and used to prepare working solutions in Tris-buffer (0.1 M, pH 8.2-8.6) with a concentration of the lyophilized product of 40-60 mg/ml. Extracts were prepared in the same way from the brain, muscles, kidneys, and liver of the hens and from adenohypophyses of Central Asiatic turtle Testudo horsfieldi.

Extract from whole adenohypophyses was fractionated by electrophoresis in agar gel in a 0.1 M Tris-buffer system, pH 8.2-8.6. The agar was cut into four parts, ground in the frozen state, mixed with Freund's adjuvant, and injected subcutaneously into rabbits in a dose of 5-6 ml of the mixture once a month. The complete course of immunization of six rabbits required 360 mg of the lyophilized extract. Active antisera were obtained after the second reimmunization.

Methods of immunoelectrophoresis, immunodiffusion, and indirect immunofluorescence were used [1]. The electrophoretic mobility of the antigens was calculated relative to the mobility of human albumin [13]. The cellular localization of the antigens was investigated in chick embryos at the 13th, 15th, and 18th days of development. The pituitary glands, removed with adjacent tissues, were fixed in Bouin's fluid or 1% acetic acid solution in ethyl alcohol, and embedded in paraffin wax [12]; sagittal sections 5 μ thick were cut.

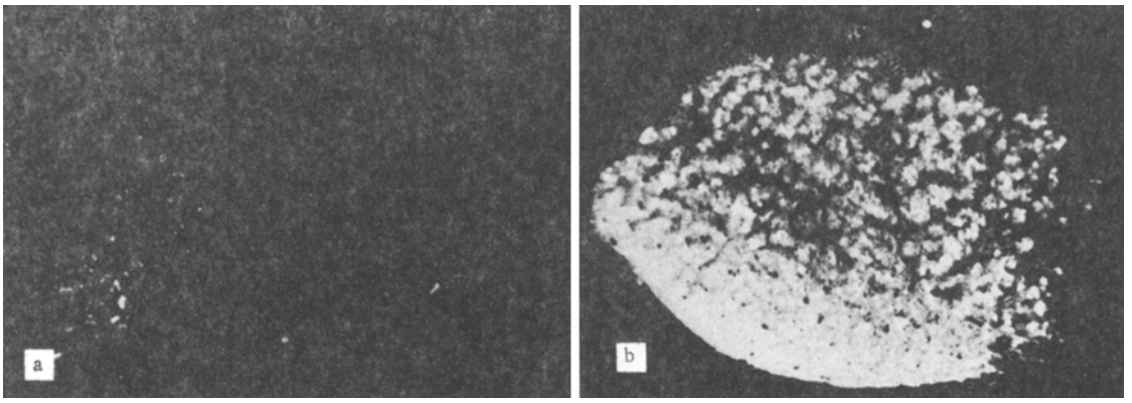

Fig. 2. Localization of A1 in adenohyophysis of 18-day chick embryo: a) cephalic lobe; b) caudal lobe. Indirect immunofluorescence, 30×.

EXPERIMENTAL RESULTS

All antisera against the electrophoretic fractions of the hens' adenohypophyses contained antibodies against serum antigens. After exhaustion with hens' blood serum only two of them, obtained against the cathodal fraction of adenohypophysis, continued to react during immunoelectrophoresis with extract of adenohypophysis, to form 4 or 5 precipitation lines. Both antisera revealed an antigen A1 in the adenohypophysis, forming a long precipitation band when high concentrations of the lyophilized extract were used, comparable in length with the precipitation band of human serum globulin. Exhaustion of the antisera with extracts of hens' brain, muscles, and kidneys led to a decrease in the number of precipitation bands found in reactions with the adenohypophysis, and after exhaustion with liver extracts only one precipitation band (A1) remained. It can be concluded from these results that A1 is a tissue-specific antigen of the hen's adenohypophysis. An identical antigen was also found in the adenohypophysis of Testudo horsfieldi, evidence of its weak species-specificity, but was not found in the rat adenohypophysis.

The distribution of A1 in the hen's adenohypophysis was investigated in experiments in which adenohypophyses were divided into cephalic, middle, and caudal parts. Immunoelectrophoresis revealed A1 in each of these parts. The immunochemical identity of the cephalic A1 and caudal A1 was confirmed by the merging of the corresponding precipitation bands in the immunodiffusion test. At the same time, the analysis showed significant differences in the electrophoretic properties and quantitative content of A1 in the cephalic and caudal lobes of the hens' adenohypophysis.

During immunoelectrophoretic tests with extracts from the caudal and middle parts of the adenohypophysis, using the lyophilized material in concentrations of 60-40 mg/ml, long precipitation bands were formed, evidence of the considerable heterogeneity of A1 in these parts. In tests with extracts from the cephalic part

TABLE 1. Relative Electrophoretic Mobility of Tissue-Specific Adenohypophyseal Antigen A1 in Hens and Chick Embryos

Test object	$M \pm m$	P_1	P_2
Hens' adenohypophysis:			
cephalic lobe	23±1,2 (18)	<0,001	—
caudal lobe	54±1,0 (27)	—	<0,001
Embryonic adenohypophysis:			
19 days of develop.	50±0,4 (4)	—	—
15 " "	33±1,0 (8)	<0,001	<0,001
13 " "	28±0,8 (4)	—	—

Legend. P_1) Significance of differences from mobility of A1 from caudal lobe, P_2) significance of differences from mobility of A1 from cephalic lobe; number of determinations shown in parentheses.

of the adenohypophysis, using the same concentration of lyophilized material, shorter and straighter precipitation arcs were formed, at the level of the cathodal half of the precipitation bands of A1 from the caudal lobe (Fig. 1). The relative electrophoretic mobility of A1 from the caudal lobe was twice that of A1 from the cephalic lobe (Table 1).

During serial dilution of the extracts a visible precipitation band was formed in reactions with the cephalic part of the adenohypophysis only in the presence of sufficiently high concentrations of lyophilized extract, namely 60 and 40 mg/ml; with a concentration of 30 mg/ml the reactions were negative. A1 was detected in the caudal part of the adenohypophysis over a wider range of dilutions – from 60 to 1 mg/ml. With a reduction in the concentration of lyophilized material in the extract of the caudal lobe the precipitation bands formed by Al became shorter and formed a regular arc. However, the essential point is that the relative electrophoretic mobility of A1 was unchanged and differed from the mobility of A1 from the cephalic lobe.

During embryonic development Al was found after the 13th day. In reactions with pituitary homogenates from 13-, 15-, and 19-day embryos the arrangement of the precipitation bands (Fig. 1) points to a progressive increase in relative electrophoretic mobility of Al in this period, and on the 19th day it was similar to the mobility of Al from the caudal lobe of the hens' adenohypophysis.

For the immunohistochemical detection of A1 fixation of the pituitary glands with 1% acetic acid in ethyl alcohol proved to be the optimal method, for after fixation with Bouin's fluid specific fluorescence was absent. In 13-day embryos cells containing A1 were found only in the lower sector of the caudal lobe; on the 15th day of development and in 18-day embryos, on the other hand, massive fluorescence of the cells was observed in the caudal lobe; in 18-day embryos, moreover, A1 was found in solitary cells located at the periphery of the cephalic lobe (Fig. 2).

It can be concluded from these results that the tissue-specific antigen discovered in the hens' adenohypophysis is characteristic of the caudal lobe. Considering the relatively late appearance of this antigen in embryogenesis, the dynamics of changes in its electrophoretic mobility and cellular localization is evidence that the adenohypophysis continues to differentiate after the 13th day of embryonic development. The discovery of two forms of the antigen, with high and low electrophoretic mobility respectively, in the hens' adenohypophysis may perhaps indicate that in the definitive adenohypophysis cells containing this antigen differ in their level of differentiation.

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