EFFECT OF ANTILENTICULAR ANTIBODIES ON FORMATION OF EYE ANLAGEN OF CHICK EMBRYOS

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The action of antibodies against two lenticular antigens (α- and δ-crystallin) on the cranial part of 18-h chick embryos led to complete inhibition of development of the eye. During the action of antibodies on the cranial part of 24-h embryos, development of the eye was disturbed to some extent and inhibited. If antibodies were injected into the yolk, less marked disturbances of development were observed.

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In the course of embryonic development of tissues and organs of animals, besides morphological differentiation, differentiation of the antigenic properties of the tissues is observed [1, 2, 5, 7, 10, 12]. It is considered [5] that antigens exert some action on the course of morphogenetic processes. For this reason, one approach to the elucidation of the morphogenetic role of antigens is to study the action of tissue antibodies blocking the corresponding antigens on the development of tissues and organs containing these antigens [6, 8]. Experiments on chick embryos [4, 9, 11] have shown that developmental anomalies of the eye of chick embryos take place as a result of the action of antilenticular antibodies. However, all investigations of this character have been undertaken, or even at later stages. Only in one investigation was an attempt made [11] to study the action of antibodies against the individual crystallins of the lens.

In the present investigation the role of antigens (crystallins) of the lens was studied at different stages of formation of the eye by blocking them with antibodies against α- and δ-crystallins.

EXPERIMENTAL METHOD

Experiments were carried out on chick embryos of the Russian White breed at 18 and 24 h of incubation. The lens was used as the test object. Antisera were obtained in rabbits. A saline tissue extract of the lens of an adult chicken, made up in a dilution of 1 : 5, was injected once, intraperitoneally, into a rabbit in a dose of 5 ml. Blood was taken on the 11th day after injection. The resulting antisera were identified by the ring-precipitation test, by Ouchterlony's double diffusion test, by Osserman's identity test, and by immunoelectrophoresis [10]. The results showed that antisera against the lens of adult hens reacted with identical antigens in the ring-precipitation test in a dilution of 1 : 200,000. Analysis of these antisera by immunoelectrophoresis showed that they form two precipitation bands: one against α-crystallin and one against δ-crystallin. In Ouchterlony's test, when comparing antiserum against the lens of an adult chicken with highly concentrated (1 : 2) antigens from the brain, liver, heart, kidney, and serum of an adult chicken, only one very feeble precipitation band appeared, and only with antigen from the brain. Ouchterlony's test showed that δ-crystallin gives a band of identity with antigen from adult chicken brain.

Intact embryos of the same period of incubation and embryos treated with blood serum of unimmunized rabbits served as controls. Subsequent analysis showed that development of the eye of chick embryos took place under the influence of normal serum in the same way as development of the eye in intact em-

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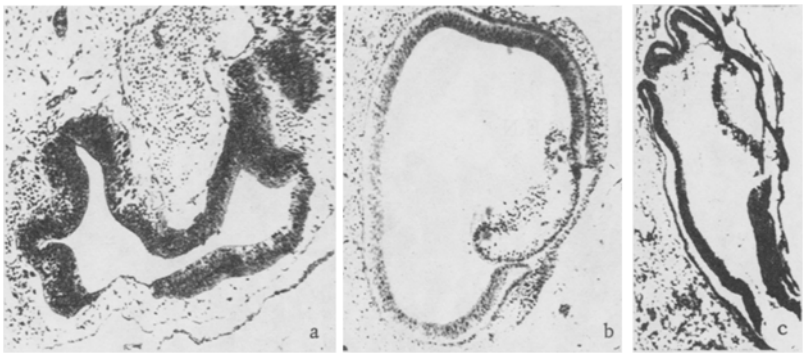

Fig. 1. Action of antibodies for 48 h on 18-h chick embryos: a) Cranial end of embryo; b) control; c) yolk. Carazzi's hematoxylin, counterstained with eosin, 70×.

bryos. For this reason, during the description of the subsequent tests, only chick embryos under the influence of normal serum will be described as the control.

Before the experiment eggs incubated for the required time were examined in the ovascope and the position of the air chamber noted on the shell. Next, using ophthalmic scissors, windows were made in the region of the air chamber in the alcohol-sterilized eggs. The shell membrane was removed with ophthalmic forceps and the vitelline membrane over the embryo detached. Next, a piece of filter paper, measuring 1×1 mm^2, soaked in antiserum, was placed on the cranial part of the embryo, or an injection of 0.1 ml antiserum was given into the yolk. The windows were covered with shell, paraffin wax was poured on, and the eggs were replaced in the incubator. The embryos were inspected 48 h after injection of antiserum. The embryos were fixed in Bouin's fluid, embedded in paraffin wax, and histological sections were cut to a thickness of 5-6 μ. The sections were stained with Carazzi's hematoxylin and counterstained with eosin.

EXPERIMENTAL RESULTS

In cases when the cranial end of 18-h chick embryos was treated with antiserum adsorbed on filter paper, after further incubation for 48 h considerable disturbances in development of the eye were observed in the embryos (Fig. 1a). Whereas in control embryos at this period of incubation (18 + 48 h) the lens with the primary lenticular fibers were formed (Fig. 1b), in the experimental animals formation of the lens, or even of the optic cup could not be observed in any of the five cases. Formation of both optic cup and lens was observed 48 h after injection of antiserum into the yolk of embryos at the same period of development, but destruction of the primary lenticular fibers was present in the lenses of these embryos, the neural and pigmented layers of the primitive retina were much thinner than in the control, and their cells were more loosely arranged (Fig. 1c).

In the control chick embryos at 24 h of incubation, treated with normal serum for 48 h, the formation of primary lenticular fibers was observed in the lens (Fig. 2a), the optic cup consisted of two layers, and the neural and pigmented layers were well-defined and in close contact with each other. In the eyes of the experimental embryos at the same period of development (24 + 48 h), treated with antiserum adsorbed on filter paper and applied to the cranial part of the embryo, only invagination of the lens placode had occurred, the optic cup consisted of only one layer, and the pigmented layer was absent (Fig. 2b). When antiserum was injected into the yolk of the chick embryos (Fig. 2c), deformation of the optic cup was observed, the neural layer was ill-defined, and destruction of the presumptive lenticular fibers took place in the pinched off lens vesicle.

It can thus be concluded from these results that antibodies exert a substantial effect on the developing eye tissues of the chick embryo. The character of action of the antibodies depends on the age of the embryos and the method of administration of the antibodies. If antibodies are injected into embryos at earlier stages of development (18 h of incubation), their action is more powerful (complete absence of development both of the optic cup and of the lens). The same treatment applied to embryos at later stages of development (24 h) led to only slight anomalies in the development of the eye. Antibodies applied directly to cranial part of the embryo and acting at the moment of application have a stronger effect than antibodies

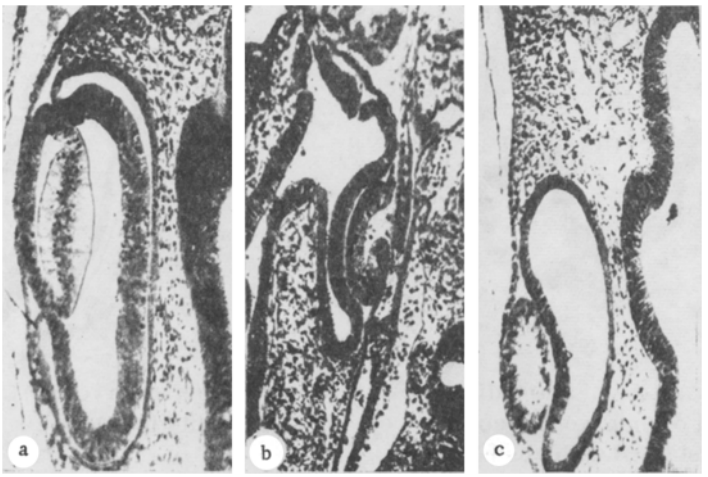

Fig. 2. Action of antibodies for 48 h on 24-h chick embryos: a) Control; b) cranial part of embryo; c) yolk. Carazzi's hematoxylin, counterstained with eosin, 70×.

injected into the yolk, in which case they reach the cranial part of the embryos not immediately, but after a certain interval, and also in lower concentration.

The results of these experiments can be regarded as evidence that antibodies exert an effect on morphogenetic processes by blocking the synthesis of the corresponding antigens. This suggests that the tissue antigens play a definite role in organ differentiation. It is difficult at present to say whether these antigens influence the course of organogenesis, by bringing about the "morphogenetic immunity response" [3], or whether, as definite chemical substances (in this case crystallins), they act on biochemical processes associated with the regulation of morphogenesis [5].

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