

During investigation of the antigenic differentiation of the lens in the course of the individual development of vertebrates it has been found that besides organ-specific antigens other types of antigens are present in the lens. In particular, stage-specific and species-specific antigens have been found in the lens of duck embryos [5-7], and species-specific in the lens of fishes and hens [1, 9, 13]. The lens of mice also contains several antigens not characteristic of this organ alone. These antigens have been called common organ antigens or interorgan antigens [2], by analogy with the interorgan antigens described for chick embryos [12].

This paper describes an immunological investigation of the interorgan antigens of the lens in mice.

#### EXPERIMENTAL METHOD

Serum was taken from 5 rabbits immunized with extract from the lens of noninbred mice and from 2 rabbits immunized with the blood serum of mice. Immunization was carried out by the scheme described earlier, using Freund's adjuvant [4]. The titer of antisera was not less than 1:1000.

The antisera thus obtained were used to investigate for the presence of interorgan antigens of the lens in the tissues of adult mice, mouse embryos of 9-15 days of development, and also adult rats and hens. Tissue extracts were prepared in Tris buffer (pH 8.4, 0.1 M) with tissue and buffer in the ratio of 1:10.

The methods used for testing were Ouchterlony's precipitation reaction in agar and immunoelectrophoresis. To prepare the 1.5% agar gel, Difco agar and Tris buffer (pH 8.4, 0.1 M) were used [13]. The electrode compartment of the apparatus were filled with the same buffer.

#### EXPERIMENTAL RESULTS

The sera obtained by immunization of the rabbits with lens antigens from mice reacted not only with extracts of the lenses. In some cases a positive reaction was also observed with extracts from other organs and tissues. Three antisera (Nos. 2, 15, and 55) reacted with extracts both from the lenses of the mice and from other organs. The results of the reactions of these sera in immunoelectrophoresis are given in the table, which shows that the anti-lens serum formed from 1 to 3 precipitation bands with the tissue extract, but not one of them reacted with the blood serum of the mice. Meanwhile the rabbit sera (Nos. 3 and 4) obtained against the blood serum of the mice did not react with extracts from the mouse lenses. Hence, as a result of crossed reactions, no antigens identical with the antigens of the blood serum could be found in the lens.

During the study of the formation of the lens antigens in embryogenesis, and especially during analysis of the distribution of antigens in the tissues of the embryo in connection with morphogenetic transformations, it is very important to differentiate between the specific antigens of the lens and antigens of the other type. An investigation was made of extracts prepared from the heads (without the anlagen of the eyes), the trunks, and the rumps of mouse embryos of the 9th-15th days of development, by means of Ouchterlony's reaction. The anti-lens serum No. 55 used in these reactions formed two precipitation bands in the agar with all the extracts. Crossed reactions showed that these antigens were identical with antigens revealed by this same serum in extracts from the lung and liver of adult mice. These results show that interorgan antigens are present in the tissues in the earliest stages of development of embryos.

It is important to know which of the antigens of the lens are interorgan antigens, and identical with the antigens found in other tissues. For this purpose Osseman's reactions were carried out [11]. In immunoelectrophoresis an extract from the lenses of adult mice and anti-lens sera (Nos. 14 and 55) were compared with extracts from

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Number of Antigens Detected by Anti-lens Serum in Extracts from Organs of Adult Mice

Organs and tissues of a mouse from which extract was obtained	Anti-lens serum		
	№ 55	№ 2	№ 15
Brain	1	3	1
Heart	2	3	2
Muscles	—	—	1
Lung	3	2	2
Kidney	1	1	2
Liver	2	3	3
Spleen	2	2	—
Testis	3	2	2
Blood serum	—	—	—

the lung and liver of adult mice and also with an extract from 10-day embryos. One such reaction is illustrated in Fig. 1. As a result of these experiments two interorgan antigens of the lens were identified. In immunoelectrophoresis one of these antigens appeared in the zone of mobility of the  $\beta$ -crystallins but rather closer to the anode than the organ-specific antigens of this fraction. The other antigen occupied an intermediate position between the  $\alpha$ - and  $\beta$ -crystallins.

Antigens identical with the interorgan antigens of the lens were observed not only in the organs of the mice. Antiserum No. 7, obtained by immunization of a rabbit with extracts from the lenses of mice, contained antibodies mainly against one of the interorgan antigens of the lens tissue. It may be seen from Fig. 2 that this same antigen was also found in the organs of the rat—in the lung and spleen. The results of experiments carried out with this serum showed that the analogous antigen was found in the liver and lens of the rat but was absent from the hen's lens. Similar results were obtained in reactions with serum No. 55, but in extracts from the organs of the mice it revealed

up to three interorgan antigens (Fig. 3). However, only one of these antigens could be found in the lens and the other organs of the rats. This antigen was not present in the hen's lens, as in the preceding case also.

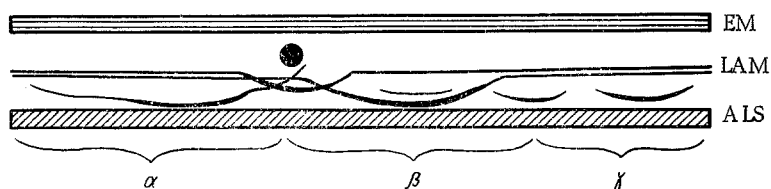


Fig. 1. Identification of interorgan antigens of the mouse lens in immunoelectrophoresis (Ossermann's reaction). EM—extracts from 10-day mouse embryo; LAM—extract from lenses of adult mice; ALS—anti-lens serum No. 14;  $\alpha$ ,  $\beta$ ,  $\gamma$ —zones of agar occupied by corresponding fractions of crystallins.

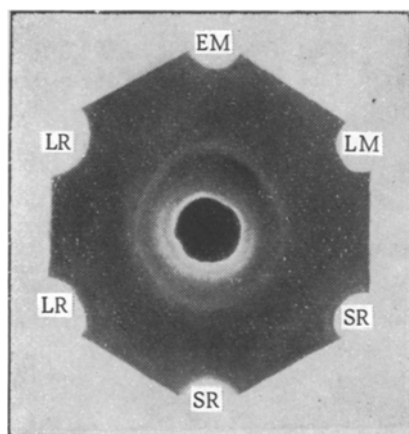


Fig. 2. Reaction of anti-lens serum No. 7 with extracts from tissues of mice and rats. EM—extracts from 10 day mouse embryos; LM—extract from lungs of adult mice; SR—extract from spleens of adult rats; LR—extract from lungs of adult rats.

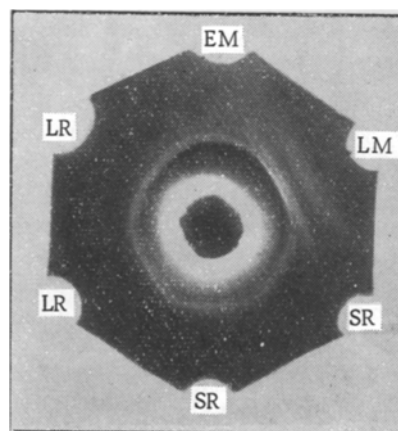


Fig. 3. Reaction of anti-lens serum No. 55 with extracts of tissues of mice and rats.

These results show that the lens of noninbred mice contains, besides organ-specific antigens, antigens similar to those of other organs and tissues. These results, like those of earlier investigations [5, 8-10, 12, 13], demonstrate the common antigenic properties of the lens and the other tissues of the body. On the other hand, they show that, besides species-specific, organ-specific, and stage-specific antigens [3, 5], there is a large group of interorgan antigens, the presence of which must be taken into account during the study of the antigenic structure of developing tissues and organ.

#### LITERATURE CITED

1. V. S. Apekin, *Byull. éksp. Biol.*, No. 1, 77 (1964).
2. V. M. Barabanov, In the book: *Proceedings of a Conference of Junior Scientists of the Institute of Experimental Biology* [in Russian], Moscow (1965), p. 8.
3. E. O. Vyazov, *The Immunology of Embryogenesis* [in Russian], Moscow (1962).
4. M. G. Zaks and M. M. Sokolova, *Doklady Akad. Nauk SSSR*, 139, No. 6, 149 (1961).
5. B. V. Konyukhov, *A Morpho-immunological Study of Organs of Animals in Embryogenesis*. Author's abstract of candidate dissertation. Moscow (1966).
6. B. V. Konyukhov, *Byull. éksp. Biol.*, 31, No. 4, 66 (1956).
7. B. V. Konyukhov, *Byull. éksp. Biol.*, 44, No. 12, 96 (1957).
8. R. A. Flickinger, E. Levi, and A. E. Smith, *Physiol. Zool.*, Vol. 28 (1955), p. 79.
9. W. Krayenhoff-Sloot, *Exp. Eye. Res.*, Vol. 2 (1963), p. 307.
10. D. J. McCallin and J. Langman, *J. Embryol. exp. Morph.*, Vol. 12 (1964), p. 77.
11. E. F. Ossermann, *J. Immunol.*, Vol. 84 (1960), p. 93.
12. A. M. Schechtman, *Proc. Soc. exp. Biol. (New York)*, Vol. 68 (1948), p. 263.
13. J. Swaan, *Immunochemical Analyses of the Eye Lens during Development*. Amsterdam (1963).