antigen-antibody system and that inability to detect these antibodies in the past may be attributed primarily to their being present only in exceedingly small concentrations and not necessarily to their being "incomplete" or "univalent" antibodies (*7*). Further experiments are planned to elucidate the nature of the hemagglutination factor detected in allergic serums.

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27 December 1956

Formation of Lentoids by Dissociated Retinal Cells of the Chick Embryo

The capacity for self-differentiation of the embryonic chick eye has been studied by culturing the excised rudiment or its constituent tissues *in vitro* (*1, 2*), or as chorio-allantoic grafts (*3, 4*). Within the limitations imposed by the experimental conditions, the isolated eye tissues were found to be capable of developing histotypically with a remarkable degree of normality. It was noted, however, that when they were isolated from the ocular environment, some of the tissues occasionally developed in a direction different from that which they originally manifested, being thus able to give rise to more than one of the structural components of the eye. Thus, transformation of retinal tissue into tapetum and transformation of pigmented tapetum cells into sensory retinal cells were observed in cultures and grafts of embryonic chick eye tissues (*2, 4*). Such developmental modifications may be experimentally provoked by damage to the ocular tissues—that is, by a disruption of the existing intercellular and intertissue relationships (*5*). Apparently the effects of this damage and the ensuing processes of repair and reorganization give rise to conditions under which cells may assume a new association with different histogenetic properties and become diverted into an altered developmental course. Recent observations on the behavior *in vitro* of dissociated cells of embryonic chick sensory retina support this possibility; under suitable conditions such discrete cells in suspension aggregated and reestablished tissue continuity, but, instead of resuming their prior development as sensory structures, they frequently formed lentoid tissue.

The neural retinal tissue of 6- or 7-day embryos was stripped out of the dissected eyes. Special care was taken not to include pigmented tapetum cells and to discard the iris with the adjoining retinal tissue. The sheets of sensory retinal cells thus obtained were then dissociated into a suspension of discrete cells by procedures described earlier, based on the treatment of the tissue with cation-deficient solutions and trypsin (*6*). The suspended cells (Fig. 1*a*) were then cultivated *in vitro* under conditions suitable for the formation of aggregates and their differentiation (*6*). Watch-glass cultures of undissociated retinal tissue served as controls.

Dissociated retinal cells aggregated to form rosettes—that is, small groups of cells arranged concentrically around a lumen (Fig. 1*b*). When grown in organ cultures such rosettes continued to develop as a sensory tissue. However, if numerous clusters of rosettes were heaped together and cultured for several days under conditions of overcrowding, many of them underwent changes leading to their transformation into lentoid tissue: the cell nuclei became lighter staining and enlarged, the amount of cytoplasm in each cell increased considerably, and the cells elongated or became pear-shaped. The cells continued to proliferate without, however, retaining the rosette pattern, so that eventually the lumen of the rosette became filled with closely packed, elongated cells. In this form the clusters were histologically identifiable as lenslike bodies or lentoids (Fig. 1*c, d*). In the living cultures such lentoids could be easily spotted by their spheroid shape and their bright translucence. Each lentoid was usually surrounded by a layer of small cells, resembling, in position, early lens epithelium, but, in this case, they were of retinal origin. Since each such culture consisted originally of numerous rosettes, various phases of their transformation into lentoids could be found side by side.

A consistent feature of these lentoid-forming cultures was the presence of numerous loose and rounded retinal cells, most of which showed cytolytic and karyolytic changes. These cells originated in rosettes which, owing to the conditions of the culture, had distintegrated. The appearance of these cytolyzed cells preceded, in time, that of the lentoids, and if the conditions of the culture were such as to prevent the formation or the

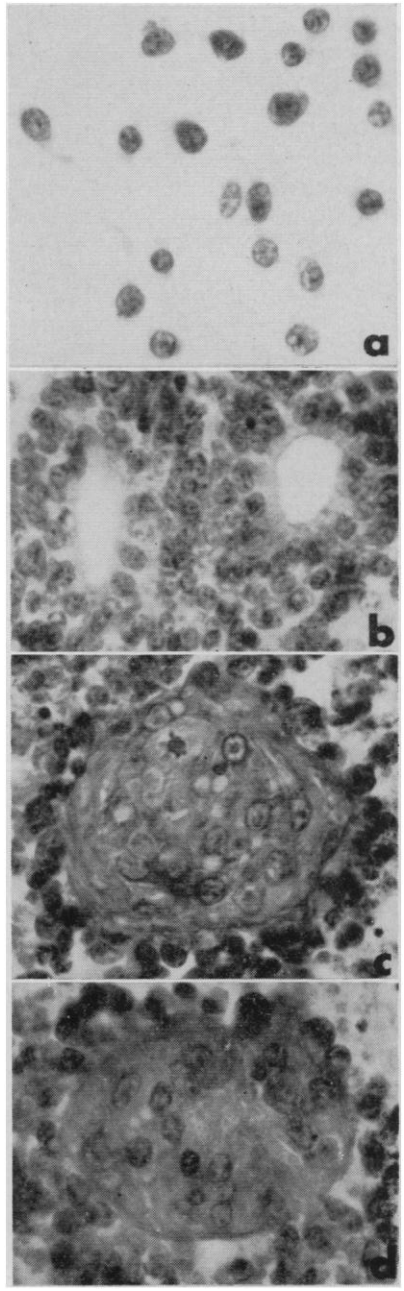

Fig. 1. (*a*) Smear of a cell suspension of neural retina tissue from a 7-day chick embryo (×760); (*b*) rosette-shaped cell aggregates formed in 2-day suspension culture (×620); (*c, d*) lentoids formed in 4-day culture of rosettes on a plasma clot (×620).

accumulation of these cells, no lentoids were formed.

The lentoids could be maintained *in vitro* for from 3 to 4 days, during which time they continued to grow in size, by cell division and through enlargement of the cells. The complete development of their cells into lens fibers was not observed, nor was it expected to take place, since it had been established that the culture environment was unsuitable for the terminal development of lens fibers or for their maintenance in an unaltered state (*7*).

In cultures of undissociated retinal tissue, lentoids were found only in a few isolated instances. They were present only in areas where rosettes had also formed. In all cases the lentoids were accompanied by cytolyzed cells, and their presence could easily be related to injury to the tissue and to its disruption owing to manipulation.

It has been established that the dissociated cells of a variety of embryonic tissues resume, following aggregation, their histotypic structure and course of development in accordance with their origin (*6*). In their ability to assume and pursue, following dissociation and aggregation, an altered course of development, retinal cells provide an interesting exception to the other types of cells studied. The situation in which the transformation into lentoids takes place indicates two sets of preceding and probably prerequisite conditions: the reassociation of the cells in a new pattern (rosettes) and the presence of numerous cytolyzed retinal cells. It should be possible to examine the bearing of each of these conditions on the process of this transformation and, eventually, to decide to what extent it is initiated by stimuli external or intrinsic to the affected cells.

In conclusion, retinal cells of the chick embryo, in being able to become transformed *in vitro* into lentoids, provide an additional example of a tissue which, although apparently "determined" and advanced in its specialization, may, under appropriate circumstances, alter its original course of development (*8*).

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Thermoperiods and Production of Apothecial Initials in the Fungus Sclerotinia trifoliorum

Sclerotinia trifoliorum Erik. responds to diurnal thermoperiods as indicated in Table 1. When the organism is incubated at constant temperatures, very few apothecial initials, the precursors to the sexual fruiting stage, occur. Other fungi might very well respond in a similar way; they are generally incubated at constant temperatures. The response is similar to that of many other living organisms (*1*). No previous reports, to our knowledge, are available on the influence of thermoperiods on the sexual fruiting stage of a fungus.

Production of the sexual stages of fungi under controlled conditions has been very difficult in many genera and species (*2*). In the past, in the genus *Sclerotinia,* apothecial initials and subsequent apothecia have been produced in culture, but these results could not be repeated with reliability (*3*).

In the experiments described here (*4*), the organism was grown in the dark on ground whole-wheat medium (50 g/lit) in 1.5-percent agar and preincubated at 24°C for 3 weeks in Petri dishes. All plates were sealed immediately with paper masking tape. This is not necessary, but it reduces contamination from repeated handling. Cultures were incubated, after preincubation, at several temperatures for definite time periods by moving the plates manually. Series A cultures (Table 1) were incubated for 8- and 16-hour periods; series B for 4- and 20-hour periods. Both A- and B-series cultures were subjected to temperatures of 7°, 15°, 21°, and 24°C in all possible combinations. Similar culture plates were held continuously at the same temperatures.

From the data, it can be seen that several diurnal thermoperiods influence production of apothecial initials. More than 100 apothecial initials were produced per

Table 1. Effect of cycling thermoperiods on the production of apothecial initials in *Sclerotinia trifoliorum* Erik.

Incubation (°C)					No. of apothecial initials			No. of sclerotia
4 hr	8 hr	16 hr	20 hr	24 hr	13 day	21 day	27 day	
				Series A				
	7	15			0	0	31	202
	7	21			0	72	400	235
	7	24			0	2	34	190
	15	7			0	31	194	234
	15	21			0	7	48	290
	15	24			0	10	67	384
	21	7			0	2	35	179
	21	15			16	242	303	305
	21	24			0	0	0	255
	24	7			0	12	158	259
	24	15			1	48	245	186
	24	21			0	0	0	190
				7	0	0	0	226
				15	0	2	13	209
				21	0	0	34	232
				24	0	0	0	209
				Series B				
7			15		0	0	101	215
7			21		61	188	563	305
7			24		0	15	66	359
15			7		0	0	0	360
15			21		7	81	105	209
15			24		0	0	0	212
21			7		0	0	3	214
21			15		11	56	66	255
21			24		0	0	5	324
24			7		0	0	0	192
24			15		4	64	131	217
24			21		0	23	83	218