fibrils were still loose aggregates at 37°C. With increasing time of incubation the gels grew more insoluble, and became 80 percent irreversibly aggregated in 48 hours at 37°C.

The recently formed gels, 15 minutes old, exhibited no shrinkage, but dissolved abruptly at 52°C (Table 1). Older gels exhibited shrinkage before complete dissolution. Results were the same in phosphate buffer and sodium chloride. Gels incubated at 37°C for 1 year underwent about 50 percent shrinkage at temperatures 4° to 5°C above the "younger" preparations and failed to show any visible dissolution even at 80°C after 1 hour. Small increases in shrinkage and denaturation temperature occurred after incubation periods ranging from 1 to 28 days (Table 1). Shrinkage was initiated in native tendons 6° ± 1°C higher than that for reconstituted collagen. In all cases they went into solution completely at 58° to 59°C in both phosphate and sodium chloride suspending media.

Characteristics of denaturation of collagen dissolved in dilute acid and neutral solutions (0.15 to 0.2 percent protein) are described in Fig. 1. The temperature at which one half of the total change of rotation is reached is T_D, and T_M is the temperature at which the change in rotation ceases. The curves are identical in shape with those reported by others (*3*, *5*). The T_D is lower by about 10°C for denaturation at *p*H 4.2 in acetate than at *p*H 7.6 in phosphate. The T_M was much more variable and higher in the lower concentrations of arginine, in one experiment reaching a maximum of 56°C in 0.1*M* and ranging between 46° and 52°C. In the experiment reported in Fig. 1, the rotation fell appreciably more slowly after the initial rapid drop at 42°C in the sample containing 0.1*M* arginine than in those containing larger amounts of arginine. This may be an artifact caused by turbidity, since this region of the curve was accompanied by an appreciable fall in light transmission indicating the formation of aggregates. In the absence of arginine, gelation occurred at about 37°C preventing further observation. Arginine did not influence T_D. Unfortunately arginine is not effective in inhibiting gelation in NaCl medium, hence the necessity of working in phosphate. However, reconstituted gels and tendons incubated in NaCl with or without arginine revealed little difference in behavior compared with preparations in neutral phosphate.

There was a difference of about 21°C between the denaturation temperatures of dissolved collagen in acetate, *p*H 3.5 to 4.2, and the shrinkage temperature of the intact tissue, a value close to that reported by Doty and Nishihara (*5*).

That the denaturation temperature of collagen in solution is lower by 10°C than that of fibrils is consistent with the interpretation of Flory and Garrett (*3*) of concentration dependence of denaturation. Increases in shrinkage temperature of reconstituted collagen as compared with denaturation temperature in the dispersed state, ranged from 6°C after 15 minutes to 12°C after 1 year of incubation, a change which might be explained by slow progressive increase in the concentration of intrafibrillar protein. The high resistance of the 1-year-old gels to dissolution on heating to extreme temperatures suggests the formation of strong intermolecular bonds which maintain the overall gel fabric in spite of the collapse of molecular structure.

It is probable that the higher shrinkage temperature of the native tendons as compared to reconstituted fibers represents the mechanical restraint of the noncollagenous ground substance and perhaps circumferential fibril networks.

The shrinkage and denaturation temperatures of newly formed as well as old fibrils, as shown here, are well above the physiological range. Such reconstituted fibrils are not susceptible to digestion by the common proteases at 37°C (*6*). It seems unlikely that exposure of any of the known collagen fractions to body temperatures in vivo predisposes it to proteolytic attack. The recent detection of collagenolytic activity at neutral *p*H and physiologic temperatures in amphibian tissues, and in mammalian bone and uterus (*6*, *7*) indicates a more direct enzymatic attack in collagen resorption.

Note added in proof. In all experiments, denaturation temperature, T_D, in 0.15*M* acetate at *p*H 3.5 to 4.2, and shrinkage temperature, T_S, for whole tendons at neutral *p*H were consistently lower by 2° to 4°C than the values generally reported in the experimental literature. This does not in any way affect the significance of the data.

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Regenerating Tissues from the Cockroach Leg: A System for Studying in vitro

Abstract. *Regenerating leg tissue of* Leucophaea maderae *shows considerable activity in vitro. Migrations of ameboid and fiber-like cells from the explant take place, vesicles are produced, and interaction occurs between tissues. The system described facilitates the study of these activities in vitro.*

The success of Grace (*1*) in establishing strains of cells from the larva of *Antheraea eucalypti* has sparked new interest in the culture of insect tissues. It has been difficult, however, to find tissues of paurometabolous insects that will respond to culture in vitro.

Embryos and embryonic tissues survive well in vitro (*2*), but do not continue their development over any length of time. Postembryonic tissues survive well (*3*) but, to date, differentiation has been reported to occur only in the tissues of holometabolous insects. Tissues from the regenerating leg of the nymphal cockroach *Leucophaea maderae* not only survive well, but also continue to develop in vitro, thus providing a system which can be subjected to experimental procedures.

The growth of the regenerating leg was described by Bodenstein (*4*). When the leg of a nymphal cockroach is removed at the trochanter-femoral joint,

a regenerative blastema appears just proximal to the closed wound. This grows at the expense of the old muscle tissue to form a regenerate which becomes a new leg at the succeeding molt. Cowden and Bodenstein (*5*) have studied the differentiation of the regenerating tissue as it occurs in vivo.

For study in vitro, the two mesothoracic legs of 5th-instar nymphs were removed at the trochanter-femoral joint 2 days after molting. The stumps were opened from 10 to 21 days after removing the leg and the regenerating tissues were dissected out. These tissues were trimmed, rinsed, and placed in culture chambers. The culture medium was a modified version of that used by Grace (*1*). The best results were obtained with the ionic ratios altered to match those recommended by Ludwig *et al.* (*6*); the heat-treated homologous blood plasma could be omitted if desired or replaced with 1 percent bacteriological peptone (*7*). At a *p*H of 6.5, plasma-free cultures maintained activity for as long as 40 days.

Activity in the explanted tissues occurred at both the cellular and tissue levels. At the cellular level, the activities of migration, pinocytosis, and phagocytosis were common, as reported previously for other insects. In Fig. 1*A*, plasmatocyte-like cells are shown phagocytosing dead blood cells. The phagocytes varied in shape from nearly round to spindle-shaped and even stellate configurations. They migrated by pseudopod formation or by rounding up, detaching from the glass surface, and floating about in the medium. They formed cell sheets both at the glass and free-air interfaces. A second type of cell emerged from the cell reticulum beneath the epithelium of the explant. These cells were fiber-like with dendritic pseudopodia at the ends, and migrated along the glass surface (Fig. 1*B*). When the epithelial cells deteriorated, these cells often remained behind as a network of living cells. A third type of cell with a characteristic "signet ring" configuration was observed in rapidly growing cell sheets.

A more highly organized level of activity was seen in the production of vesicles by the explant tissue. These were hollow, spherical structures which, when mature, consisted of a single layer of thin epithelial cells, which became interlaced with attenuated, fiber-like cells.

One type of vesicle developed within the explant itself and migrated to the surface (Fig. 1*C*). This particular vesicle first appeared as a swelling within the explant, after 7 days in vitro. Within 48 hours, it had reached the proportions shown in Fig. 1*C*. By 60 hours it had more than tripled in size, and consisted of a layer of epithelial cells interlaced with fiber-like cells and covered with a thin membrane of gelatinous secretion. Vesicles of this type either remained connected to the explant or were budded off to become separate from it. A second type of vesicle formation occurred in the cell sheet at the edge of the explant. Fig. 1*D* shows an early vesicle of this type, which first appeared as a clump of "signet-ring" cells at the edge of the explant. As these cells increased in number, a cavity gradually developed in the center of the mass, thus producing the small vesicle which, as shown in Fig. 1*D*, lies entirely outside the jelly-like layer covering the parent epithelium. The formation of vesicles of both kinds was observed repeatedly.

Both the vesicles and the early stages of the developing regenerate consisted of fluid-filled sacs of epithelial cells which were underlaid by webs of fiber cells. The possibility that both of these result from the same kind of organized activity is under investigation.

One activity common to both the vesicle and the early regenerate was the passage of culture medium into the tissue sac, possibly by an active-transport

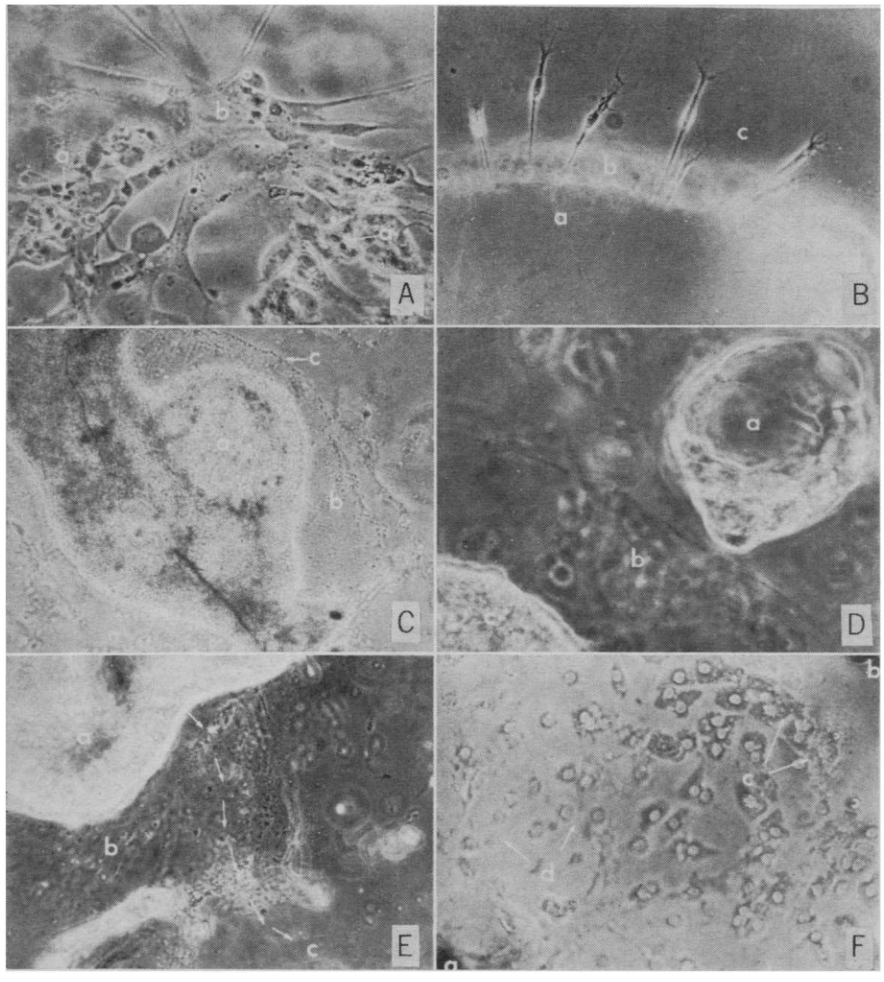

Fig. 1. Photomicrographs of cell cultures from regenerating tissues of cockroach legs (dark contrast phase). *A*, Dead blood cells (*a*) being engulfed by migrant cells from blastema explant (*b*) (× 200; 13 days in vitro). *B*, Fiber-like cells migrating from regenerate epithelium. (*a*) Cavity of regenerate; (*b*) epithelium; (*c*) culture medium (× 200; 3 days in vitro). *C*, Explant of regenerate (*a*) developing vesicle; (*b*) gelatinous secretion, (*c*) granular material at surface (× 100; 9 days in vitro). *D*, Small vesicle (*a*) developing in the cell sheet outside of blastema explant (*c*) which is invested in a gelatinous sheath (*b*) (× 200; 5 days in vitro). *E*, Regenerate explant (*a*) which has healed over. Arrows mark path of flow of culture medium through gelatinous sheath (*b*) into surrounding medium (*c*) (× 200; 12 days in vitro). *F*, Cell sheet extending from blastema explant (*a*) toward muscle explant (*b*). Vacuolated cells near muscle (*c*) contrast sharply with normal cells (*d*) (× 100; 8 days in vitro).

process. The vesicles were quite fragile, and frequently, when the medium was being replenished, they ruptured and collapsed. Sometimes, however, the lesion healed over, and the vesicle swelled up again within a matter of a few hours and continued to grow. In one instance, the cut surface of an entire explant was seen to heal over except for a small opening. After 12 days in vitro, a sluggish stream of fluid, made visible by particles of debris, was seen flowing out of this opening (Fig. 1*E*). This phenomenon was observed over a period of hours for three successive days. The similarity to the behavior of the vesicles was striking.

Another type of organized activity was the secretion of a gelatinous material from the surface of the epithelium, areas of which assumed a mulberry-like appearance with individual cells protruding from the surface (Fig. 1*C*).

The gelatinous material containing scattered dark granules appeared at the surface of the explant and built up into a thick layer, with the granular material concentrated at the surface. Occasionally, better-defined flakes and fibers could be seen forming at the surface of the layer. This process, which morphologically resembled the secretion of cuticular material, frequently occurred in the same location as vesicle formation (Fig. 1, *C* and *D*).

If a drop of fresh homologous blood was placed adjacent to an explant, an interaction between these tissues appeared by the 4th day. Plasmatocytes emerged from the blood clot at a point directly opposite to the blastema and migrated toward it. Cell migration was stimulated in the blastema, with the greater proportion of the cells emerging on the side nearest the drop of blood. It is interesting to note that while drops of whole homologous blood stimulated activity within the blastema, raw homologous blood plasma (15 drops/2.5 ml) added to the medium was highly toxic to the blastema cells. Apparently the blood cells play an active role in this interaction.

Negative interaction between muscle explants and blastema often appeared about the 6th day as a pronounced vacuolation of the migrant cells with frequent deterioration and death of those migrants nearest to the muscle explant. This was most pronounced when the two explants were not in contact (Fig. 1*E*). When the two tissues were in contact, or were both part of the same explant, the interaction was less obvious.

It appears that this system, in which an active, regenerating tissue was cultured and could be subjected to experimental manipulation, is suitable for studying regeneration in the tissues of paurometabolous insects.

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Pure Maple Syrup: Nutritive Value

Abstract. *Variations in concentrations of sugar, nitrogen, phosphorus, potassium, calcium, and magnesium of sap from sugar maple* (Acer saccharum, *Marsh.) trees are related to the time of sap collection and result in variation of the same components in pure maple syrup. Thirty milliliters (one fluid ounce) of pure maple syrup may contain 3 to 6 mg of phosphorus, 10 to 30 mg of potassium, 40 to 80 mg of calcium, and 4 to 25 mg of magnesium.*

Recent investigations on the relation of fertility of soils supporting sugarbushes to the mineral composition and yields of sap from sugar maple (*Acer saccharum,* Marsh.) have indicated that there are considerable amounts of several elements of importance in human nutrition in pure maple syrup.

The methods for the collection and analysis of the sap have been reported (*1*). Table 1 presents the average yield of sap and the average concentrations of sugar and nutrient elements for each yield from four trees sampled periodically during one season (1960). These trees are 150 years old and are supported by Mardin and Lordstown channery silt loam soils in central New York. The total tapping season in 1960, controlled by weather conditions, was from 28 March to 24 April.

Though the particular relation of time to the yield of sap and concentrations of sugar and nutrients varies within and between seasons because of weather conditions (*2*), the general seasonal trends are indicated in Table 1. Thus, the peak of the sap yield and of the sugar concentration tends to increase as the tapping season progresses. Calcium and magnesium concentrations tend to increase during the season to the "buddy" sap stage, which is a stage late in the season when the physiological processes in the tree result in a change in the color and taste of sap, making it less suitable for maple products (*3*); then the calcium and magnesium markedly decrease. Phosphorus and potassium concentrations increase during the first half of the season, decrease during the second half to the "buddy" sap stage, and then increase strikingly.

Table 1. Average yields of sap, and average sugar and nutrient element concentrations (volume basis) of sap and syrup during the 1960 tapping season of four sugar maple trees in central New York.

Sap yield per tap-hole (lit.)	Sap sugar conc. (%)	Sap syrup (*v/v*)	N (%)		P (ppm)		K (ppm)		Ca (ppm)		Mg (ppm)	
			Sap	Syrup	Sap	Syrup	Sap	Syrup	Sap	Syrup	Sap	Syrup
						31 March						
5.0	3.6	24/1	0.72	0.010	3.3	79.2	10.0	240.0	49.0	1176.0	5.0	120.0
						2 April						
9.7	3.5	25/1	0.78		3.3	82.5	12.5	312.5	54.0	1350.0	9.8	245.0
						8 April						
7.4	4.7	18/1	1.35		3.8	68.4	20.0	360.0	81.0	1458.0	21.0	378.0
						12 April						
9.1	3.7	23/1	2.16		3.5	80.5	19.3	443.9	86.0	1978.0	22.0	506.0
						16 April						
7.3	3.3	26/1	2.06		3.4	88.4	12.0	312.0	96.0	2496.0	28.0	728.0
						21 April						
Trace	3.2	27/1	3.96	0.054	6.8	183.6	36.0	972.0	76.0	2052.0	17.0	459.0