starting the computations, each of the vectors λ_r may be chosen arbitrarily.) In the next stage of the iterations, the λ_x just solved for becomes one of the λ_r's, and another vector becomes the λ_x to be solved for. In the small problems which have been worked thus far, with m no greater than 4, convergence has been reasonably rapid, depending in large measure on the apparent "cleanness" of the factorial structure.

As an illustration of results obtained, Table 1 compares the biquartimin solution with that obtained by Thurstone (*1*, p. 229) by graphical methods for his "box problem." Corresponding transformation vectors from the two solutions are about 3° apart.

The principle utilized by the quartimin criterion could be applied easily to the special case where one requires orthogonality. This has not yet been done; at any rate, it would seem that the criterion of simple structure should alone determine to what extent any given set of data approaches orthogonality. Like other oblique solutions, the biquartimin criterion allows complete freedom in this respect.

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19 July 1957

Chemoprophylaxis with Diazouracil of Poliomyelitis in Mice

One approach to the chemotherapy of virus diseases has been the use of various antimetabolites to interfere with nucleic acid metabolism (*1*). Since the publication of the reports on the action of benzimidazole against poliomyelitis in mice (*2*), investigation of compounds of this nature has been pursued in this laboratory (*3*) as a possible method for chemoprophylaxis. One of the substances tested was diazouracil, which had been found to have some activity against certain viruses (*4*). This report presents evidence about the effectiveness of diazouracil in the prevention of paralytic poliomyelitis in mice.

Table 1. Chemoprophylaxis of poliomyelitis with diazouracil in mice. Mice were inoculated intraperitoneally with an estimated 10 ID_{50} of MEF_1 poliomyelitis virus. Diazouracil treatments (10 mg/kg day, intraperitoneally) were given for 4 days, beginning one day before virus inoculation.

Treated No. surviving/ No. inoculated	Control No. surviving/ No. inoculated	Survival index
	Sample No. 1	
11/19	3/20	5.0
6/20	2/20	2.0
8/20	2/19	1.7
7/19	1/20	1.6
	Sample No. 2	
6/20	0/20	1.7
8/19	5/20	1.9
	Total	
46/117 (39%)	13/119 (11%)	

In these studies, mice of the Webster strain weighing less than 12 g were inoculated intraperitoneally with 0.2 ml of a 10 percent suckling-mouse brain suspension of the MEF_1 strain of type II poliomyelitis virus, approximately 10 ID_{50}. Mice were treated intraperitoneally with diazouracil (*5*) at the rate of 10 mg/kg day for 4 days beginning the day before virus inoculation; however, on the day of virus administration, treatment was given subcutaneously. Control animals were treated similarly with equal volumes of buffered saline. Mice were examined daily for paralysis throughout an observation period of 21 days.

Data from several experiments with diazouracil are presented in Table 1. The results are expressed as the ratio of the number of animals surviving on the 21st day to the number of animals inoculated. A survival index was calculated from the ratio of the harmonic mean of the survival time of the treated group to that of the control group, with a favorable response in terms of prevention or delay indicated by ratios greater than 1 (*6*). In all experiments, diazouracil reduced the incidence or delayed the onset of paralysis in mice inoculated with poliomyelitis virus. Thus, in the first experiment, treatment with diazouracil reduced the incidence of poliomyelitis from 85 percent (three survivors of 20 mice inoculated) to 42 percent (11 of 19 surviving), with harmonic mean survival times of 4.0 and 20.2 days, respectively. When the results of these tests were combined, it was found that only 13 of 119 control animals survived, compared with 46 of 117 treated animals—a difference significant at the 1 percent level (*7*). When treatment with diazouracil was begun on the day of virus inoculation or thereafter, it was less effective. No protection was observed when intraperitoneal treatment with diazouracil was started the second day after virus infection or when diazouracil was given orally at the rate of 100 mg/kg day for 4 days beginning the day before virus inoculation.

In contrast to its action in mice, diazouracil did not protect monkeys. When infected orally with the Mahoney strain of poliomyelitis virus, 6 of 6 monkeys in each of two control groups developed paralysis, as did a group which was treated intraperitoneally with four daily doses of 5 mg of diazouracil per kilogram each, beginning the day before virus inoculation, while in a group treated intravenously with five daily doses of 2.5 mg/kg, the morbidity was 5 of 6.

Although the effectiveness of diazouracil is compatible with the assumption that analogs can be used to interfere with the nucleic acid metabolism involved in virus replication, it remains to be demonstrated that this is the mechanism of the chemoprophylactic action of the compound against poliomyelitis in mice.

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New Low Chromosome Number for Plants

Previously, the lowest chromosome number reported for plants was $X = 3$. This number occurs in *Crepis, Crocus,* and *Ornithogalum* (*1*). In the process of a biosystematic study of the *Blepharodon* section of *Haplopappus* several species have been found to have low chromosome numbers. Of particular interest, however, is *Haplopappus gracilis* (Nutt.) Gray. This is a small annual composite that ranges from southern

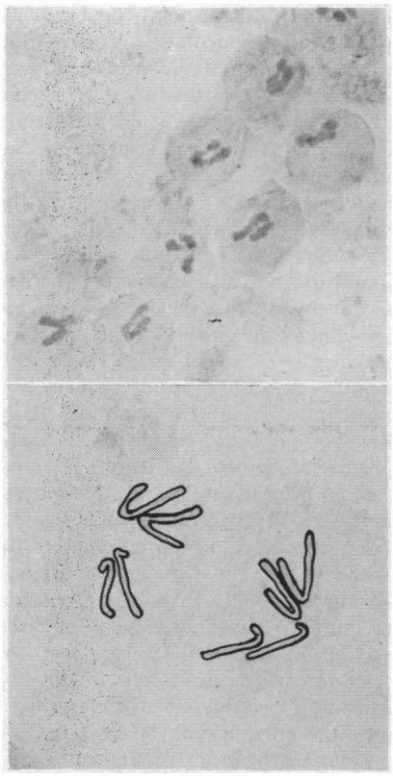

Fig. 1 (Top). Early first anaphase of meiosis showing two pairs of chromosomes. Fig. 2 (Bottom). Late mitotic anaphase showing chromosome number and morphology. The chromosomes have been outlined with India ink.

Colorado and Utah to the state of Durango in Mexico, and west to the eastern border of California (*2*). Seeds of this species were germinated in the greenhouse in March, and the plants flowered in July.

For cytological study, immature heads were fixed for 48 hours in a mixture of two parts of absolute ethyl alcohol to one part of propionic acid. Because of the small size of the disc flowers, the anthers were not dissected out. Instead, entire corollas were macerated and then squashed in iron propionocarmine stain. Usable slides were made permanent by withdrawing the stain from one side of the cover glass with filter paper while a Venetian turpentine and alcohol mixture was introduced from the other side.

In a study of meiosis of two plants of *H. gracilis,* only two pairs of chromosomes were observed at the beginning of the first anaphase (Fig. 1). Other stages of meiosis were studied and found to be normal. The plants produced approximately 100 percent normal pollen.

Several stages of mitosis were observed in the floral tissue. In a mitotic anaphase (Fig. 2) four chromosomes can be seen near each pole. These are morphologically distinct. One pair of homologs has the centromere located in a near-median position with only slightly unequal arms, while the other set has a subterminal centromere with correspondingly unequal arms. For reference in future studies of *H. gracilis,* the chromosomes with the near-median centromere are designated as *A* and those with the subterminal centromere as *B*. The length of chromosome *A* at mitotic anaphase in 12.5 μ, and that of *B* is 7.5 μ.

Apparently the basic chromosome number for the *Blepharodon* section of *Haplopappus* is $X = 2$, as found in *H. gracilis*. This is the lowest chromosome number known in plants at the present time. In related species of *Haplopappus* I have found $n = 4$ in *H. spinulosus* ssp. *typicus* Hall (*3*), $n = 4$ and 6 in *H. spinulosus* ssp. *cotula* (Small) Hall, and $n = 8$ in *H. nuttallii* Torr. and Gray (*4*). It is interesting to note that Hall (*2*) considered *H. gracilis* to be morphologically the most advanced annual member of its section. It thus appears that evolution of this species has occurred on the diploid level.

In addition to being an object of curiosity, *H. gracilis* may prove to be an excellent cytogenetic tool. Seeds of the species germinate easily, and the plants grow well under greenhouse conditions. Preliminary tests in hybridization indicate that the species is self-sterile. The preceding characteristics plus the fact that *H. gracilis* has only two relatively large pairs of chromosomes at meiosis, and thus two linkage groups, should make it a very suitable plant for experimental study.

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20 August 1957

Biological Synthesis of Cellobiose

A cellobiose phosphorylase from *Clostridium thermocellum* acts upon cellobiose to produce equimolecular quantities of glucose and glucose-1-phosphate (*1*). The enzyme can be liberated from bacterial cells that have been grown on either cellulose or cellobiose by grinding them with alumina or glass beads. The reaction catalyzed by this enzyme is reversible and, under conditions similar to those used for the biological synthesis of sucrose (*2*) and maltose (*3*), will yield cellobiose from a mixture of glucose and α-D-glucose-1-phosphate.

In a representative experiment, a mixture containing phosphorylase and the substrates for cellobiose synthesis was prepared. It contained the following: 0.45 ml of maleate buffer at *p*H 7.6; 0.70 ml of enzyme, 17 mg of protein per milliliter; 0.15 ml of 0.5*M* sodium fluoride; 0.15 ml of 0.1*M* magnesium sulfate; 0.15 ml of 0.14*M* sodium thioglycollate; 0.45 ml of 0.1*M* glucose; and 0.45 ml of 0.1*M* glucose-1-phosphate. One milliliter of this mixture was placed in each of two tubes. One tube was immediately immersed in boiling water for a period of 3 minutes to inactivate the enzyme. The second tube was incubated at 37°C for 6 hours, and then the enzyme was inactivated by heating the tube in boiling water. The mixtures were freed of salts by shaking the tubes with small amounts of ion-exchange resins (Chempro C-20 and Duolite A-40), and then chromatographic examinations for sugars were made. The fluids removed from the resins were evaporated to dryness at room temperature in a vacuum desiccator. The sugars in the residue were taken up in 1 ml of 95-percent ethanol at room temperature. The sugars in the alcoholic solution were separated and tentatively identified by paper chromatography by use of S and S 589 green ribbon paper and a descending butanol-pyridine-water solvent (*4*). A control solution containing 0.5 μmole each of glucose and cellobiose was placed on the same paper. The solvent was permitted to drip from the edge of the paper to obtain good separation of glucose and cellobiose. The paper was dried, and the sugar spots were developed by treating the paper with an isopropyl alcohol solution of phthalic acid and aniline (*5*) followed by heating for 10 minutes at 100°C.

Glucose and cellobiose separated well; glucose but no cellobiose was present in the zero-time tube; and both glucose and cellobiose were present after 6 hours' incubation of glucose and glucose-1-phosphate in the presence of cellobiose phosphorylase.

The synthesis of cellobiose was further demonstrated in a similar experiment in which larger quantities of glucose, glucose-1-phosphate, and enzyme were used. Proteins were removed from the reaction mixture by treatment with trichloroacetic acid. The mixture was then made alkaline, and the glucose phosphates were precipitated by the addition of four volumes of cold ethanol. The alcohol was evaporated, and the partially purified sugars were taken up in water. A sufficient quantity (about 10 mg) of cellobiose was obtained to prepare an osazone (*6*). The cellobiosazone was separated