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*).

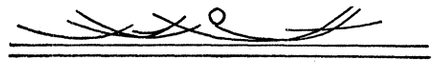

Fig. 1. Tracing of the immunoelectrophoretic pattern of *P. vinckei* antigens exposed to homologous rabbit antibody. The preparation was developed in a moist chamber for 48 hours, then stained with Amido Black. (About × 1.8)

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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References and Notes

1. G. H. Beale and J. F. Wilkinson, *Ann. Rev. Microbiol.* **15**, 263 (1961).
2. S. Krascheninnikow and E. L. Jeska, *Immunology* **4**, 282 (1961).
3. H. Kott and S. Adler, *Trans. Roy. Soc. Trop. Med. Hyg.* **55**, 333 (1961); H. Chun-Hoon and R. Samuels, *J. Protozool.* **7** (suppl.), 6 (1960); S. Adler and J. Adler, *Bull. Res. Council Israel Sect. E* **4**, 396 (1955).
4. M. E. Rose, *Immunology* **2**, 112 (1959).
5. P. Grabar and C. A. Williams, *Biochim. Biophys. Acta* **10**, 193 (1953).
6. S. Adler and A. Foner, *Bull. Res. Council Israel Sect. E* **9**, 1 (1961).
7. D. E. Hughes, *Brit. J. Exptl. Pathol.* **32**, 97 (1951).
8. I. W. Sherman and R. W. Hull, *J. Protozool.* **7**, 409 (1960).
9. O. H. Lowry, J. J. Rosenbrough, A. L. Farr, R. J. Rewdal, *J. Biol. Chem.* **193**, 265 (1951).
10. R. J. Wieme, in *Studies on Agar Gel Electrophoresis* (Arscia, Brussels, 1959), p. 87.
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.

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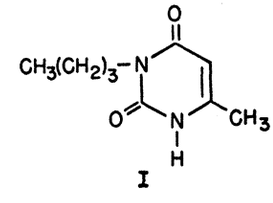
Substituted Uracil Herbicides

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:



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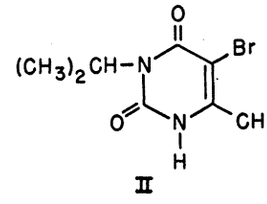

II

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-

Table 1. Phytotoxicity (percentage kill) of uracils applied at 2 lb/acre.

Crab-grass	Sor-ghum	Wild oats	Nut-sedge	Mus-tard	Bean
3-Butyl-6-methyluracil (I)					
100	70	100	0	100	100
5-Bromo-3-isopropyl-6-methyluracil (II)					
100	100	100	100	100	100
5-Bromouracil					
0	0	0	0	0	0
6-Methyluracil					
0	0	0	0	0	0
Uracil					
0	0	0	0	0	0

lized from 20-percent aqueous ethanol. Melting point, 158° to 159°C. Analysis calculated for $C_8H_{11}BrN_2O_2$: C, 38.88; H, 4.49; Br, 32.34. Found: C, 39.02; H, 4.64; Br, 31.96.

These two substituted uracils are unusually phytotoxic to many plants. Some examples are given in Table 1. Compound II is active against a wider range of plants than compound I. Compound I has some interesting selective properties. For example, it has provided kill of many annual weed species without damage to peas and peanuts, even when applied at twice the rate needed to kill the weeds. Compound II has not shown this particular type of selectivity.

Since compound II is effective on a wide range of both annual and perennial weeds, it is particularly attractive as an industrial herbicide where it is desirable to kill all plants. It is especially phytotoxic to perennial grasses which have been previously difficult to control economically.

The approximate lethal dose by oral administration to the male white rat is at least 7500 mg/kg for compound I and 3400 mg/kg for compound II, as determined by the Haskell Laboratory for Toxicology and Industrial Medicine of the Du Pont Company (*2*).

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References and Notes

1. O. Mumm and E. Gottschaldt, *Chem. Ber.* **55**, 2068 (1922).
2. Helpful discussions have been held with Drs. G. D. Hill, R. S. Johnson, B. C. Pratt, and H. S. Rothrock. This report is contribution No. 749 of the Industrial and Biochemicals Department and Central Research Department, E. I. du Pont de Nemours and Company.

26 December 1961

Patterns of Gene Pleiotropy in Morphogenetic Processes

Abstract. Selection for an increase and decrease in the expression of the *ocelli-less* mutant in *Drosophila subobscura*, variable for the number of ocelli and bristles, has revealed the pleiotropic nature of the mutant. In addition to modifying a morphogenetic system responsible for the formation of ocelli and bristles, it also modifies another morphogenetic system determining the positions of these structures, at the extreme range of the mutant expression.

This investigation is an attempt to deduce the nature of the development processes and their genetic control by studying the range of adult variation as the end product of these processes. The pattern chosen for study was that of the macrochaetae and ocelli (Fig. 1, top) on top of the head in *Drosophila*. The number and arrangement of these structures is highly uniform in Drosophilidae, but several mutants are known to alter their number and arrangement.

The sex-linked recessive mutant, *ocelli-less,* in *Drosophila subobscura* removes the head bristles and ocelli. In the foundation population, homozygous for the mutant, the flies had varying combinations of bristles and ocelli. The three pairs of orbital setae and the pair of vertical setae II were not much affected by the mutant. In a few flies the bristles were repeated (two or more bristles lying close together at a site normally occupied by one) unilaterally as well as bilaterally. The ocelli in some of the flies were slightly displaced from their normal positions, and sometimes their sizes were also affected. Since a positive correlation was found between the presence of bristles and of ocelli, the degree of expression of the mutant was measured by giving a unit "score" for the presence of each of the structures studied. Earlier work (*1–3*) had shown that by selective breeding for higher score it was possible to obtain a population which, although carrying the mutant, contained a high proportion of wild-type individuals. Continued selection, after the wild-type phenotype had been reached, increased the frequency of (i) repeated bristles, (ii) repeated ocelli, (iii) neomorphs, a novel pair of bristles which is normally absent in Drosophilidae but is present in a family closely related to it, Aulacigasteridae, (iv) additional central bristles at irregular sites, and (v) of additional ocelli at irregular sites. These repeated and additional structures at both specific and irregular sites were found to be highly correlated with genes for higher score.

In the other direction, selection for lower score eliminated the central structures (three ocelli, ocellar and postvertical setae) after which little progress was made. In the later generations of selection, however, flies showed depressions on the margins of the head, on one side or both sides, which varied in the degree of expression. Such depressions were highly correlated with genes for lower score.

In these experiments (*1–4*), the three pairs of orbital setae were not included in the scoring system, and, in later experiments (*2–4*), the pair of vertical setae II was also not scored, as they were not much affected by the mutant. With the elimination of central structures in the downward selected line, however, a curious phenomenon was observed. In some of the flies in which central structures were absent, additional marginal bristles at irregular

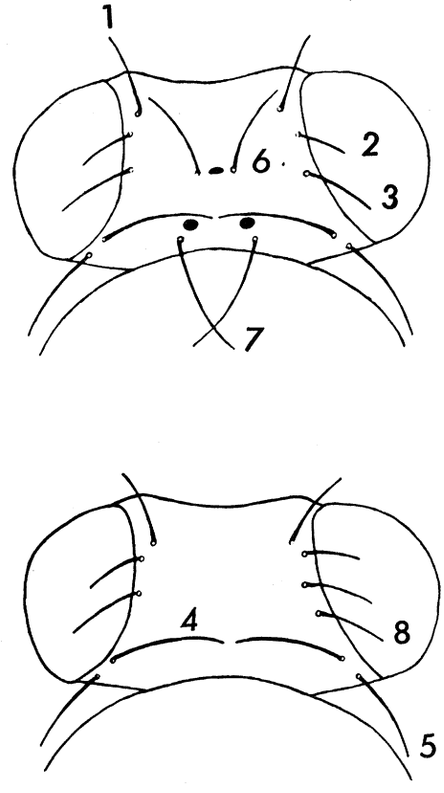

Fig. 1. (Top) The wild-type pattern of macrochaetae and ocelli in *Drosophila subobscura*. (Bottom) The pattern of macrochaetae in the *ocelli-less* population selected for lower number of structures; 1–3, orbital setae; 4, vertical seta I; 5, vertical seta II; 6, ocellar seta; 7, postvertical seta; 8, additional bristle; •, ocelli.