incorporation into the anterior (chordal) and posterior (somitic) mesoderm seems to support this idea. We would also like to focus attention on the fact that the axial mesoderm which plays an important role in the induction of the nervous system in the chick embryos appears to be the target tissue for FSH[3].

Résumé. On a étudié par autoradiographie l'incorporation de la glycine-1-C^{14} par l'embryon du poussin. L'incorporation était la plus élevée dans le tissu neural et puis dans le notochorde et les somites. Après traitement avec le FSH, une incorporation plus grande s'est produite dans les mésodermes chordal et somitique. On propose qu'il peut s'agir d'un effet morphogénétique. Il est particulièrement significatif que le mésoderme axial, qui joue un rôle si important dans l'induction du système nerveux, paraît être également le point d'action de l'hormone.

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[4] Beit Memorial Research Fellow.

A Contribution to the Study of Lens Regeneration Capacity in Chick Embryos

Several investigators[1–7] have reported lens regeneration from the retinal or pigment layers in the lenectomized eyes of chick embryos. However, not all these reports are easy to evaluate and, in fact, recognition of the possible existence of this capacity in the avian embryos by some of the recent reviewers[8–10] is not without reservations. MCKEEHAN[11] reported completely negative results and has also seriously questioned the affirmations of other authors. Although GENIS-GÁLVEZ[12] obtained a number of cases of positive lens regeneration, he was persuaded not to interpret them in this way; but he also did not deny the possible existence of this ability in the chick embryos. The question being still open, the present study was undertaken specifically to investigate if lenses would regenerate in lenectomized eyes of chick embryos.

Embryos of White Leghorn fowl of approximately 3, 4 and 6 days incubation ages were used in this study. The embryo, taken from the egg, was placed in warm saline and its extra-embryonic membranes were removed. The ectoderm was then peeled away from over the eye region and the lens removed with the help of fine forceps or glass or platinum needles. Special care was taken to remove the lens in one piece. The lenectomized eye was then isolated from the head and cleansed of the adhering mesenchyme as much as possible. It was then explanted on about 1.5 cm^3 of Spratt's saline-agar-albumen medium in a moist chamber and incubated for 48–100 h at 100 °F. The control eyes were explanted in an identical way, except that the lenses were not removed from them. All operations were performed under sterile conditions. The explants were fixed in Bouins', sectioned at 8 μ thickness and stained with hematoxylin-eosin.

The controls were kept to find out if the culture conditions were suitable to maintain explants in a healthy condition and support further differentiation to any extent. The results showed that it was so, except for the eyes donated by 6-day-old embryos in which necrosis and degeneration set in after 2 days of cultivation in vitro. This was also true for the lenectomized eyes. The explants frequently became flattened and neural retina developed evaginations into the vitreous chamber. Flattening and retinal evaginations were much more pronounced in the experimental eyes than in controls.

Thirty-one out of 42 lenectomized eyes from approximately 3-day-old embryos survived cultivation for 72 h. In several cases one or both the iris borders, as seen in the sections, were thickened and bent inwards into the cavity of the eye-cup. These swollen regions were often devoid of pigment and possessed columnar cells with elongated nuclei. Definite lens formation was found, however, only in 6 cases, in 3 of which the connection of these bodies with the pigment layer was quite certainly present. These 3 cases showed unmistakable evidence of lens regeneration from the iris margin. They, in fact, represented 3 different stages in lens regeneration.

In case No. E72-14, shown in Figure 1, the lens-like structure is still nothing more than the rather thickened edge of the iris in which the pigment has disappeared and some lens fibres have been secreted in the middle. It is a small, solid structure, slightly inclined towards the interior of the eye. Its cellular continuity with the pigment layer is quite obvious. Case No. E72-9 (Figure 2) shows a more advanced stage in which the regenerating lens has assumed a distinctly globular shape with a core of fibre secreting cells. This also is a solid body whose cellular continuity with the iris border of the pigment layer is very clear. Case No. E72-11 (Figure 3) shows a still more advanced stage in the regenerative development of the

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lens. The large lens-like body fills almost the entire cavity of the eye-cup. It has become folded upon itself so that a small space has become enclosed within it. A very thin cellular bridge, only 16 μ in thickness, connects this lens with the pigment layer.

There was a large well-developed lens in case No. E72-4, which occupied the entire vitreous chamber of the eye as shown in Figure 4. The lens contained a central cavity, which, however, was not quite completely closed and in it there were present a number of degenerating cells. Serial sections did not show clearly whether this lens was connected to any other tissue layer of the eye, although there were some vague indications of it. This case can be interpreted as a stage in lens regeneration when the lens, growing out of the iris, has become folded to enclose some external space, which now forms its central cavity, and has also almost lost its connection with the parent tissue.

A lens-like body was present in case No. E72-8 also, but here again no histological connection of this body with any of the eye tissues could be ascertained. Finally, a small lentoid body was found situated on the external surface of the highly flattened and distorted eye in case No. E72-19. It had absolutely no connection with any other eye tissue and could probably be a remnant of the original lens accidentally left behind during operation.

Twenty-four lenectomized eyes from 4-day-old and 15 from 6-day-old embryos survived cultivation in vitro for 48–100 h. Except for 1 of these, none showed even the slightest sign of any lens formation anywhere in the eye. The exceptional case showed a small vesicle formed by evagination of the iris margin towards the outside of the eye. This outgrowth had no pigment and its cells were columnar with elongated nuclei. No lens fibres could be detected inside this vesicle. The author is not inclined to consider this as some early stage in lens regeneration. At best, it could be a very doubtful case.

In spite of the small number of positive cases, the evidence is qualitatively strong in support of the conclusion that lens regeneration is possible in chick embryos and does occur when such operated eyes are cultivated in vitro. This conclusion finds support in the earlier findings of BARFURTH and DRAGENDORF[1], DORRIS[4], VAN DETH[5] and especially GENIS-GÁLVEZ[12], whose studies are really relevant. Although GENIS-GÁLVEZ interpreted his results in a different manner, he did not deny the possibility of some of the lens formations in his material being due to regeneration from the iris border. In fact, some of his

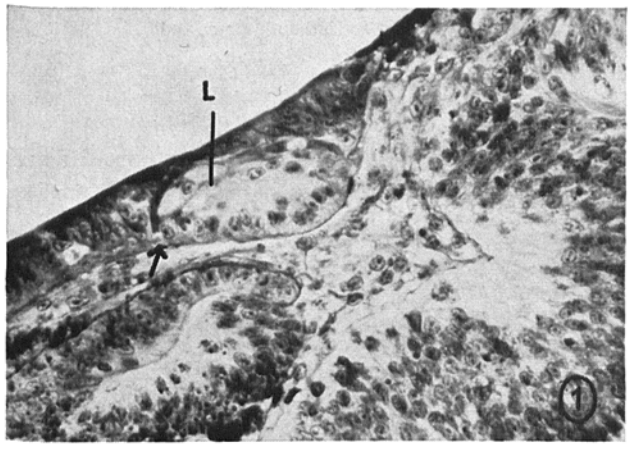

Fig. 1. Photomicrograph of a section through eye No. E72-14, cultivated in vitro for 72 h after lenectomy. L, regenerating lens. The arrow indicates its connection with the iris.

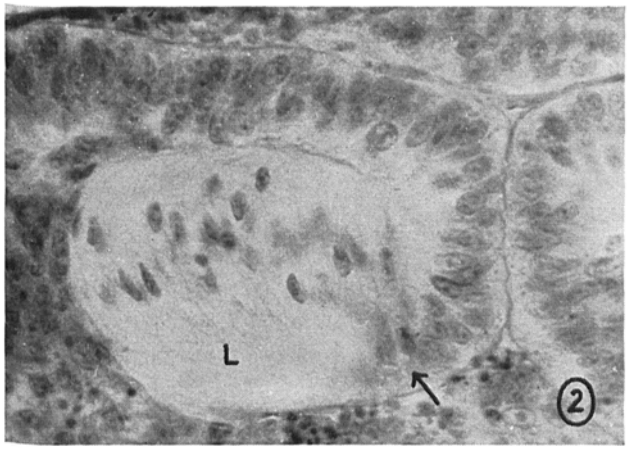

Fig. 2. Photomicrograph of a section through eye No. E72-9, cultivated in vitro for 72 h after lenectomy. L, regenerated lens. The arrow indicates its connection with the iris.

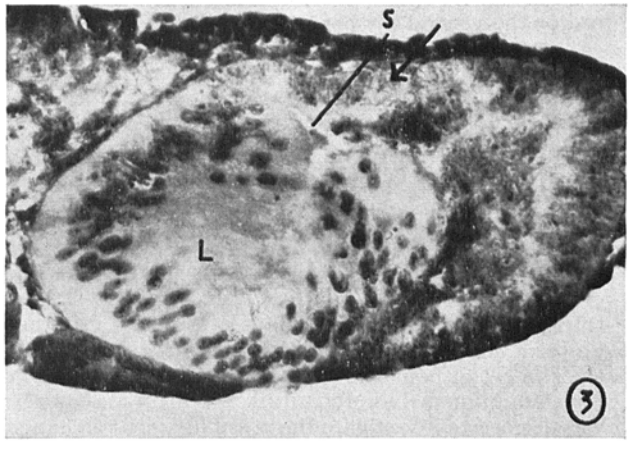

Fig. 3. Photomicrograph of a section through eye No. E72-11, cultivated in vitro for 72 h after lenectomy. L, regenerated lens; S, space becoming enclosed by folding of the lens. The arrow indicates connection of the lens with the iris.

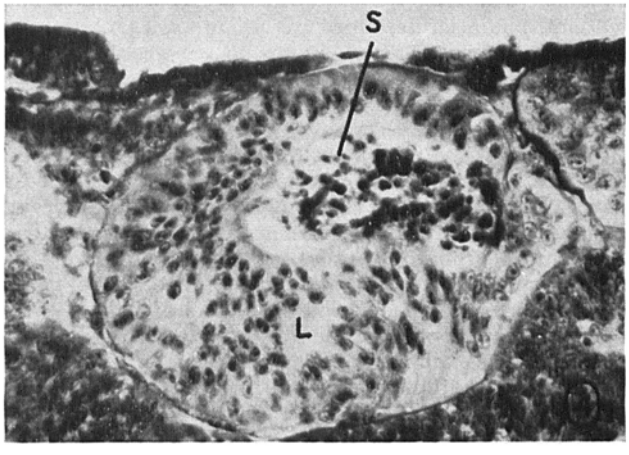

Fig. 4. Photomicrograph of a section through eye No. E72-4, cultivated in vitro for 72 h after lenectomy. L, regenerated lens; S, space enclosed by folding of the lens.

figures appear to be convincing evidence of lens regeneration.

Positive cases of lens regeneration were found only in eyes taken from embryos of about 3 days of age. An analysis of the results obtained by earlier authors shows that in their experiments also lens regeneration occurred when the eyes belonged to embryos of less than 3 days of age. Although VAN DETH[5] has reported a few cases of lens regeneration in 4-day-old embryos, his figures are not convincing at all. A similar case was found in the present series of experiments also; but, as mentioned earlier, it cannot be considered more than a very doubtful case. It is suggested that perhaps the lens regeneration capacity in the pupillary margin is present in the chick embryos up to about the end of the third day of incubation and it is lost thereafter. This would also explain the completely negative results obtained by MCKEEHAN[11]. The 72 h stage may be a critical one with respect to lens regeneration capacity. The loss, however, may not be irrevocable and the capacity can perhaps be restored under experimental conditions[13].

It has been stated[5] that in chick embryos the lens is regenerated as a solid plate from the iris border. This plate later becomes folded and encloses some of the outside space to form its own cavity secondarily. The present observations also support this interpretation. The present author, however, is unable to confirm whether it is the dorsal or the ventral iris border which is involved in lens regeneration in these embryos. According to a previous report it is the ventral border[5].

Extremely low frequency of lens regeneration in even young chick embryos still remains to be explained. It is stated[13] that even in *Triturus*, where frequency is high and lens regeneration predictable, it often fails if fibroblasts fill up the vitreous chamber of the lenectomized eye, thus preventing the retinal influences from reaching the iris. Probably a similar reason may operate in the case of chick embryos also[14].

Zusammenfassung. Die Linsenregeneration beim Hühnchen tritt nur auf, wenn die Embryonen am 3. oder 4. Bebrütungstage operiert worden sind. Wenn die Operation später stattfindet, tritt keine Regeneration auf.

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Department of Zoology, University of Rajasthan, Jaipur (India), 19th June 1967.

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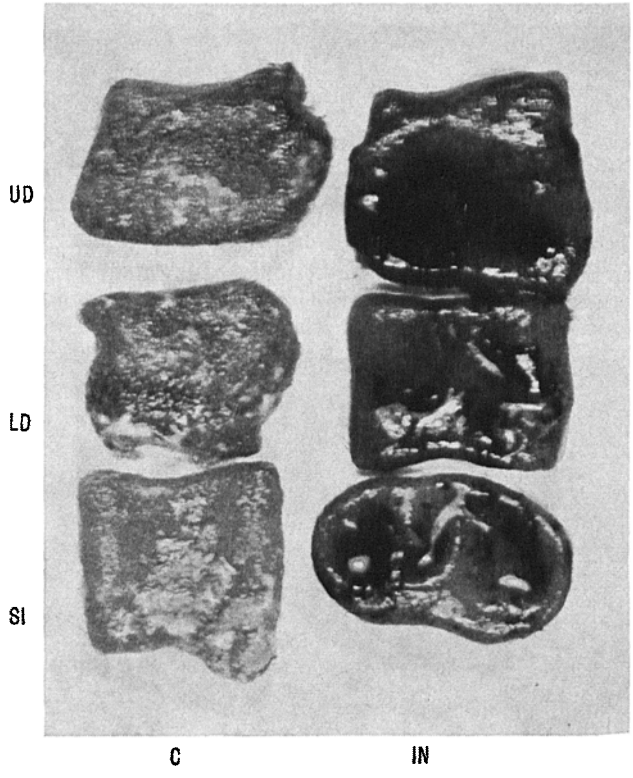
[14] The author gratefully acknowledges the support received from Dr. D. J. MCCALLION and Dr. R. A. LIVERSAGE of the Department of Zoology of the University of Toronto (Canada) where the experiments were conducted. The remaining part of the work was done at the University of Rajasthan, Jaipur, India.

Changes in Permeability of the Mucosa during Intestinal Coccidiosis Infections in the Fowl

During the fourth and fifth days of a severe infection of *Eimeria acervulina*, the mucosa appears inflamed and haemorrhagic, although actual loss of blood does not often occur (MOREHOUSE and MCGUIRE[1], PRESTON-MAFHAM[2]). It was decided to investigate the possibility that a change of permeability of the mucosa and a consequential loss of serum protein into the lumen of the gut might be associated with this inflammatory reaction.

An experiment was performed to follow the distribution of a dye, Pontamine sky blue, over the period of the infection. The use of this dye as a marker for serum proteins, has been described by HALPERN, LIACOPOULOS and LIACOPOULOS-BRIOT[3]. Eight- to ten-week-old cockerels were used for the experiment and the disease was initiated with a dose of 10 million sporulated oocysts. A 2% solution of Pontamine sky blue in Krebs-Ringer bicarbonate saline was injected into the brachial vein of both infected and control birds at a dose of 2 ml/kg body weight. A period of 20 min was allowed for the dye to circulate and the birds were then killed by an overdose of sodium pentobarbitone and the intestine removed for examination. Observations were made upon a total of 31 infected birds over the period of infection from 1–170 h.

The first appearance of the dye in the lumen of the gut was in a bird examined 48 h after infection. All of the birds examined after this time, with one exception at 52 h, showed some degree of dye loss. The amount of dye loss, as judged visually, was small up to 72 h but increased from this time to a maximum over the period from 90–120 h. Dye losses diminished gradually after this time and beyond 144 h was slight and mainly from the small



A comparison of the mucosal surfaces of the intestine of a norma control and an infected fowl, both injected with Pontamine sky blue. C, control; IN, infected bird; UD, upper duodenum; LD, lower duodenum; SI, small intestine from above the yolk stalk.