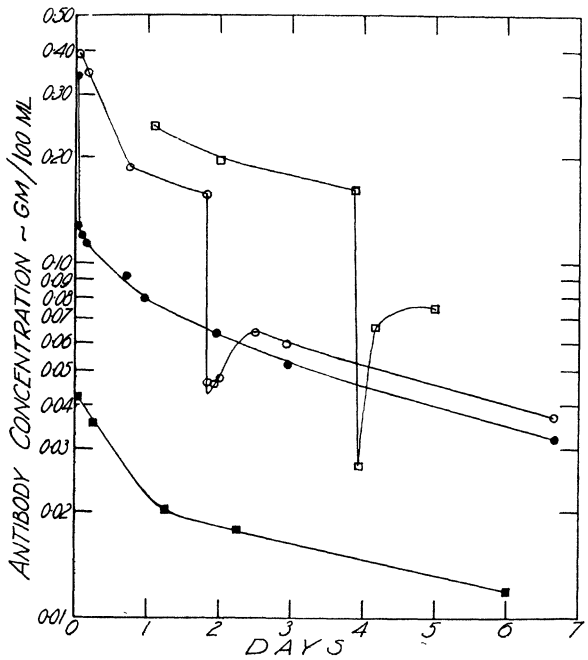

FIG. 1. All individual points on these curves represent the average values for the rabbits within a given experimental group. □ Expt. 1 A; ○ Expt. 2 A; ● Expt. 2 B; ■ Expt. 3.

venously in 1.0-ml volume, slowly. This amount of polysaccharide was calculated as enough to remove most but not all of the antibody circulating at that moment, the complex presumably being removed from the circulation in a short period of time. As can be seen, the level of circulating antibody fell immediately after administration of the specific antigen. A secondary rise soon occurred, however, and the circulating antibody then resumed its previous rate of logarithmic decline.

B. Two rabbits were given 15 ml of the same antiserum intravenously and then, after 20 min, 2.2 mg of polysaccharide III were injected. An immediate fall in circulating antibody occurred; the antibody then assumed a logarithmic rate of decline proportionate and parallel to the curve for the 4 rabbits in Expt. 2 A, and closely approximating it after the secondary rise in circulating antibody had taken place in the latter rabbits.

Expt. 3. Effect of reinfusion of antibody removed after equilibrium reached. To determine if the initial rapid fall in circulating antibody, observed in the rabbits of Expt. 2 A before the decline became exponential, was dependent upon the disappearance of antibody molecules with markedly shorter half-lives, serum derived from blood removed from the rabbits of Expt. 1 A during exchange transfusion was injected into 2 other rabbits after 3-fold concentration by negative pressure dialysis and centrifugation twice at 4000 rpm at 0° C for 30 min. Twenty-five milliliters of this serum were given intravenously to 2 rabbits after removal of 25 ml blood. The same initial rapid fall was observed. Since the donor rabbits had been in equilibrium with respect to circulating antibody, the derived serum presumably should have contained little or no antibody of very short half life. Hence it seems reasonable to conclude that the initial rapid fall following infusion of a plasma protein is, as has been previously postulated, an equilibration of the circulating protein with the extravascular protein pool.

The half life of the rabbit antibodies in these experiments varied between 5 and 7 days, which agrees with the values already reported (*14, 15*).

It would appear from the data that preformed plasma protein present in the extravascular pool can move rapidly into the circulation upon depletion of the specific protein in the latter compartment. Thus, the extravascular plasma protein is in dynamic equilibrium with the intravascular plasma protein; once equilibrium is established, a decrease in the mass of a specific plasma protein in one compartment results in the movement of that plasma protein to that compartment until equilibrium is again attained.

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A Homogeneous Cell Preparation from Soybean Leaves[1, 2]

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A typical dicotyledonous leaf is composed of three major types of tissue: epidermis, minor venation, and mesophyll. From the data of Wylie (*1*) these tissues appear to be present in roughly equal amounts. The specific biochemical contributions of the individual tissues to the general physiology of the leaf are essentially unknown (except, of course, for the photosynthesis of the mesophyll). Whereas epidermal cells may be obtained frequently merely by stripping, no procedure exists for obtaining mesophyll cells in

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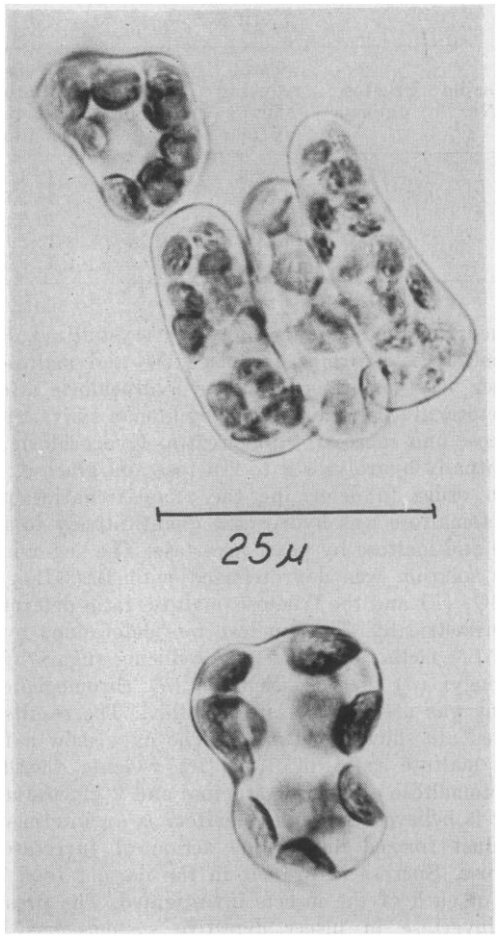

FIG. 1.

quantity. We find that soybean leaves (var. Hawkeye) will yield a tissue-free mesophyll preparation when carefully ground with 0.25 *M* sucrose, 0.05 *M* phosphate (pH 6.8) at *ca* 4° C. A test tube homogenizer with a loosely fitting pestle is used and the resulting suspension is filtered through bolting silk (*ca* 100 threads/in.). The filtrate is centrifuged at very low speed (*ca* 80 rpm) for several minutes. Upon discarding the supernatant and resuspending the cells in a nutrient solution, a tissue-free preparation of parenchyma cells is obtained (Fig. 1).

An illuminated, fresh cell preparation is capable of fixing $C^{14}O_2$ at a rate corresponding roughly to one-fifth that of a normal leaf on the basis of chlorophyll concentration. The intact excised leaf and the cell suspension show qualitative equivalence in the formation of 80% ethanol-soluble products (Fig. 2) and starch-C^{14}, but they are vastly different in their abilities to synthesize protein. While a leaf will form 10–15% of the total ethanol-insoluble material as protein in 30 min, the free-cell preparation will form none. The latter system has been subjected to various time, temperature, and nutrient conditions, without any evidence of radioactivity in the protein. That this is not an artifact arising from the method of preparation was shown as follows. An intact excised leaf was exposed to $C^{14}O_2$ in the light for 2 hr. A cell suspension was then made by our usual technique, analysis of which showed a large fraction of the radioactivity in the protein.

However, the failure of the cell preparation to form protein is not correlated with the inability to form amino acids from photosynthate. In the 30-min photosynthesis the cells had accumulated *ca* 35% of their 80% ethanol-soluble radioactivity in the form of the amino acids alanine, glutamine, aspartic acid, glycine,

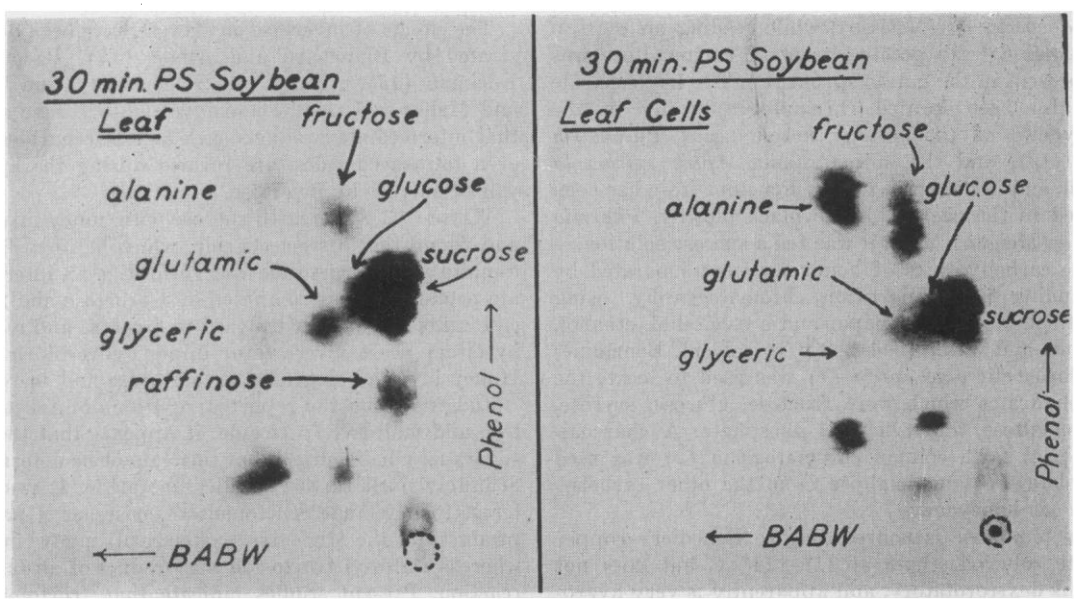

FIG. 2.

and serine.[3] It is apparent that the cells are unable to form protein from these amino acids. The system is thus unique in that it demonstrates protein synthesis to be a more sensitive reaction than photosynthesis. Unfortunately, another physiological malfunction is the rapid decrease in the rate of photosynthesis with the age of the preparation, without visible sign of deterioration. The loss in the ability to assimilate $C^{14}O_2$ is essentially logarithmic, only 20% of the original rate remaining after 1 hr at 23° C (in weak light prior to stronger illumination). It is, of course, possible that the same factors operating in negating protein synthesis are involved in the degradation of the photosynthetic system.

The preparation is intriguing as a possibility for the study of the nutrition and function of a specific type of tissue in leaves, akin to that of a highly specific organ in an animal.

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Manuscript received April 27, 1953.

[3] These are also the only amino acids formed by an intact leaf within the same time period. The fact is that even without the simultaneous formation of all the essential amino acids, protein synthesis (probably partial turnover) occurs in excised leaves. This question will be discussed in a subsequent publication.

Fructomaltose, a Recently Discovered Trisaccharide Isolated from Honeydew

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A trisaccharide, fructomaltose, has been isolated from the honeydew produced by the citrus mealy bug, *Pseudococcus citri* (Risso), while feeding on the sap of etiolated Irish potato sprouts. Fructomaltose was not present in the potato sprout sap. The trisaccharide has also been located chromatographically in the honeydews of the cottony maple scale, *Pulvinaria vitis* (L.), and the spirea aphid, *Aphis spiraecola* Patch, and in honeybee honey. Fructomaltose has been located in the excreta of the black blowfly, *Phormia regina* (Meigen), when it was fed a sucrose solution.

The carbohydrates of honeydew were separated by descending paper partition chromatography, using Whatman No. 1 filter paper and a *n*-butanol, ethanol, acetone, and water (5–4–3–2 v/v) solvent. Benzidine-trichloroacetic acid spray (*1*) was used to locate the carbohydrates which were fructose, glucose, sucrose, fructomaltose, and glucose-1-phosphate. A charcoal-infusorial earth column chromatogram (*2*) was used to isolate the fructomaltose from the other carbohydrates of honeydew.

Fructomaltose is nonreducing to Benedict's copper sulfate solution, chars at 118–124° C but does not melt, is dextrorotatory, and apparently is very hygroscopic. A satisfactory rotation value has not been obtained and the sugar has not been crystallized. Fructomaltose is hydrolyzed to fructose and maltose by either yeast invertase or dilute hydrochloric acid, to fructose, glucose, and sucrose by human saliva, and to glucose and sucrose by pancreatin. Hydrochloric acid eventually hydrolyzes it to fructose and glucose.

TABLE 1

Sample No.	Fructose, μg/mm	Total reducing sugars, μg/mm	Maltose, μg/mm	Weight ratio Fructose/maltose
1	17.5	52.9	34.5	1: 1.97
2	8.2	25.0	16.8	1: 2.05
3	13.2	44.5	30.9	1: 2.27
4	8.0	24.6	16.6	1: 2.07
5	14.0	45.8	31.8	1: 2.27

In order to determine the monosaccharide ratio, fructomaltose was hydrolyzed quantitatively to fructose and maltose by yeast invertase. The sugar-invertase solution was deproteinized with $Ba(OH)_2$ and $ZnSO_4$ (*3*) and the fructose/maltose ratio determined colorimetrically. The fructose was determined by the Roe (*4*) method and the total reducing sugars by the Somogyi (*5*) method. Nelson's (*6*) chromogenic reagent was used in the latter method. The results obtained are shown in Table 1. The data show a fructose/maltose ratio of 1 : 2, by weight; therefore, fructomaltose contains 1 fructose and 2 glucose units.

It is believed that fructomaltose is an intermediate product formed during the action of invertase on sucrose. Sucrose is present in the normal food supply of each of the insects investigated. The presence of invertase in insect digestive systems has been demonstrated by many investigators including Sarin (*7*), Bertholf (*8*), Phillips (*9*), Herford (*10*), and Fraenkel (*11*).

The effects of in e on sucrose have been demonstrated by Blanch nd Albon (*12*), Bacon and Edelman (*13*), A[illegible] and Bacon (*14*), and White and Maher (*15, 16*). These investigators have shown that intermediate products such as trisaccharides, and even tetrasaccharides, are formed during the hydrolysis of sucrose by invertase.

White (*15, 16*) treated sucrose with honey invertase and found that a trisaccharide, which he identified as α-maltosyl-β-D-fructoside was formed as an intermediate product. It was composed of 1 fructose and 2 glucose units and was hydrolyzed to fructose and maltose by either yeast invertase or dilute hydrochloric acid. Honey invertase degraded it to glucose and sucrose.

Judging from the reported reactions of fructomaltose and maltosyl fructoside, it appears that the two sugars may be identical, but this cannot be determined definitely until further studies are made. It is of interest to note that fructomaltose arises as a natural product in the digestive systems of many insects, whereas maltosyl fructoside is a product of an *in vitro* reaction. Present studies indicate that fructomaltose may be expected to arise in the digestive system of any