showed that the disease decreased the net assimilation rate of the plant and suggested, as one interpretation, that photosynthesis was slowed. Our experiments show that the disease interferes with the photochemical activity of the chloroplasts. This could decrease the amount of photosynthate available, which could well result in a decreased sugar yield. The effects of pathological conditions on the metabolic properties of chloroplasts have apparently not been investigated.

Kausche and Ruska (*3*), and Black, Morgan, and Wyckoff (*4*) published electron micrographs that indicated the presence of tobacco mosaic virus within the chloroplasts of infected tobacco plants. More recently Leyon (*5*) published electron micrographs of preparations from leaves infected with beet yellows virus that showed filamentous particles associated with the chloroplasts. Leyon suggested that the filaments represent the virus and that at least some of the virus was formed within the chloroplasts, although Nixon and Watson (*6*) argued that these filaments represent only a small part of the anomalous material in infected plants.

These experiments (*7*) were carried out with chloroplast fragments from leaves (*8*) of control and virus-yellows inoculated sugar beet plants (var. U.S. 75). Chloroplast fragments were prepared and stored as previously described (*9*). Chloroplast activity was measured

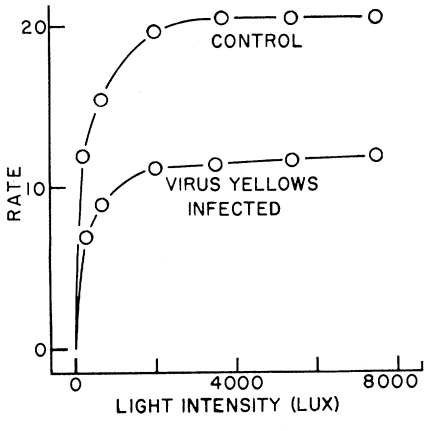

Fig. 1. Curves showing the effect of light intensity on the photochemical activity of chloroplast fragments isolated from control and virus-yellows-infected sugar beet leaves. The rates are expressed as millimoles of ferricyanide reduced per hour, per milligram of chlorophyll; the incident light intensities are expressed in lux. The reaction system was 0.0005*M* in potassium ferricyanide, 0.01*M* in potassium chloride, 0.10*M* in potassium phosphate buffer of *p*H 6.80, and 0.17*M* in sucrose. The reaction system (3.0 ml) contained chloroplast fragments equivalent to 100 mg/lit ($1.1 \times 10^{-4}M$) chlorophyll. Illumination was provided by reflector-type incandescent bulbs; the reaction was run in flowing tank nitrogen at a temperature of 15°C.

by a potentiometric technique (*10*) as a function of light intensity in order to determine whether the virus affected the rate-limiting photochemical reaction, the rate-limiting dark reaction, or both (*11*).

The results of a typical experiment with plants raised under normal fertility are shown in Fig. 1. The slope of the curves at zero light intensity is proportional to the rate-limiting photochemical reaction; the asymptote approached by the curves at infinite light intensity is a measure of the rate-limiting dark reaction. The curves show that the rates of both processes are decreased in the chloroplast fragments from the infected plants. For more quantitative determinations, the data may be plotted in the form (light intensity)/(velocity) versus (light intensity). In this form straight lines are obtained in which the rate constant k_L for the limiting photochemical reaction is proportional to 1/intercept, and the rate constant k_D for the limiting dark reaction is proportional to 1/slope as discussed elsewhere (*11*). The values of the rate parameters as calculated in this way by the method of least squares are as follows: for control plants, $k_L = 0.12$, $k_D = 22.8$; for infected plants, $k_L = 0.07$, $k_D = 12.5$. Thus, both of the rate-limiting chloroplast reactions are decreased by approximately 50 percent (on a chlorophyll basis) in the infected plants.

These preliminary experiments indicate that the beet yellows virus could decrease photosynthesis and sugar production in the plant by a direct action on the chloroplasts rather than by some indirect effect on the photosynthetic mechanism or by interference with the translocation of carbohydrate in the phloem as has been shown in the case of the curly top virus of sugar beet. The results further indicate that the degree of yellowing of the infected leaves is not necessarily related to the severity of chloroplast injury, for the decrease in chloroplast activity noted is calculated on a chlorophyll basis.

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Hemagglutination after Immunization with Schistosome Antigens

Boyden (*1*) and recently Stavitsky (*2*) reported the adsorption of protein antigens on sheep erythrocytes treated with tannic acid and their subsequent hemagglutination by specific antiserums. This technique was adapted to the antigen-antibody system in schistosomiasis. Agglutination of living cercariae of *Schistosoma mansoni* has been previously reported in the serology of schistosomiasis by Liu and Bang (*3*), Standen (*4*), and Stirewalt and Evans (*5*). In our investigations agglutination was observed in some but not all samples of normal, full-strength, inactivated serum of man, horse, cow, sheep, steer, goat, dog, hamster, rabbit, and pig. In the serums of 13 vertebrate species immunized with frozen cercariae of *S. mansoni, in vitro* agglutination of living cercariae was observed during the course of immunization (*6*). The agglutinin titer for living cercariae varied from 1:8 to 1:128, and a more sensitive and specific technique for the detection and titration of these antibodies was desirable.

The method of conducting the hemagglutination test (*7*) followed Boyden (*1*). Sheep cells in Alsever's solution were washed in buffered saline (*p*H 7.2) and incubated with a 1:20,000 dilution of tannic acid for 10 min at 37°C. The packed tanned cells were then exposed to 5 vol of a 1:5000 dilution by weight of lyophylized cercariae of *S. mansoni* (0.001 mg of lyophylized cercariae per 5 ml of saline buffered at *p*H 6.4) for 15 min at room temperature. Coated cells were adjusted to a 2-percent suspension in 1:250 normal rabbit serum. Serial dilutions were made in 1:100 normal rabbit serum. One drop of antigen-treated cells was added to each tube with an Ives dropping pipette. The test was read after 2 hr and after the cells had remained at room temperature (23°C) overnight.

Hemagglutination titers were obtained for the serums of 16 rabbits immunized by six intravenous injections administered twice weekly for 3 wk with various stages of the life-cycle of *S. mansoni* and *Schis-*

Table 1. Hemagglutination by rabbit serum after immunization with antigens from stages of the life-cycle of *S. mansoni* and *S. douthitti*.

Antigen	No. injected	Hemagglutination titer
Male worms, *S. douthitti**	230, 585	1:20, 1:80
Female worms, *S. douthitti**	234	1:40
Male and female worms, *S. douthitti**	218	1:160
Male and female worms, *S. douthitti*†	3 ml of 1:100 by weight	1:40
Female worms, *S. mansoni**	345	1:160
Immature female worms, *S. mansoni**	115	0
Male worms, *S. mansoni**	530	1:160
Cercariae, *S. mansoni**	12,000, 180,000	1:20, 1:80
Cercariae, *S. mansoni*†	6 mg in saline	1:20, 1:40
Cercariae, *S. mansoni*‡	18,000, 21,000	1:80, 1:160
Miracidia, *S. douthitti*§	1900	0
Spleens of mice infected with many eggs of *S. douthitti*†	14.4 ml 1:100 by weight	1:640

* Saline homogenate. † Lyophylized antigen.
‡ Frozen cercariae in aquarium water. § Living miracidia in aquarium water.

tosomatium douthitti (Table 1), and for the serums of six hosts immunized twice weekly for 3 wk with various doses of frozen cercariae of *S. mansoni* (Table 2). Control serums of all the afore-mentioned hosts were negative by hemagglutination. All serums in Table 1 were tested a minimum of four times, and the titers recorded are averages. Since the number of rabbits immunized with a particular antigen is small, the titer for each animal immunized is listed separately.

One rabbit immunized with frozen cercariae of *S. mansoni* showed a titer of 1:80. This animal was reimmunized 3 mo later, and the titer rose to 1:640. Agglutinins were not detected in rabbits immunized with living miracidia of *S. douthitti* and with immature female worms of *S. mansoni*. Agglutinins were not detected in the serum of a rabbit immunized with an antigen prepared from a frog lung trematode (*Haematoloechus* sp.). The titer of 1:640 obtained after immunization with a lyophylized mouse spleen-egg antigen was unexpected, since this serum showed no *in vitro* activity against living cercariae and very little activity in immobilizing miracidia (*8*).

Although saline extracts of frog lung trematodes have been used as skin-testing antigens in schistosomiasis (*9*), antibodies could not be detected by hemagglutination after immunization. The hemagglutination test for this series of serums was more sensitive than the CHR (Cercarienhüllen Reaktion of Vogel and Minning, *10*) in detecting antibody; with one exception (spleen-egg antiserum), they were less sensitive than the miracidium immobilization test (*8*).

In Table 2, the titers of serums for agglutination of living cercariae of *S. mansoni* are compared with hemagglutination titers. *In vitro* agglutination tests with cercariae of *S. mansoni* were made in nine-depression Pyrex spot plates. Into each depression 0.5 ml of each serum dilution, plus 1 drop of Penicillin g (4000 units/ml), 1 drop of Streptomycin sulfate (0.5 mg/ml), and 1 drop of concentrated cercariae were added. Serums were diluted with 10-percent Ringer's solution, and control tests were made simultaneously with the diluent. Spot plates were placed in a moist chamber at room temperature (24° to 26°C), and readings were made the following day. Except for the chicken and pig, hemagglutination titers were much more sensitive.

As is noted earlier, the inactivated serums of several normal mammals agglutinated living schistosome cercariae. These serums were not titrated at this time. Approximately 1 year later, after these serums had been thawed and frozen many times, the normal and immune serums of the horse, cow, goat, and pig were retested. Agglutinins in normal serums were observed only in the horse, at a titer of 1:8, and in all immune serums at the same or higher titer (Table 2). The agglutination of cercariae in normal serum is probably caused by a nonspecific agglutinin, since all normal serums were negative by hemagglutination.

The hemagglutination titer obtained from day to day for a single serum may vary more than twofold. Fifteen hemagglutination tests of a single immune horse serum were made over a period of several months. Except for the first two tests where the titer dropped from 1:12560 to 1:320, the titers of 12 other tests fluctuated between 1:640 and 1:2560 (three tubes). The average titer for all tests was 1:1280. This titer was obtained in six of the 15 tests. Experiments with cells of different sheep, batches of normal rabbit serum, and lots of tannic acid by various manufacturers indicated that these variables were not responsible for fluctuation in the titer. The sensitivity of the test may vary with the solubility of the cercarial antigen used to coat the sheep red cells, since several batches of cercarial antigen were used. Work is in progress to obtain a soluble cercarial fraction that may be superior to the present antigen, and preliminary hemagglutination studies with human serums are being conducted.

Table 2. Comparison of agglutination and hemagglutination titers in animals immunized with cercariae of *S. mansoni*.

Host	No. of cercariae injected	Agglutination titer	Hemagglutination titer
Chicken	6,000	1:64	1:80
Cow	40,500	1:256	1:1280
Goat	26,000	1:64	1:5120
Horse	40,500	1:32	1:1280
Pig	25,000	1:128	1:320
Rabbit	18,000	1:32	1:160

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Effect of DDT on Enzymatic Oxidation and Phosphorylation

Despite numerous attempts to elucidate the mode of action of DDT [2,2-bis(*p*-chlorophenyl)-1,1,1-trichloroethane] on adult houseflies, it must be recognized that this phenomenon has not yet been explained. Sacktor (*1*) reported that DDT inhibited cytochrome oxidase of houseflies, and suggested (*2*) that one of the explanations for the resistance of adult flies to the insecticide might be the greater oxidase activity developed in resistant strains. Recently Morrison and Brown (*3*) summarized much of the literature on this subject and reported DDT inhibition of cytochrome oxidase from American cockroaches. In the course of our search for DDT-sensitive reactions of adult houseflies we have discovered an effect of DDT that appears to be different from any reported previously (*4*). This effect consists of DDT-inhibition of oxidation of citric acid cycle intermediates, and of oxidative phosphorylation, when oxidation and phos-