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).

[3] D. J. PIZZARELLO and J. G. KLOSS, Experientia *23*, 589 (1967).

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

Experiment No. Treatment	1	2	3	1	2	3	1	2	3	1	2	3
	No. of eggs			Died during incubation			Deformities at hatching Legs			Feathers		
None	9	16	12	1	1	1	0	0	0	0	0	0
Puncture at 48h only	10	0	0	1			0			0		
at 48 h and 6 days	0	16	11		4	4		0	0		0	0
PEG; 0.1 ml at 48 h and 0.05 ml at 6 days	0	0	11			4			3 0			0
PEG and ethanol; 0.1 ml at 48 h and 0.05 ml at 6 days	10	0	0	1			0			0		
	0	16	0		3			0			0	
DPPD in 0.05 ml PEG at 6 days	0	0	12			6			0			0
DPPD in 0.05 ml of PEG and ethanol at 6 days	0	16	0		8			0			0	
PDAB in 0.1 ml PEG at 48 h	0	0	12			6			6			3
PDAB in 0.1 ml PEG and ethanol at 48 h	9	15	0	2	5		6	8		4	7	
PDAB at 48 h and DPPD at 6 days	0	20	35		11	18		7	15		5	11

after 6 days. We did it this way because our orientation studies showed PDAB is absorbed slowly from yolk, taking several days to get into the chicks. DPPD is quickly absorbed, and we gave it when we thought PDAB was exerting its major effects. Before and after injections, shells were wiped with 70% ethanol and injection sites were sealed with clear fingernail polish. Controls consisted of untreated eggs, eggs punctured through shell and yolk but not injected, and injections of solvents for PDAB and DPPD. All eggs were candled daily and the dead recorded. Deformities were scored among those that survived to hatching.

Results. Three experiments were done. The groupings used and results obtained are in the Table. PDAB caused shortened bones in the legs, both thighs and drumsticks were affected. All were obvious but measurements of both treated and control birds showed that the shortening was, on the average, about one third. It deformed feathers by giving them a curled, stubby appearance over all the body except the spine and crown of the head.

Simply puncturing the eggs more than once killed between 25 and 37%, nearly all after the second injection. None, however, were deformed. PEG alone in either 1 or 2 injections killed no more than the corresponding number of punctures and deformed none. Substituting ethanol for part of the PEG seemed to have a significant sparing effect, but too few data are available to be certain. DPPD killed more chicks than simple puncture regardless of solvent, but none were deformed. PDAB, however, seemed more lethal if dissolved in PEG alone than in a mixture with ethanol. The combination of DPPD and PDAB was neither more nor less lethal than DPPD by itself.

Only PDAB deformed the chicks, but the results of these experiments do not indicate a free radical mechanism is operating since DPPD offered no detectable protection. However, we cannot reject that mechanism based on this work because several factors may have influenced the outcome. Although we tried to time the injections properly, DPPD may not have been in the affected organs in time to prevent the PDAB effect. Insufficient quantities of DPPD may have been used to modify the effects of PDAB enough for us to detect it by simple leg measurements or subjective judgment of feathers. Finally, ethanol, although used in minute quantities, appeared to play an unexpected protective role both against PAG and PDAB that we cannot explain without further work.

At present we are studying cellular and organ uptake of DPPD with respect to time of injection so more accurate results can be obtained.

Résumé. Les embryons de poulet traités au para-diméthylaminoazobenzène (PDAB) sont déformés. Le traitement au N,N'-diméthyl-*p*-phénylène diamine (DPPD) n'immunise pas contre ces déformations, car dans ce cas, les radicaux libres formés par le PDAB n'entrent pas en jeu.

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