Let us note that the synthesis of DNA of plantlets incubated in the presence of tritiated thymidine or hydrolized *E. coli* DNA^{-3} did not seem affected in any way by the change of temperature between development and incubation. Moreover, plants incubated in a solution of ^{3}H-thymidine synthesized during the same time about 10 times less radioactive DNA than the amount found after absorption of *E. coli* DNA^{-3}H.

The fact that under our experimental conditions in certain circumstances temperature affected the depolymerization of the foreign DNA translocated without influencing, in the least, the synthesis of endogenous DNA proves that the 2 phenomena are distinct[8].

Résumé. Nous avons étudié l'influence de différentes températures sur la translation de l'ADN d'origine bactérienne chez les plantules de tomate. La température n'a aucune influence en elle-même. Néanmoins des variations de température diminuent la dépolymérisation de l'ADN étranger migré dans la plante.

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26 January 1968.

[8] This work was done under a Euratom/C.E.N. contract, and was supported by grants from the Belgian 'Fonds de la Recherche Scientifique Fondamentale Collective'.
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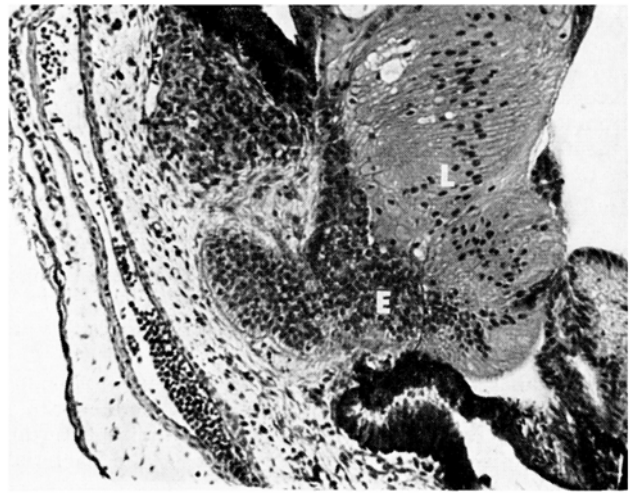
[10] Boursier au 'Fonds national suisse de la recherche scientifique'.

The Question of Lens Regeneration from Parts of the Optic Vesicle in the Chick Embryo[1]

Numerous experiments have demonstrated that, in certain Urodeles, a new lens forms from iris tissue after removal of the original lens from the eye (STONE[2]). Limited studies suggest that lens regeneration from iris tissue does not occur in adult reptiles, birds or mammals (STONE[3]). New lenses are occasionally formed in the optic vesicle of the chick embryo after removal of the original lens but their origin is not clear. Although DETH[4] and REINBOLD[5] have described regeneration of lenses from the optic vesicle in tissue culture experiments the evidence is inadequate. MCKEEHAN[6] found no evidence of lens regeneration following removal of the lens in ovo. The present work was undertaken to further explore the origin of new lenses in the eye of the chick embryo.

The embryos used in these experiments were obtained from White Leghorn eggs supplied by a commercial hatchery. With the aid of fine tungsten needles the lens was excised from the optic cup of embryos of 48–72 h of incubation at 38°C (stages 17–20, HAMBURGER and HAMILTON[7]) following 2 procedures. In some cases the ectoderm was removed from the side of the head, the lens excised and the optic cup cut out. In other cases the lens was removed through a slit in the ectoderm and the optic cup together with ectoderm was cut out. In all cases the optic cup was as free as possible of mesenchyme. The optic cups were cultured either on the chorio-allantois of host embryos or on a modified agar medium (WOLFF and HAFFEN[8]). The explanted optic cups were subsequently recovered, fixed, sectioned and stained for microscopic examination. Of 112 optic vesicles alone cultured in this way none showed any evidence of new lens formation. They did, however, show some degree of metaplasia similar to that described by MCKEEHAN[6]. Of 159 explants of optic vesicles and ectoderm a large number contained lenses or lentoid structures (Figure) frequently clearly associated with the ectoderm. The frequency of lens formation is shown in the Table.

The results of our experiments show clearly that a new lens appears in the explanted optic vesicle after excision of the original lens only if ectoderm is included with the explant and then only if the explant is taken from an embryo of less than 72 h of incubation (Table). The possible origins of new lenses in the optic vesicle are limited to: regeneration from lens fragments left behind after the operation; regeneration from part of the retina; or by induction in remaining competent head ectoderm. In the present experiments care was taken to remove the lens intact and to prevent fragmentation of the lens. It is un-



Optic vesicle and ectoderm of a 60 h embryo, after removal of the original lens, cultured for 5 days on the chorioallantois. A large lens (L) has formed adjacent to ectoderm (E).

[1] This work was supported by a grant from the National Research Council of Canada to D. J. McCallion.
[2] L. S. STONE, J. exp. Zool. *164*, 87 (1966).
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[4] J. H. M. G. VAN DETH, Acta neerl. Morph. *3*, 151 (1940).
[5] R. REINBOLD, Archs Anat. microsc. Morph. exp. *47*, 341 (1958).
[6] M. S. MCKEEHAN, Anat. Rec. *139*, 227 (1961).
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[8] ET. WOLFF and K. HAFFEN, Tex. Rep. Biol. Med. *10*, 463 (1952).

likely that the lenses subsequently observed developed from lens fragments. Since no lenses were observed to develop in any of 112 optic vesicles explanted without ectoderm it may be concluded that regeneration from the optic cup does not occur. The remaining possibility is that new lenses are induced in the head ectoderm by the optic cup. The ectoderm retains its lens competence for up to 5 days of incubation (DETH[4]) and the optic cup retains its inductive capacity for at least some hours after the original lens induction.

Since MCKEEHAN[6] has already written a critical review of the literature it is only necessary to comment on 2 pertinent reports. DETH[4] removed the lens from the eye of chicks of 2–5 days of incubation and cultured the optic cup in vitro. Although he considered the head ectoderm to be removed from the region of the eye he states that ectoderm was explanted with the optic cup and ectoderm is shown in his Figures. He found only 11 lenses or lentoid bodies in 114 explants 7 of which had no connection with the iris. Furthermore the time interval for the appearance of these lenses (48 h or less) is much shorter than the time required for lens regeneration. REINBOLD[5] removed the lens from the eye of chicks of 48–50 h of incubation and cultured the blastoderm in vitro for 1–3 days. His cultures also included head ectoderm. In 27 cultures 15 formed no lenses. Of the others only 10 were examined histologically.

Frequency of lens formation in cultures of optic vesicles and ectoderm

Age of chick in h	No. of cultures	No. of explants with lenses	Frequency of lens formation
48	30	21	70%
60	45	15	33%
68	33	6	20%
72	49	0	0

He found 9 lenses or lentoid bodies of which at least 4 were continuous with the head ectoderm. In this study, also, the time interval for the appearance of lenses is very short. It is probable that the lenses described by these authors arose either by reconstitution of lens fragments or by induction in competent head ectoderm. MCKEEHAN[6] removed both the lens and the presumptive cornea from the eye of 42 embryos of 3–5 days of incubation and obtained no lenses whatever. The age of his embryos together with the absence of head ectoderm could explain his results.

The question is whether a removed lens is replaced in an orderly predictable manner from another tissue of the eye by a process of regeneration. The evidence presented by previous studies does not demonstrate that such regeneration occurs, especially in view of MCKEEHAN's negative results. We believe that our results strongly suggest that new lenses form as a result of induction in still competent ectoderm and that this explanation could account for some or all of the lenses described by DETH and REINBOLD.

Résumé. Après excision du cristallin des yeux d'embryons de poulet de 48–72 h d'incubation les vésicules optiques étaient cultivées in vitro ou sur le chorio-allantoïde d'autres embryons. Elles étaient cultivées en quelques cas seules et en autres cas avec l'ectoderme céphalique. Nouveaux cristallins ne se formaient que si l'ectoderme céphalique était inclus avec la vésicule optique et si les vésicules étaient prélevées des jeunes embryons. On peut conclure que les nouveaux cristallins se forment dans l'ectoderme céphalique par induction. Nous n'avons trouvé aucune évidence de régénération du cristallin des tissus de l'oeil.

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Effects of Paradimethylaminoazobenzene and the Antioxidant N,N'-Diphenyl-*p*-phenylene Diamine in Developing Chicks[1]

EMANUEL and LIPCHINA have suggested that the carcinogen Paradimethylaminoazobenzene (PDAB) exerts its effect by a free-radical mechanism[2]. Since many carcinogens are mutagens and embryo deforming, and since we are interested in embryo deforming effects of free radicals[3], we decided to see (1) whether PDAB deforms developing chicks and (2) if so, whether the antioxidant N,N'-diphenyl-*p*-phenylene diamine (DPPD) could modify its effect.

Methods. Fertilized White Leghorn eggs obtained from Truslow Farms, Chestertown, Maryland were incubated at 38 °C, between 62 and 67% humidity, and rotated 3 times daily.

PDAB was dissolved, 6 mg in either 0.1 ml polyethylene glycol (PEG) or in 0.1 ml of a mixture of 9 parts PEG and 1 part absolute ethanol. The latter was tried because PDAB is more soluble in it. While this is a large dose our preliminary orientation experiments showed it was the smallest dose that produced detectable deformities, probably because so little is removed from yolk during embryonic life. DPPD[4] was dissolved, 0.1 mg in 0.05 ml PEG or PEG and ethanol in the mixture described above.

Before treatment of any kind, shells were sterilized for 30 min in formalin vapors generated by a mixture of 2 g $KMnO_4$ in 50 ml 37% formaldehyde. PDAB and DPPD were administered by making a small hole in the shell over the air sac and injecting through it into the yolk. PDAB was given after 48 h of incubation and DPPD

[1] Supported in part by a grant from the Forsyth Cancer Service.

[2] N. M. EMANUEL and L. P. LIPCHINA, Acta Un. Int. Cancr. *20*, 103 (1964).

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[4] Obtained through the courtesy of the U.S. Rubber Company.