Table 1. The effect of horizontal rotation at 0.25 rev/min and dark periods of different lengths on the flowering responses of *Xanthium pennsylvanicum*. All plants subjected to dark periods of 7.75, 8.25, or 8.75 hours were vegetative (stage 0) and are not shown.

Dark period (hr)	Flowering stage			
Rotated plants				
9.25	0	0	0	0
9.75	0	0	0	0
10.25	0	1	4	4
Stationary plants				
9.25	4	4	1	4
9.75	5	6	5	4
10.25	6	5	7	6

es from the rotating axis. The rotational velocity was 0.25 rev/min. The average light intensity at the plant surface was approximately 800 ft-ca, with a variation between 350 and 1000 ft-ca. The timing of the light-dark treatment was controlled by General Electric time clocks. The temperature of the experimental room was controlled by refrigeration. The air was circulated continuously by an electric fan. Temperature in the room ranged from 24° to 27°C during the experiments. The method reported by Lincoln *et al.* (*3*) was used to assess the flowering response.

Forty-eight plants, each having 6 to 8 mature leaves, were selected for uniformity from 200 plants. Each of the 12 treatments was repeated four times, for a total of 24 experimental plants and 24 controls. The control plants

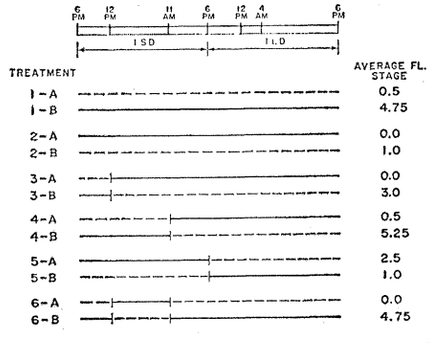

Fig. 1. Effect on the flowering response of *Xanthium pennsylvanicum* of rotation around a horizontal axis at 0.25 rev/min at different periods. Light treatment given during the rotation treatment is shown by open bar (light, 800 ft-ca) and cross-hatched bar (dark). The time is indicated above the bar. Light treatment of 3 long days given before the short-day (SD) treatment is not shown. All the plants were moved to the long-day (LD) greenhouse at the end of the rotation treatment. Dotted lines, plants being rotated; solid lines, stationary upright condition. Flowering response measurement is according to Lincoln *et al.* (*3*).

were placed on a bench near the rotator. At this time, the lights in the experimental room were on. The plants were subjected to treatment as follows: 2 long days and 3 days of variable light and dark followed by 1 long day. They were then taken to the long-day greenhouse and grown there until dissection.

During the 3 days of variable light and dark there were six day-lengths: 7.75, 8.25, 8.75, 9.25, 9.75, and 10.25 hours of dark, respectively. All the dark treatments started at 2:00 A.M. The variable dark periods were provided by removing the plants in total darkness at appropriate times and transferring them to another room having the same temperature as the first. Both the rotated and the control plants were placed upright under lights in this second room. When the last treatment group (10.25 hours of dark) had been transferred to this room, all the plants were watered and then returned to their former positions, either on the rotator or on the bench in the experimental room.

Table 1 shows that flowering was suppressed in plants that were rotated. All plants subjected to a 9.75-hour dark period and rotated were vegetative, while the corresponding stationary controls had an average flowering stage of 5.0. Even with a 10.25-hour dark period, flowering of the rotated plants was much reduced.

To ascertain the effects of rotation before and rotation during the dark period, plants were removed from the rotator at various times during the treatment. At the same time, other plants were placed on the rotator in reciprocal treatments (see Fig. 1). The light treatment consisted of 3 long days, 1 short day, and 1 long day. The inductive period during the short-day treatment was 11 hours. The average flowering response of the stationary control plants was 4.75 (treatment 1-*B*), while that of plants rotated throughout the experimental period was 0.5 (treatment 1-*A*). The results of the various rotation treatments show that floral initiation appears to be most sensitive to rotation prior to the inductive dark period.

It is possible that there is a disturbance in the transport of metabolic products which affects the preinduction phase. The strong epinastic response of leaves seems to indicate a change in the auxin distribution pattern. Brain (*4*) has reported a marked change in the auxin diffusibility of rotated plants. The reduction in flowering by rotation may be due to disturbances of the polar transport, which may lead to an increase of auxin in the leaves. Thurlow (*5*) has shown that spraying indoleacetic acid on *Xanthium* inhibits flowering (*6*).

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21 March 1962

Antigenic Structure of *Plasmodium vinckei*

Abstract. An erythrocyte-free preparation of the erythrocytic stages of *Plasmodium vinckei* was made from infected mouse blood and disintegrated in a Hughes press. Rabbit antisera yielded a series of precipitation arcs against plasmodial extract when examined by immunoelectrophoresis. No arcs developed when uninfected mouse erythrocytes or stromata were tested against the same antiserum.

In contrast to the antigenic analysis of bacteria, which has progressed rapidly both in breadth and in depth in recent years, analysis of protozoan cells is still in its infancy. Alone among the protozoa, the serotypes of *Paramecium* and *Tetrahymena* have been extensively investigated (*1*). Recent discussions of interspecies specificity deal chiefly with free-living and parasitic ciliates (*2*) and with parasitic flagellates (*3*). Among intracellular parasites the antigenic structure of *Eimeria stiedae* has been analyzed (*4*). To our knowledge, the separate antigenic components of intracellular stages of parasitic blood protozoa have not yet been demonstrated.

The present report records the antigenic fractionation of the erythrocytic forms of one of the rodent malarias, *Plasmodium vinckei*, with the aid of immunoelectrophoresis, according to techniques first described by Grabar and Williams (*5*).

We employed a strain of *P. vinckei* 100 percent fatal to white mice (*6*). In

our experiments the parasites invaded more than 60 percent of the red blood cells by day 4 and killed mice by day 7 after inoculation. Blood from 20 mice at peak infection was collected into 3.8-percent sodium citrate by cardiac puncture under ether anesthesia. The pooled cells were washed by centrifugation in about 50 ml of phosphate-buffered saline at *p*H 7.2. The buffy coat of leukocytes was discarded, and the sediment consisted essentially of normal and parasitized erythrocytes.

Erythrocytes were lysed by suspending the cell sediment in 40 volumes of Eastman saponin, 1:10,000 in saline, at 37°C for 15 minutes. After centrifugation at 9000 rev/min for 3 minutes, the parasite sediment was resuspended in fresh saponin, and incubated for an additional 10 minutes. The parasites were then washed four times by centrifugation at the same speed in large volumes of buffered saline. The parasite sediment proved infective to mice. The staining properties of the parasites in Giemsa films were unimpaired, with excellent differentiation of cytoplasm and nuclei. No red cell stromata were microscopically detectable.

When centrifuged at 11,000 rev/min for 5 minutes, the parasites sedimented as a thick, brown paste. This was ground with the aid of a Hughes press (*7*) chilled to −40°C. The product consisted of about 2 ml of muddy, brownish fluid, which, on centrifugation in the cold, yielded a small solid button composed of blackish-brown parasitic pigment—a relatively insoluble (*8*) metabolic by-product of the digestion of hemoglobin by plasmodia, which accumulates in the cytoplasm of the developing parasite—and a clear but opalescent liquid phase wih a light brown tinge. This liquid, which constituted our antigen, contained no microscopically recognizable formed elements. Its protein content, estimated with Folin phenol reagent (*9*), proved to be 5 to 15 mg/ml.

Antisera were produced in rabbits against antigen prepared as described and administered on the day of preparation. On day 1, each rabbit received 0.3 ml of antigen intravenously and 1.0 ml of antigen in complete Freund's adjuvant intramuscularly. On day 14 a booster injection of 0.1 ml of antigen without adjuvant was given into the footpad of each hind leg. On day 34 the rabbit was bled for serum by cardiac puncture, and the serum was stored at −20°C.

Antigenic fractionation was carried out by immunoelectrophoresis on microscope slides. Our instrumentation was similar to that used by Wieme (*10*) in his microelectrophoretic studies. Slides were coated with 1 percent agar in barbiturate buffer at *p*H 8.6 and ionic strength 0.04; and electrophoretic runs were done at 140 volts for 20 minutes. The distance between the well containing the antigenic preparation and the antiserum trough was 2 mm. Antigenic components migrated from the point of zero mobility in both directions to an approximately equal degree (*11*).

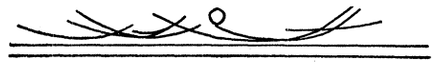

Fig. 1. Tracing of the immunoelectrophoretic pattern of *P. vinckei* antigens exposed to homologous rabbit antibody. The preparation was developed in a moist chamber for 48 hours, then stained with Amido Black. (About × 1.8)

After electrophoresis of the antigenic preparation and introduction of antiserum into the troughs, slides were kept in moist chambers at 25°C for 24 hours. By this time most of the precipitation arcs had developed, but one was delayed until 48 hours. Slides were then washed in buffered saline for 3 days to remove unprecipitated serum proteins, then stained with Amido Black (see Fig. 1).

Controls done with normal rabbit serum were uniformly negative, which indicates that the precipitating factors developed in response to our inoculations. Controls testing the antiplasmodial antiserum against washed mouse red cells or stromata, both ground in the Hughes press, were also negative, demonstrating that our plasmodial preparation was uncontaminated with red cell components capable of producing precipitin in rabbits. We conclude that the arcs developed in response to plasmodial antigen, and that erythrocytic forms of *P. vinckei* therefore contain a series of distinct antigenic factors, each capable of eliciting the production of precipitating antibody in rabbits (*12*).

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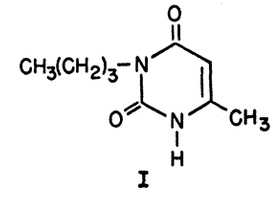
11. We attribute the bidirectional migration to endosmotic flow, since the same material all migrated in the direction of the anode when run through a Tiselius apparatus.
12. This study was supported by grant E-2859 from the National Institute of Allergy and Infectious Diseases, U.S. Public Health Service. It represents part of the Ph.D. thesis of one of us (D.S.) at the Hebrew University. We gratefully acknowledge the devoted help of Miss Miriam Bitton.

11 June 1962

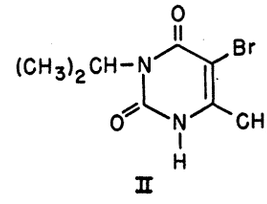
Substituted Uracil Herbicides

Abstract. Explorations in the pyrimidine series have led to the discovery of highly active herbicides, of which 3-butyl-6-methyluracil and 5-bromo-3-isopropyl-6-methyluracil are examples.

We have discovered a group of substituted uracils that are highly phytotoxic to a wide variety of plants. These compounds have low mammalian toxicity, and thus promise to become economically important herbicides. Examples are the new uracils:



I



II

Synthesis of compound I was effected by reacting butyl isocyanate with methyl 3-aminocrotonate (*1*) to form methyl 3-(3-butylureido)crotonate, which was cyclized without isolation by heating in 6-percent sodium hydroxide. The uracil I was precipitated by acidification and recrystallized from heptane/2-propanol. Melting point, 182° to 183°C. Analysis calculated for $C_9H_{14}N_2O_2$: C, 59.32; H, 7.74; N, 15.37. Found: C, 59.34; H, 7.73; N, 15.02. Synthesis of compound II was carried out in the same manner with isopropyl isocyanate. The alkyl uracil was brominated in acetic acid to give compound II, which was recrystal-