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.

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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^3RNA 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 between the cells concerned with the phagocytosis of antigen and those cells capable of synthesizing antibody has been demonstrated, the mechanism of transfer of cyoplasmic substances from machrophages 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

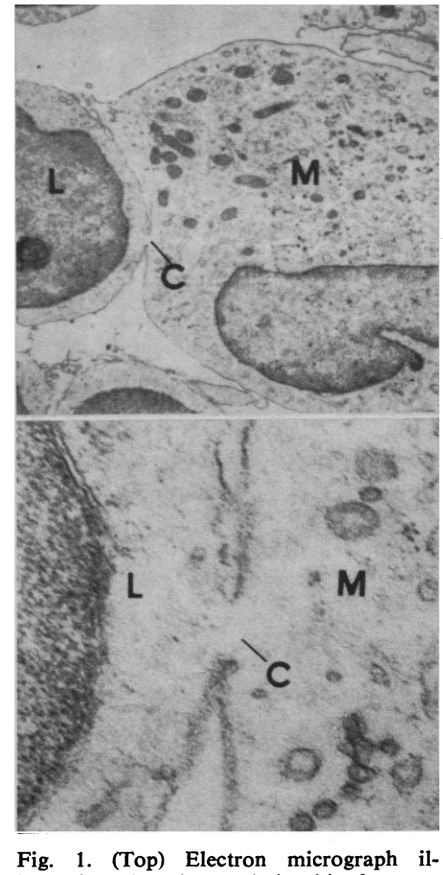

Fig. 1. (Top) Electron micrograph illustrating the close relationship between a macrophage (*M*) and a lymphocytic cell (*L*). The connection is shown at *C* (× 6750). (Bottom) Higher magnification of the connection (× 45,000).

embedded for optical and electron microscopic examination.

Clusters of lymphocytic cells could be found surrounding macrophages in both lymph nodes and spleen from all animals, but with a greater degree of frequency in the immunized animals. These clusters were scattered throughout the medullary areas of the lymph nodes and in the red pulp of the spleen, particularly in those areas of the red pulp adjacent to the mantle layer and the cords of the white pulp. It must be emphasized that the majority of the lymphocytic cells were not in such an arrangement. At an optical level, fusion of the cytoplasm of the macrophages with the surrounding lymphocytic cells could not be demonstrated. However, examination of these cells in the electron microscope revealed areas of direct communication between the cytoplasm of the macrophages and some of the immediately adjacent lymphocytic and plasma cells (Fig. 1). The cytoplasmic membranes of the two cells formed a continuous structure. Within this connecting corridor of cytoplasm small particles of the size of ribosomes could be resolved. There was no evidence of interchange of any other structures in these preliminary examinations. The transfer of antigenic molecules between macrophages and lymphocytes was not seen when horse ferritin was used as an electron-dense antigen. In fact, heavy-metal, labeled antigen was not identified in any of the known antibody-producing cells.

Direct communication between the macrophage and its satellite lymphocytic cells was not a common occurrence. Only one or two of the lymphocytic cells in the cluster showed this direct physical connection with the macrophage. In the immunized animals there were more cytoplasmic connections. The small number of cytoplasmic connections in any given "clone" may be the result of poor sampling. Extensive serial or closely adjacent sectioning has not been satisfactorily accomplished. However, these bridges may last for only a short time, as shown by Aronson (*7*) in the case of cytoplasmic bridges between phagocytic cells.

This evidence, added to that of others, suggests that there is a transfer of cytoplasmic content from the macrophage to lymphocytic cells, and that this exchange may be concerned with the transfer of ribosomal particles. It is reasonable to presume that the connections between these cells would allow for an easy transfer of such material.

At least two morphologically different cells can interact with the macrophage in lymphoid tissue. It is interesting to speculate on this point, since there is evidence that morphologically different types of cells produce 19*S* and 7*S* antibody, a large mononuclear cell having the features of a cell of the lymphocytic series and the plasma cell (*10*).

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References and Notes

1. In this report the term *macrophage* applies to those cells of the reticuloendothelial system in the spleen and lymph nodes that are capable of phagocytosis. Some authors prefer the term *histiocyte*.
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18 December 1963

Wasting Disease Induced in Young Mice by Administration of Cortisol Acetate

Abstract. *A single injection of cortisol acetate into young mice induced a wasting syndrome similar to that observed in runt disease and in the post-thymectomy syndrome. The course of the disease was less severe if the dose of the drug was decreased or if the animals were older at the time of injection.*

Wasting syndromes occur in newborn mice injected with allogeneic (*1*) lymphoid cells (runt disease) (*2*), in F_1 hybrids injected with parental lymphoid cells (*3*), in lethally irradiated mice injected with allogeneic bone marrow cells (secondary disease) (*4*), and in mice thymectomized shortly after birth (*5*). These syndromes are all characterized by progressive weight loss, ruffling of the fur, diarrhea, and death. A pathological finding that is common to all of these types of wasting is profound atrophy of the lymphoid organs. It has been suggested that this lymphoid depletion results in widespread metabolic dysfunction leading to the fatal wasting (*6*).

Although adrenal corticosteroids are known to cause lymphoid depletion (*7*), the role of these hormones has not been delineated with relation to the wasting diseases. In this study we investigated the effects of cortisol acetate (hydrocortisone acetate) on mice of various ages, the conditions under which a wasting disease can be produced, and the correlation between this and other types of wasting disease on the basis of morphologic criteria.

Mice of various strains, mostly C3H, were used throughout the experiment. Cortisol acetate in aqueous suspension was administered subcutaneously in the neck region. Animals were either allowed to pursue the natural course of the disease or were killed at varying time intervals after drug administration.

A single injection of 0.25 mg of cortisol acetate into 1-day-old mice resulted in a wasting syndrome strikingly similar to that developed in runt disease and in the post-thymectomy syn-

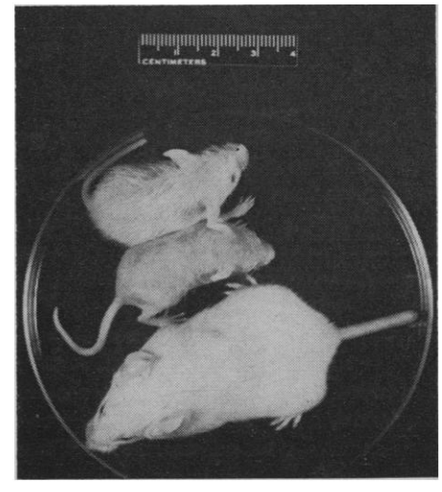

Fig. 1. Littermates (Swiss mice) age 27 days. The two mice with wasting disease were treated with 0.25 mg of cortisol acetate at 5 days of age; the third mouse served as an untreated control. Note thinned skin, ruffled fur, and scarce hair of treated mice.