

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. 1E). 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. 1C).

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. Plasmotocytes 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. 1E). 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.

EDWIN P. MARKS

JOHN P. REINECKE

Biology Department, Washburn University, Topeka, Kansas

References and Notes

1. T. D. C. Grace, *Nature* **195**, 788 (1962).
2. J. G. Carlson, *Ann. N.Y. Acad. Sci.* **95**, 932 (1961); J. W. Larsen, *Ann. Entomol. Soc. Am.* **56**, 720 (1963).
3. M. E. Martignoni and R. J. Scallion, *Biol. Bull.* **121**, 507 (1961); W. E. Beckel, *Nature* **177**, 534 (1956); M. Luscher, *ibid.* **160**, 873 (1947).
4. D. Bodenstein, *J. Exptl. Zool.* **129**, 209 (1955).
5. R. Cowden and D. Bodenstein, *Embryologia (Nagoya)* **6**, 36 (1961).
6. D. Ludwig, M. K. Tracey, M. L. Burns, *Ann. Entomol. Soc. Am.* **50**, 244 (1957).
7. Nutritional Biochemicals Corp., Cleveland, Ohio.

27 December 1963

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

Based on the approved standards for maple syrup, sap is concentrated by evaporating the water until a specific gravity of the liquid is 1.32 and the amount of total solids is 65 percent. Of these solids, 95 percent is sucrose. Thus, the numbers of unit volumes of sap to make one unit volume of syrup depend upon the concentration of sugar in the sap (4). Table 1 presents the ratios, by volume, of sap to sugar and concentration of nutrient elements in the syrup. Except for nitrogen, the concentrations of nutrient elements in the syrup are in direct proportion to the ratios of sap to sugar (by volume). Boiling the sap in the syrup-production procedure results in virtually complete volatilization of the nitrogen.

The results of the analysis for phosphorus, potassium, calcium, and magnesium in the sap may be converted to information of the nutritive value of pure maple syrup, based on a specific volume of syrup:

$$X \frac{(86)}{S} (0.001) = Y,$$

where X is the nutrient element concentration, parts per million of sap volume; 86 is the volume in liters of sap of 1 percent sugar concentration needed to yield 1 liter of syrup of standard density; S is the concentration of sugar, percent of sap volume; 0.001 is to convert concentration in parts per million to content of the nutrient element in milligrams per milliliter of syrup volume; and Y is the nutrient element content, milligrams per milliliter of syrup of standard density.

Pure maple syrup has nutrient elements useful in human diets. Syrup produced from sap collected in the latter portion of the tapping season is higher in nutrient elements than syrup from sap collected early in the season. Pure maple syrup may have approximately the same calcium content as an equal volume of whole milk.

ALBERT L. LEAF

Department of Silviculture, State University College of Forestry, Syracuse University, Syracuse, New York

References and Notes

1. A. L. Leaf and K. G. Watterston, *Forest Sci.* 10, in press.
2. K. G. Watterston, A. L. Leaf, J. H. Engelken, *Soil Sci. Soc. Am. Proc.* 27, 236 (1963).
3. K. C. Holgate, *N.Y. State Agr. Expt. Sta. Geneva, N.Y., Bull. No. 742* (1950).
4. H. Kriebel, *Proceedings of the Fifth World Forestry Congress, 1960* (The Congress, 1962), vol. 2, p. 750.
5. I thank S. W. Potter, Jr., J. H. Engelken, K. G. Watterston, A. R. Talli, J. B. Hart, Jr., and D. M. Riordan for assistance.

28 October 1963

Cytoplasmic Interaction between Macrophages and Lymphocytic Cells in Antibody Synthesis

Abstract. *A direct cytoplasmic connection between macrophages and potential antibody-producing cells has been demonstrated in lymph nodes and spleen. This was observed in the tissues from both immunized and nonimmunized rabbits.*

The nature of the interaction between macrophages (1) and cells potentially capable of producing antibody (cells of the lymphocytic series and plasma cells) in the immune response has been a matter of conjecture for some time (2). Structural units in lymph nodes from immunized animals, consisting of macrophages surrounded by cells of the lymphocytic series, have been observed (3, 4). "Clones" of lymphocytic cells closely surrounding a large phagocytic cell have been described in suspensions of lymph node cells (3, 5), and in suspensions of macrophages incubated with lymph node cells from the same or different animals (5, 6). Recently Aronson (7) demonstrated bridge formation and the flow of cytoplasmic contents between phagocytic cells from several sources. However, despite the close proximity of the phagocytic cells and those of the lymphocytic series, no direct structural or functional connection between these two classes of cells has been demonstrated.

It has also been shown that antibody formation can be initiated in vitro. If phagocytic cells are exposed to the antigen and then the filtrates from these cells are added to lymph node cells maintained in tissue culture some antibody synthesis takes place (8). Fishman *et al.* (5) have shown that when RNA containing tritiated cytidine that was obtained from macrophages is incubated with cells from lymph nodes, cells of the lymphocytic series incorporated the tritium-labeled RNA in their cytoplasm. These authors noted that when H^3 RNA from macrophages was added to a lymph node suspension that contained both lymphocytic cells and macrophages "clones" were present in which lymphocytic cells in close proximity to macrophages were labeled to a greater degree than those not in contact with the macrophages. These experiments were interpreted on the basis of the proposals of Garvey and Campbell (9), that RNA or an RNA-antigen complex may be transferred from antigen-stimulated macrophages to antibody-producing cells. Since both a geographic and a biologic relationship be-

tween the cells concerned with the phagocytosis of antigen and those cells capable of synthesizing antibody has been demonstrated, the mechanism of transfer of cytoplasmic substances from macrophages to the cells of the lymphocytic series is considered here.

Lymph nodes and splenic tissue were obtained from albino rabbits of both sexes under sodium pentobarbital anesthesia. Samples were taken from rabbits immunized with horse ferritin (cadmium free), diphtheria toxoid, complete Freund's adjuvant, and a combination of Freund's adjuvant and diphtheria toxoid; samples were also taken from rabbits that had not been previously immunized. Portions of the lymph nodes and spleen were fixed and

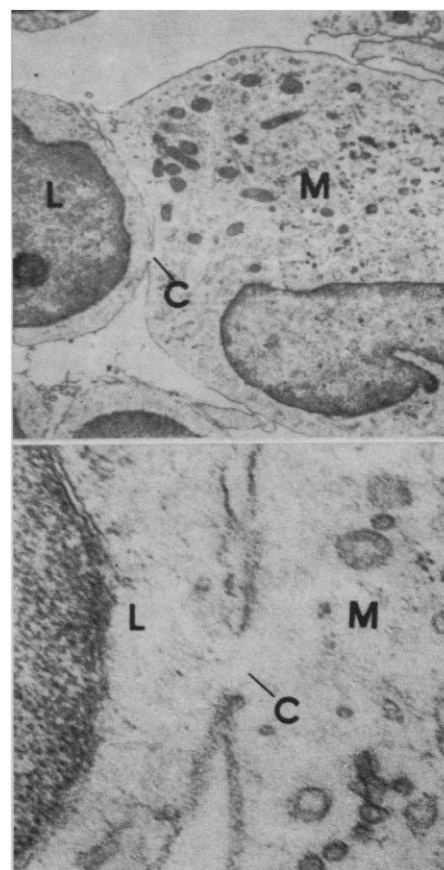


Fig. 1. (Top) Electron micrograph illustrating the close relationship between a macrophage (M) and a lymphocytic cell (L). The connection is shown at C ($\times 6750$). (Bottom) Higher magnification of the connection ($\times 45,000$).