EXPERIMENTAL BIOLOGY

STUDY OF ANTIGENIC PROPERTIES OF TISSUES AND ORGANS DURING ONTOGENESIS

PART II. ORGAN SPECIFICITY OF THE CRYSTALLINE LENS

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It has been shown by a number of authors [6,7,8,9,10,12, and others] that the crystalline lens of adult animals possesses well-marked organ specificity; this is evidenced in the similarity of the antigenic properties of the proteins of the crystalline lenses of different vertebrate species [10]. The majority of workers ascribe this similarity to the circumstance that the lens of all animals fulfills one function only, viz. that of transmitting light.

Some papers have recently been published on the antigenic properties of the lens during ontogenesis [3,4,5]. Thus, Burke, Sullivan, Petersen, and Weed [3] found that the specific antigens of chick lens cannot be detected by the reaction of complement fixation earlier than the 160th hour of incubation, by which time the lenses of the embryos do not differ in their basic morphological features from those of adult birds. Similar findings were obtained for the crystalline lens of frogs. From their experimental results the authors drew the conclusion that the antigens present in the organs of adult animals make their appearance at the stage of development at which their morphology first resembles that of the adult animal. The work of Ten Cate and Van Doorenmaalen [4] does not, however, support this conclusion. These authors found, using a microprecipitation method, that the specific antigens of hen or frog crystalline lenses make their appearance long before the lenses of the embryos assume the morphological structure characteristic of the adult animals. They were able to find these antigens at the stage of transformation of the lens rudiment into the lens vesicle.

Flickinger, Levi, and Smith [5] have recently announced similar findings.

There is still some uncertainty as to when the organospecific antigens of the crystalline lens make their appearance, and this is an obstacle to the understanding of ontogenetic development of antigenic organ specificity in general.

The researches described in this paper were undertaken with the object of elucidating this problem, using the methods of anaphylaxis and desensitization of guinea pigs [1 and others]. The techniques used for study of anaphylactic reactions were described in our previous communication [2].

The experiment for which the results are presented in Table 1 was performed as follows. The guinea pigs were sensitized by subcutaneous injection of lens tissue taken from embryos at various stages of incubation (90, 130, 192, 264, and 360 hours), and also from adult ducks. On the 21st day after the injection all the animals were desensitized to the species-specific antigens and, after checking that desensitization was complete, all guinea pigs were given intravenous injections of a saline extract of adult duck crystalline lens tissue.

As appears from Table 1, all the animals gave a positive anaphylactic reaction. Since they had all been desensitized to species-specific antigens, this reaction can only have been due to persisting sensitization to organo-specific antigens.

TABLE 1

Anaphylactic Reaction of Guinea Pigs Sensitized with Duck Embryo Lens Tissue, in Response to an Injection of Adult Duck Lens Extract

Guinea pig No.	Sensitized (subcutaneous)		Desensitized (intravenous)			Check of completeness of desensitization (intravenous)			Challenging injection (intravenous)		
	antigen	dose	antigen	dose (mg)	reaction	antigen	dose (mg)	reaction	antigen	dose (mg)	reaction
102	Suspension of 90 hour duck embryo lens tissue	80 lenses	Duck serum	200	++	Duck serum	300	—	Adult lens tissue extract	1000	++++
1241	Ditto	80 "	Ditto	200	++	Ditto	300	—	Ditto	1000	++
1211	"	80 "	"	200	++	"	300	—	"	1000	++
273	Suspension of 130 hour duck embryo lens tissue	1.6 mg	"	200	+	"	300	—	"	300	++
299	Ditto	1.6 "	"	200	++	"	300	—	"	300	++
938	"	1.6 "	"	200	++	"	300	—	"	300	++
865	Suspension of 192 hour duck embryo lens tissue	1.6 "	"	200	+	"	300	—	"	300	++
400	Ditto	1.6 "	"	200	++	"	300	—	"	300	++
968	"	1.6 "	"	200	++	"	300	—	"	300	+++
38	Suspension of 264 hour duck embryo lens tissue	1.6 "	"	200	++	"	300	—	"	300	++
690	Ditto	1.6 "	"	200	+	"	300	—	"	300	+++
238	"	1.6 "	"	200	+	"	300	—	"	300	+++
219	Suspension of 360 hour duck embryo lens tissue	1.6 "	"	200	++	"	300	—	"	300	+++
825	Ditto	1.6 "	"	200	+	"	300	—	"	300	+++
258	"	1.6 "	"	200	+	"	300	—	"	300	+++
94	Suspension of adult duck lens tissue	1.6 "	"	200	+	"	300	—	"	300	+++
529	Ditto	1.6 "	"	200	+	"	300	—	"	300	+++
229	"	1.6 "	"	200	+	"	300	—	"	300	+++
641	.	.	.	.	.	.	.	.	"	1000	—
942	.	.	.	.	.	.	.	.	"	1000	—
233	.	.	.	.	.	.	.	.	"	1000	—

TABLE 2

Anaphylactic Reaction of Guinea Pigs Sensitized with Duck Embryo Tissues, in Response to Injections of Adult Duck Lens Extract

No. of guinea pig	Sensitized (subcutaneous)			Desensitized (intraperitoneal)			Check of completeness of desensitization (intravenous)			Challenging injection (intravenous)		
	antigen		dose	antigen	dose (mg)	reaction	antigen	dose (mg)	reaction	antigen	dose (mg)	reaction
1451	Suspension of 72 hour embryos (23-25 somites)	Heads	Extract of tissues of 160 embryos	Duck serum	500	+++	Duck serum	400	—	Adult duck lens extract	1000	++
448			Ditto	Ditto	500	+++	Ditto	400	—	Ditto	1000	+++
485			"	"	500	+++	"	400	—	"	1000	++
1201		tail parts	"	"	500	+++	"	400	—	"	1000	—
1142			"	"	500	++	"	400	—	"	1000	—
1105			"	"	500	++	"	400	—	"	1000	—

It follows from the results of this series of experiments that the crystalline lens exhibits well-marked organ specificity at all the stages of development studied. It also appears that the intensity of the reaction is somewhat greater when sensitization is achieved with lens tissue from late than from early stages of embryonic development. It should also be noted that well-marked shock in guinea pigs sensitized with lens vesicles from 90 hour embryos only appeared after introduction of much larger doses of antigen than for animals sensitized with material from later stages of development. These findings suggest that organ specificity of the crystalline lens increases during embryonic development.

Morphological studies carried out by us showed that it is at this stage of embryonic growth (90-120 hours of incubation) that formation of primary lens fibers begins, with corresponding changes in the affinity of lens tissue to dyes. Whereas up to 96 hours of incubation the lens stains pink with Mallory's stain, similarly to the cells of the ectodermal epithelium, after this time it stains blue, similarly to the lens fibers of later stages of development. It is conceivable that formation of the primordial lens fibers, change in staining reactions, and enhancement of antigenic organ specificity are all interconnected processes.

Thus the data presented in Table 1 show that the lens vesicle already contains organ-specific antigens, characteristic of the adult duck, after 90 hours of incubation. These results are in agreement with those of Ten Cate and Van Doorenmaalen [4], and of Flickinger, Levi, and Smith [5]; these authors were unable to find organ-specific antigens at earlier stages of development of the lens.

The following experiment, the results of which are presented in Table 2, was undertaken with the object of finding when organ-specific antigens first appear in the lens.

One series of guinea pigs was sensitized with a suspension of tissues from the heads of embryos at the 23-25 somite stage (the embryos were decapitated immediately below the level of the brain), and a second series was sensitized with a suspension of tissues caudal to this section.

About the same amount of lens tissue was taken for sensitization of the animals of the first series as in experiments on 90-hour embryos. For this, we measured the volume of the lens rudiment at the 23-25 somite stage (72 hours of incubation), and of the lens vesicle at the 33-34 somite stage (90 hours of incubation). From these values we calculated the number of embryos which would be needed in order to give a sensitizing injection to one guinea pig. On the 21st day all the animals were desensitized to species-specific antigens, and after testing for completeness of desensitization, all animals were given a challenging injection of adult duck lens extract. Guinea pigs sensitized with duck embryo head suspensions gave a positive anaphylactic reaction, whereas those sensitized with the remaining tissues did not develop anaphylactic shock.

The data of Table 2 thus show that lens antigens are present in the heads of 23-25 somite embryos (72 hours of incubation). They are evidently located in the lens rudiment, which at this stage shows the first signs of invagination. This supposition is supported by the data of Woerdeman [11] for amphibiae; he found that lens antigens originate in the cephalic ectoderm in vitro only after the action on it of substances from the optic vesicles, and also noted that lens antigens were absent from the optic vesicle.

The results of our experiments, together with Woerdeman's data, permit of the conclusion that the organ-specific antigens of the lens originate at the stage of formation of the lens rudiment of embryos. In order finally to confirm this view, it would be necessary to apply special methods of study, which would allow of the investigation of the antigenic properties of tissues at still earlier stages of organogenesis.

It may be concluded from our experimental results that organ-specific antigens, characteristic of the crystalline lens of the adult duck, are to be found in the cephalic tissues of embryos at the 23-25 somite stage (72 hours of incubation). The antigenic organ-specificity of the lens increases as embryonic development proceeds.

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