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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22 January 1957

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

plate in the 4- and 20-hour periods when the cultures were held at 7° and 21°C, 24° and 15°, 7° and 15°C, and 15° and 21°C. The largest numbers of apothecial initials were produced in the 8- and 16-hour periods. The plates that were held at constant temperatures continuously produced 13 apothecial initials at 15°C and 34 at 21°C.

It has not as yet been possible to determine the ideal thermoperiod required by the organism because of the numerous possible combinations of temperatures and time periods; 7° and 21°C for 4 and 20 hours seems to be important because some cultures held for 7 months at a continuous 15°C produced apothecial initials in 15 days when subjected to thermoperiods. Apothecial initials have been produced in 40 days in the laboratory. In nature, a period of 7 months is required. Light is essenital for maturity of apothecial initials into apothecia (*5*).

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14 January 1957

Dissociation of Human Serum Macroglobulins

The elevated globulins of macroglobulinemic serums (*1*) are usually revealed as a single electrophoretic component with mobilities in the γ- or β-globulin range. However, on examination in the ultracentrifuge, a mixture of molecules showing strongly concentration-dependent sedimentation constants with values of 18, 25, and 32 Svedberg units (S) at infinite dilution is found to make up about 95 percent of the purified material. The appearance of macroglobulins in hyperglobulinemias associated with multiple myeloma is relatively rare. These macroglobulins have sedimentation constants below 15 S and often make up only a minor portion of the total protein (*2, 3*). Evidence has been recently presented that macroglobulins have their antigenic counterparts in normal serum (*3*), and it has been suggested that these molecules might represent discrete aggregates of serum proteins of relatively low molecular weight.

Attempts to dissociate pure macroglobulins electrophoretically by a combination of salt, *p*H, and temperature variations were unsuccessful. However, the addition of sulfhydryl compounds such as mercaptoethanol and cysteine resulted in the conversion of all macroglobulin fractions tested into a molecular entity having a sedimentation constant near that of the usual 6.5-S γ-globulin. One-percent solutions of the proteins studied were treated with 0.1*M* mercaptan in potassium phosphate buffer (ionic strength, 0.2; *p*H, 7.4) over a period of 24 to 48 hours at room temperature. Following this, the sulfhydryl compounds were removed by exhaustive dialysis for 48 to 72 hours against the phosphate buffer or against solutions of the buffer containing 0.02*M* iodoacetate or iodoacetamide. Removal of the sulfhydryl compound by dialysis results in reaggregation, but into molecular components that differ from the original material. The presence of the sulfhydryl blocking agents prevents such reassociation. These compounds have no dissociative effect on the native macroglobulins. Some typical results with an electrophoretically pure macroglobulin are shown in Fig. 1. It can be seen that the macroglobulins of *A* are converted to a single component by treatment with mercaptoethanol. Removal of the sulfhydryl reagent by dialysis leads to a marked diminution of the 6.5-S peak with the appearance of two new components sedimenting near 13 and 18 S (see Fig. 1*C*). If the sulfhydryl reagent is removed in the presence of a sulfhydryl blocking agent, in case of Fig. 1*D*, iodoacetate, the protein persists in the 6.5-S form. Such results indicate that the depolymerizations noted are the result of the breaking of disulfide bonds.

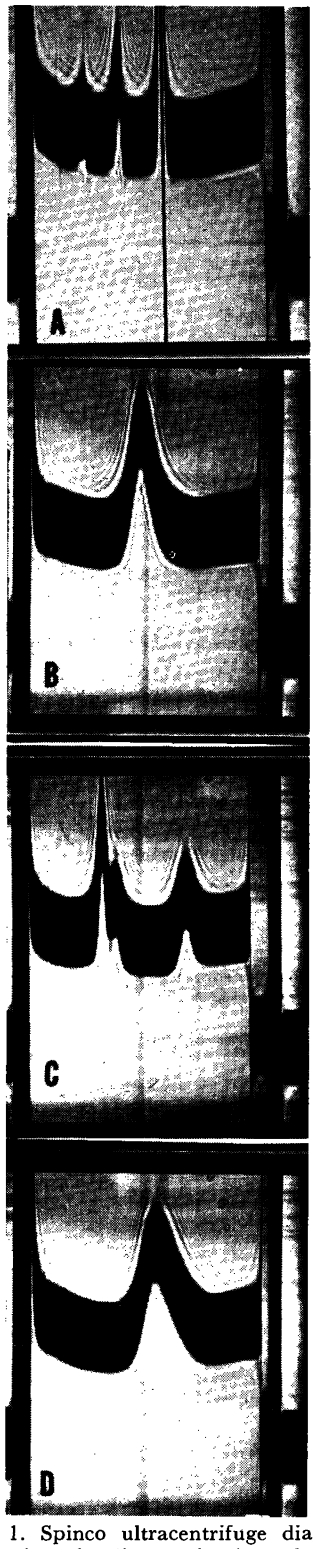

Fig. 1. Spinco ultracentrifuge diagrams (direction of sedimentation is to the left) of a purified macroglobulin fraction and the products derived from it. The times of centrifugation at 59,780 rev/min are given in parentheses. All samples contained potassium phosphate. (*A*) Parent macroglobulin (20 min); (*B*) macroglobulin after 48-hr treatment with 0.1*M* mercaptoethanol (65 min); (*C*) sample *B* after 48-hr dialysis against 0.02*M* sodium acetate (30 min); (*D*) sample *B* after 48-hr dialysis against 0.02*M* sodium iodoacetate (60 min).

A macroglobulinemic multiple myeloma serum protein (LE, *3*) was also converted to the 6.5-S component on treatment with mercaptoethanol, but unlike the 18- to 32-S type of macroglobulin, it did not spontaneously reaggregate on removal of the sulfhydryl agent. About 80 percent of the 18-S component of normal human serum which makes up nearly 2 percent of the total serum protein is converted to a smaller molecular entity by mercaptoethanol. The 9.5-S component of normal γ-globulins which forms on aging of samples is not affected by this type of treatment.

The physical and immunochemical relationships of the monomer units to the parent and reaggregated fractions are being studied. The data promote our earlier hypothesis that macroglobulins may be aggregates of normal serum globulins of molecular weight near 160,000 (*4*).

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