nuclei or their cortical connections. The observations seem to be explained best by the assumption that multiple circuits participate in the mechanism of temporal orientation, so that a lesion of a single thalamic nucleus produces only transitory disturbances.

Following lesions of other subcortical regions (midbrain in the area of the spinothalamic system; pallidum) chronotaraxis was not observed.

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Lack of Lens Induction by Eye Cup

Marko Zalokar

National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Maryland*

In amphibian embryos, trunk ectoderm can be induced to form a lens at the neurula stage; in later stages, depending on the species involved, lens potency is restricted to the ectoderm surrounding the eye and, then, is lost altogether (*1*). However, the eye cup still retains its lens-inducing ability for some time, and in *Triturus,* even in adults, the eye cup can induce a neoformation of a lens from the upper rim of the iris. In a series of publications, several Soviet authors (*2–4*) reported that the trunk ectoderm of early and late embryonic stages, and even tadpole ectoderm, could be transformed into lens tissue when transplanted into the eye cavity of the tadpole. According to later reports, the chordomesoderm and the tail skin of the metamorphosing tadpole reacted in the same manner (*5*).

Somewhat different were the experiments of Törö (*6*). He cultured embryonic chick and rat iris epithelia *in vitro*; after some 10 explant generations he implanted them back into the eyes of the young animals, after the lens had been removed. Well-formed lenses could be observed in these eyes a few days after operation. Törö supposed that the iris epithelium dedifferentiated in tissue culture and acquired the ability to be converted into a lens when exposed to the induction of the eye. The reported experiments demonstrated both lens potency in tissues that were believed unable to form a lens and strong lens-inducing properties in fully developed eyes. These interesting observations deserved independent confirmation, and we tried to repeat the experiments, using locally available amphibians and chicks (*7*).

The types of experiments performed are summarized in Table 1. In *Rana pipiens,* tadpoles with posterior leg buds, or slightly younger, served as hosts. The lens of one eye was removed through an opening in the cornea. Pieces of trunk ectoderm from the neurula or early or late tail-bud stage, were then introduced with a micropipette into the lenseless eye. In the majority of experiments, the implant could not be found in fixed preparations of tadpoles killed 1 wk or later after operation. When the implant was not expelled or resorbed, it showed an epithelial vesicle with no indication of lens formation. The cells partially lost their pigment, and the center of the vesicle contained expelled yolk and pigment granules.

Removing the lens in *Ambystoma gracile* and *A. maculatum* larvae was easy, and implanting other tissues was successful in most cases. The operative technique was similar to that previously described for *Triturus* (*8*). The hosts were larvae with three- or four-toed hind legs. Trunk ectoderm from the neurula or early or late tail-bud stage, was implanted into the eye. The implanted piece rounded into a vesicle that usually settled near the pupil. Four days after the operation, a general depigmentation of the vesicle was observed. Some cells lining the center of the vesicle still contained pigment granules, but the cells facing the eye cavity were free of pigment. The yolk granules and most of the pigment were expelled, either into the central cavity of the vesicle, or into several smaller cavities inside the vesicle wall (Fig.

Table 1. Implantation of embryonic ectoderm and iris epithelium into lensless eyes. Harrison stages for *Ambystoma,* Shumway stages for *Rana pipiens.* Iris tissue culture from 12-day-old embryos was implanted in *Gallus gallus.*

Host	Stage of donor	Duration of experiment (days)	Eyes operated	Successful implantations
Rana pipiens	20	5	6	5
		7	8	3
		14	13	2
		21	12	7
Ambystoma gracile	22	4	7	4
		7	10	4
		10	8	7
		14	12	12
A. maculatum	20	7	11	8
		14	10	8
		21	10	9
	30	7	14	14
		14	12	12
		28	20	18
	38	14	14	12
		28	14	13
Gallus gallus		9	1	1
		14	6	3
		28	4	2

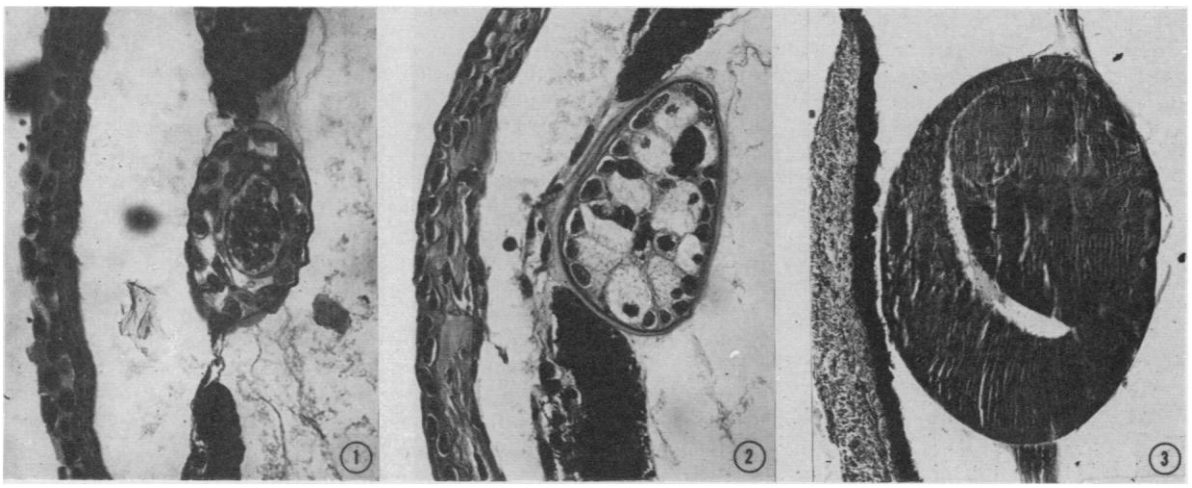

Fig. 1. Ventral ectoderm of *A. gracile* (embryo stage 22) implanted into larval eye, 14 days after operation. Fig. 2. Ventral ectoderm of *A. maculatum* (embryo stage 30) implanted into larval eye, 28 days after operation. Fig. 3. Lenslike formation observed in chick eye 28 days after incomplete lensectomy.

1). A conspicuous basal membrane surrounding the vesicle appeared in 14-day-old implants. The epithelium resembled corneal tissue more than it did a regenerating lens vesicle. After 21 days the implants remained unchanged, except that, in some cases, they contained Leydig's skin gland cells, such as are found in the ventral skin of an embryo of an age equivalent to the age of the explanted ectoderm (Fig. 2).

To repeat Törö's experiments, chick iris from 12-day-old embryos was explanted in hanging drop cultures and neutral red was added (*6*). After 8 to 10 transplantations, pure cultures of iris epithelium were secured. These iris cultures were implanted into the eyes of newly hatched chicks, after the lens had been removed. The chicks were killed and the operated eyes were fixed after indicated periods (Table 1). The implanted epithelium could be recognized easily in several preparations, adjacent to the remains of the capsula lentis, where it grew in a discoid formation several layers thick. It was heavily pigmented and showed no transformation into lens tissue. In the operated eyes, lenslike formations remarkably similar to the ones illustrated by Törö were found (Fig. 3); these could be formed only from the incompletely removed original lens. Similar lentoids were observed in control experiments when the lens was removed but no implant introduced. Eyes fixed immediately after operation contained the remains of the original lens, and these remains could be observed in operated eyes *in vivo*.

The attempt to repeat the experiments of the Soviet authors (*2–4*) and of Törö (6) gave only negative results. The trunk epithelium, implanted into larval eyes, did not form lens tissue, but it showed, temporarily, a similarity to corneal tissue. The expulsion of the yolk granules and the pigment could have been an effect of specific forces in the surrounding eye or, perhaps, an effect of a liquid medium as is observed (*9*) in the cultures of iris epithelia. The ultimate formation of Leydig's gland cells showed that no real change in the prospective potency of the implanted epithelium occurred. It is interesting to note that the polarity of the implanted epithelium was reversed: the embryonic ectoderm, when prepared for implantation, curled in small balls, leaving the original skin surface exposed; inside the eye, this same surface was covered with the basal membrane.

Our results are not in accord with those of the Soviet workers, but the possibility remains that variation of experimental material might account for the difference. Verejskaja (*10*) attempted analogous experiments with rats by implanting embryonic ectoderm into the eye cavities of young animals. All implants developed typical skin formations, but, in 98 percent of the experiments, the remains of incompletely removed lenses were observed.

Okada (*11*) and Reyer (*12*) presented an extensive criticism of the work of the Soviet authors that is supported by our negative results. It is very difficult to remove complete lenses from anurans, chicks, or rats. The remaining lens epithelium can produce lens formations (*13*) that can be easily misinterpreted as formations from the implants. As Okada pointed out, the lenses were observed 1 to 3 days after implantation by the Soviet authors, in a period of time in which no implanted tissue could be expected to give such large or complete lenses. It takes 4 days to produce a lens with fibrils from the lens placode in the frog embryo, and 7 days to regenerate the lens with fibrils in *Triturus* larvae. Any debris of leftover lenses could, on the other hand, originate these formations. There is also the possibility that the depigmented ectodermal vesicles were mistaken for young lens vesicles. Only the presence of lens fibrils would confirm the real lens nature of the tissue.

In chicks, cultured iris epithelium did not develop into a lens when it was implanted into lensectomized eyes, but lentoids were formed from incompletely re-

moved lenses. Such partial reconstitution of lenses, which is not a true regeneration, has often been reported (*13*). Törö's contrary results should be evaluated in the light of this knowledge.

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* Present address: Biology Department, Wesleyan University, Middletown, Conn.

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Ultraviolet Polymerization of Monomeric Methacrylates for Electron Microscopy

Stanley Weinreb

Department of Pathology, University of Wisconsin, Madison

Specimens to be sectioned for electron microscopic techniques are usually imbedded in monomeric methacrylate mixtures that are subsequently polymerized by the addition of a suitable chemical catalyst and the application of heat (*1*). However, because of the small quantities of monomer used, it is often inconvenient and/or difficult to dissolve the catalyst. In addition, chemically catalyzed samples have a tendency to develop undesirable bubbles during polymerization.

Massey (*2*) reported that tissue imbedded in chemically catalyzed monomeric methacrylates often suffers from a "popcorn reaction." To minimize the disadvantages of chemical polymerization, Massey suggested the use of ultraviolet polymerization of uncatalyzed monomers from which the inhibitor had been removed. Her ultraviolet source appears to have been a type RS-40 sun lamp designed for home use. However, this sun lamp also emits large amounts of infrared radiation, and its operating temperature is so high that it will char paper on contact. This necessitates special precautions against fire hazard during the long periods of continuous operation needed for photopolymerization. In addition, forced draft ventilation is usually necessary to avoid overheating of the specimen, and the effective irradiated area at the recommended distance is small and not uniform.

Because of the advantages of using photopolymerization, it was decided to develop a modified method. There are many commercially available radiation sources that can supply the near ultraviolet needed for the photopolymerization of monomeric methacrylates. Most of these, however, require special electric equipment or have such high emission values that the small samples used may boil. It was, therefore, decided to employ a lamp of relatively low emission value that would still result in photopolymerization within a convenient period of time.

The lamp chosen was a Westinghouse FS20T12 fluorescent sun lamp (*3*). This lamp is physically and electrically similar to a standard 20-w fluorescent lamp and operates in standard 20-w fluorescent-lamp fixtures. It has approximately the same emission range as the RS-40 sun lamp, an effective life of about 4000 hr, a normal operating temperature of about 100°F, and very little infrared radiation. No forced draft ventilation is necessary.

The sun lamp was utilized in conjunction with a standard 20-w fluorescent channel-strip fixture containing lamp holders, starter, and ballast (Fig. 1). A polished aluminum reflector, *R*, with a 1⅛-in. radius of curvature was attached to the unit. The reflector was designed so that it also served as a mount for the specimen holders, *H*, and backing reflectors, *BR*.

Specimens were imbedded in No. 4 gelatin capsules using a mixture of 1 part ethyl methacrylate and 3 parts *N*-butyl methacrylate (*4*) from which the inhibitor had been removed. The filled capsules, *S*, were then attached in a vertical position to a strip of cellulose tape, *T*, that had been fastened to a specimen holder, *H*. The specimen holder was then clipped

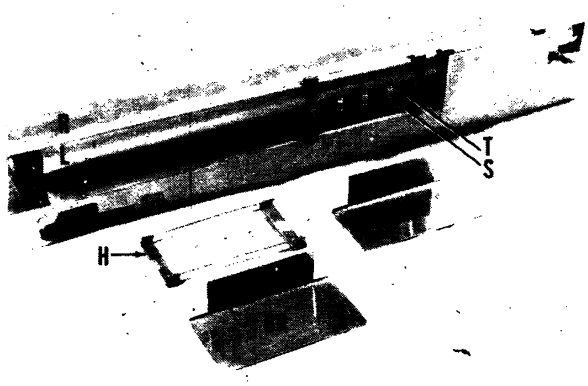

Fig. 1. Ultraviolet sun lamp assembly for the photopolymerization of monomeric methacrylates. *BR*, backing reflector; *H*, specimen holder; *L*, fluorescent sun lamp; *R*, reflector; *S*, specimen capsule; *T*, cellophane tape.