Calpodes ethlius[6] continued intermolt endocuticle growth in an ecdysone-free medium; in isolated imaginal discs of *Drosophila melanogaster*[7] and *Plodia interpunctella*[8] a pulse of ecdysone followed by cultivation in the absence of the hormone induced the deposition of a complete cuticle (including well formed endocuticle). Cuticle formation in vitro has also been observed in the presence of ecdysone; however, in such cases endocuticle was not or only incompletely deposited (for reviews, see Marks and Sowa[9] and Oberlander[10]).

The present results demonstrate that multilamellar as well as circadian-like endocuticle was deposited in cultured leg pieces which were taken from freshly molted imaginal cockroaches. It could be that in cockroaches postmolt deposition of endocuticle is triggered *before* or *during* molt. Thereafter, endocuticle deposition does not seem to depend on further hormonal stimulation. However, the leg pieces contained not only epidermal cells, but also certain quantities of haemocytes, fat body cells and other cells. The efficiency of these nonepidermal cells for the observed endocuticle deposition remains obscure, particularly with regard to the synthesis of cuticle proteins[11].

In the cultured leg pieces mainly multilamellate endocuticle was deposited. The reason for the formation of such endocuticle, which was never found in tibiae developed in vivo, is unknown. In vitro deposition of circadian-like layered endocuticle has not been observed before. Whether the frequency of double layer formation in vitro is really controlled by a circadian (temperature-compensated) clock has to be examined by further experiments.

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Imprinting of the Peking duck *(Anas platyrhynchos)* and dependence on exposure to light during ontogenesis

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Summary. Embryos of Peking ducks were either incubated in complete darkness up to hatching or were put into light one week before hatching. Control embryos were incubated under dim light conditions which corresponded broadly to the natural conditions. Under standardized imprinting conditions the controls and both groups of the light deprived ducklings showed the 'following response'. Most of the dark-incubated embryos, however, did not distinguish between imprinting and test objects of different shapes. Since most of the embryos kept in darkness only for 21 days also failed to develop the capacity for shape discrimination, there is apparently a critical period for light influences on the development of this capacity at some time during the early prenatal period.

Key words. Prenatal light deprivation; imprinting in ducks.

Visual experience influences the development of various brain regions in a variety of vertebrate species[2-6]. Observations on birds similar to those on other vertebrate groups indicate that exposure to normal light optimizes the development of behavior with respect to early learning phenomena such as the imprintability of chickens, while dark-rearing leads to behavrioral deficits[7]. However, the role of the prenatal influence of sensory input on the posthatch pattern of behavior has not attracted any attention, with the exception of acoustic stimulation of chickens in ovo[8-10].

The question investigated here was whether the influence of light during incubation of the avian embryo is essential for the structural differentiation of behavioral patterns which evolve during early postnatal life. The 'filial imprinting' of precocial birds like Peking ducks turned out to be suitable for answering this question because imprintability is influenced by visual stimuli and imprinting takes place during a 'sensitive period' in early postnatal life[11,12].

Material and method. So far we have studied 37 control animals which were exposed to light in ovo and 80 light-deprived animals. The control embryos were incubated (incubator type 'VOMO') for 28 days at 37.8 °C in dim light. Light-deprived embryos were incubated in another incubator of the same type in complete darkness. Within the group of light-deprived embryos 64 animals hatched in darkness, while 16 eggs were put into light 1 week before hatching, i.e. on the 21st day of incubation. After hatching, all ducklings were reared in diffuse light in separate plain gray boxes, which were protected from any visual stimuli. The training and testing was done under standard conditions in the imprinting apparatus which has been constructed by Pröve[1] based on the model of the Hess imprinting-apparatus[12]. The imprinting object (fig. 1) was a green ball moving and emitting sounds. About 20 h after hatching, each duckling was trained for 15 min and was then put back into its box. 24 h after the training the animal was tested with a green duck for 10 min. For the test the duckling was placed in the arena at about the same distance away from the two models while they were stationary (position a, a' in fig. 2). After the duckling had started off towards one or the other of the models, the models started to move and the duckling was allowed to follow for a few min. Then it was pushed away cautiously and the test was repeated twice after changing the position of both models and stopping the sounds. Only when the choice was correct three times did we assume that the imprinting had been successful; this is recorded in columns above the baseline of the table (fig. 3). Even if only one choice

was incorrect we assumed that the imprinting was ineffective; this is recorded in colums below the baseline of the table. Several birds did not show directed movements at all. Either they ran around crying nervously and avoided approaching one or the other model or they remained totally motionless (see small graph on top of fig. 3).

Results. There was no visible difference in behavior of controls and light-deprived ducklings during the imprinting procedure (fig. 1). On the average it took each duckling 0.5–1 min to stop crying, to approach the imprinted model (position a) and to follow it nestling and twittering (positions b–d). In the test of control animals (fig. 2 a, a'–c) 64.9% of the ducklings (see also fig. 3) approached the imprinted model (position b), chose it again in further tests or corrected for an error and followed the familiar model for at least 8 min (position c). Several ducklings of the remaining 35.1% approached the unfamiliar model once or twice and did not correct the error (21.6%) or remained totally undecided, i.e. did not follow either of the two models (13.5%, fig. 3). Both groups of light-deprived ducklings (fig. 2 a, a'–c, c') behaved abnormally in the test. They approached either the imprinting model (position b) or the unfamiliar one (position b') and followed one or the other, nestling and twittering. For the whole time of the test the duckling did not change the model which it had first chosen. As seen in figure 3 the vast majority of the deprived animals could not discriminate between the shapes of the models; i.e. 82.8% of the ducklings which were incubated in darkness until hatching and 81.3% of the deprived animals which were put into light one week before hatching. Single ducklings of both groups behaved indifferently (see graph on top, fig. 3) i.e. they changed several times from one model to the other or they remained totally undetermined. To test the significance of the data we performed the Fisher Test for 2 × 2 contingency tables, for which the values for all dark-incubated birds were combined. This test produced the chi-square value 49.17, which is significant at $p < 0.001$.

Discussion. Our experiments have confirmed that light is not required for the development of the 'following response'[13] but in ovo exposure to light seems to be required for the development of the ability to discriminate between different shapes of the models for 'filial imprinting'. We conclude that the 'following response' involves basic visual capacities which develop in several mammalian species even after long-term deprivation tests[14]. The disability in discriminating between models of dif-

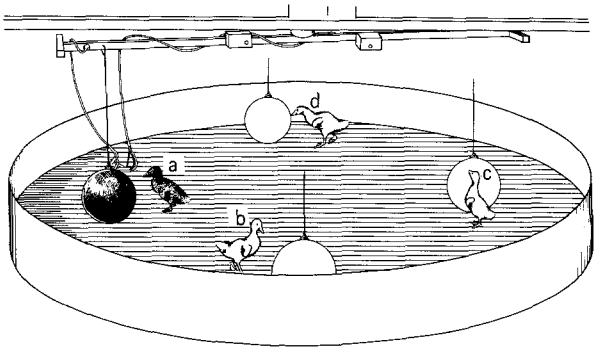

Figure 1. Training of ducklings to follow a green ball which gives intermittent sounds (20 h after hatching). The light-incubated ducklings as well as the dark-incubated ones behaved the same way: the bird approached the model (position a) and followed it nestling and twittering (positions b, c, d) for the whole time of 15 min.

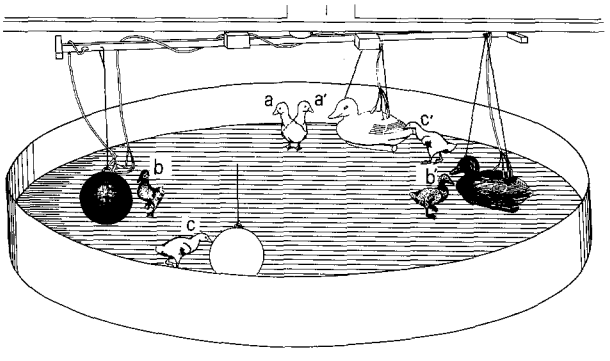

Figure 2. Test of the ducklings 24 h later. The animal was placed into the arena within sight and call of two models, the imprinting model and a test model, a green duck (position a, a'). The light-incubated ducklings generally chose the familiar model (position b) and followed it for the whole time of at least 8 min (position c). The dark-incubated ducklings, however, followed either the one or the other model (position b, b') for the whole time of the test (position c, c'), mostly without changing. Definitely, the deprived ducklings had not developed the capacity of shape recognition. Most of the embryos which were kept in dark-incubation for only 21 days also failed to acquire the capacity for shape recognition.

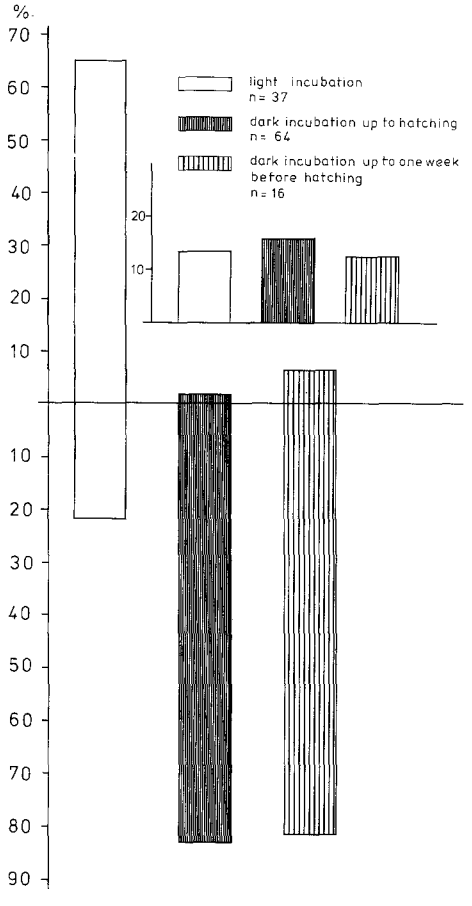

Figure 3. Quantification of successful (columns above the baseline) and unsuccessful (columns below the baseline) imprinting of ducklings under standardized conditions. 64.9% of the light incubated embryos (□) had developed the capacity of recognizing different shapes. But as many as 82.8% of the dark-incubated embryos, which even hatched in darkness (■) had not developed this capacity. The fact that even those ducklings which were set in the light again 1 week before hatching (▥) were mostly unsuccessful (81.3%), confirms that light is used during the prenatal developmental time. The small graph at the top right contains those ducklings of the three groups which did not show directed movements. Either they ran around avoiding approaching either model or they remained totally motionless.

ferent shapes also existed in the small group of dark-incubated embryos which were put into light 1 week before hatching. This indicates that the developmental defect was probably induced earlier. Further experiments are necessary to evaluate the effect of light on differentiation. Especially we need answers to questions like: which other parameters (color, size, direction, etc.) may be affected by the learning deficits? When is the critical period for light exposure during the incubation time?

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Effects of retinoic acid on ascites cells of the TA3 mouse mammary carcinoma[1]

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Summary. Retinoic acid caused a decrease in adhesiveness but no growth change in the allotransplantable TA3-Ha cell and no change in adhesiveness or growth in the strain specific TA3-St cell.The retinoic acid binding protein was detected in the TA3-Ha, but not the TA3-St, cell.
Key words. Mouse mammary carcinoma; ascites cells; retinoic acid; cell adhesiveness; cell growth.

Correlation of the presence of a retinoic acid binding protein with altered adhesiveness has been found in 2 sublines of TA3 mouse mammary carcinoma ascites cells grown in culture. The 2 ascites cell lines, TA3-St and TA3-Ha, which were derived from the same spontaneous tumor in a female strain A mouse, have been studied extensively because of their different transplantability characteristics[4,5]. The TA3-St cell is capable of growth in ascites form only in the mouse of origin, strain A; whereas, the TA3-Ha ascites cell is able to grow in some foreign species, as well as in allogeneic mouse strains.

As shown in figure 1, the addition of retinoic acid (*β*-all-*trans*-retinoic acid, Hoffmann-La Roche, Nutley, NJ) to the culture medium showed a dose-dependent decrease in the adhesiveness[6] of the TA3-Ha cell (A). No effect upon the adhesiveness of the TA3-St cell could be observed (B). The specificity of this effect upon adhesiveness in the TA3-Ha cell was shown by the sharp increase in this property upon removal of retinoic acid from the TA3-Ha culture medium (fig. 1A). No effect upon the adhesiveness of the TA3-St cell could be observed as a result of retinoic acid removal (fig. 1B).

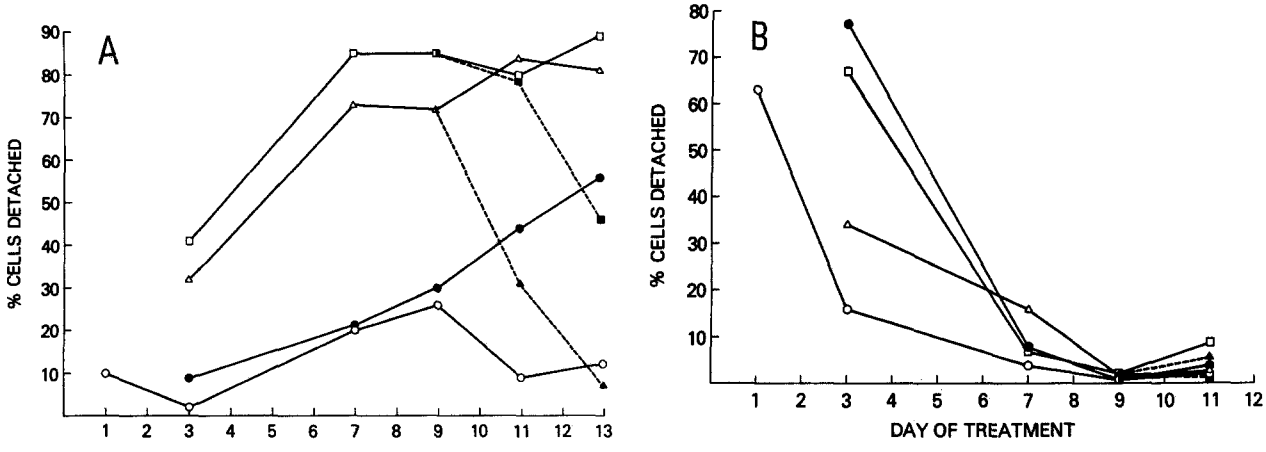

Figure 1. Time course of the effect of retinoic acid on the adhesion of TA3 cells. Cells were treated with control medium (○) or with control medium and Me_2SO (●); 1 μg (△) or 5 μg (□) of retinoic acid in Me_2SO per ml of medium. The time dependence of the effect on adhesion is shown. One half of the dishes treated with 1 μg (▲) or 5 μg (■) of retinoic acid were switched back to control medium at day 9 to show the reversibility of the effect. *A* TA3-Ha cells; *B* TA3-St cells.