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

by dissolving the sample in hot water and recrystallizing from pyridine. Its melting point was approximately 195°C. It appeared to be identical with known cellobiosazone that was examined at the same time.

This formation of a β-glucoside from an α-glucose derivative confirms the observation of Fitting and Doudoroff (*3*) that the synthesis of a disaccharide by this type of a phosphorylase involves a Walden inversion of the glucose-1-phosphate (*7*).

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12 August 1957

Mechanism of Agglutination of Macaca rhesus Erythrocytes by Human Hepatitis Serum

In an attempt to find a useful laboratory method for the assay of the human hepatitis virus, we have investigated the recently described (*1*) phenomenon of *Macaca rhesus* erythrocyte agglutination. Although this reaction was considered to be the result of a nonspecific antibody combination, we examined the possibility that it might be a direct effect of a virus.

Hepatitis sera were obtained from local hospitals and from the Communicable Disease Center, U.S. Public Health Service, Chamblee, Ga. Many of the diagnoses were proved by autopsy or biopsy, and all had extensive laboratory support. For comparison, serum was obtained from other medical cases, both with and without jaundice, and from normal blood-bank donors of the various major blood groups and Rh types.

Blood from a small group of normal rhesus monkeys was drawn into 4 percent sodium citrate, and the cells were washed with large volumes of 0.9 percent normal saline. Hemagglutination reactions were carried out in twofold serum dilutions in saline; 0.2-ml volumes, measured with calibrated pipettes, were used. Equal volumes (0.2 ml) of the washed 1 percent rhesus cells were added, and the shaken tubes were refrigerated at 4°C for 2 hours. Agglutination was determined by the settling patterns and from the cohesion of the cells when the tubes were gently tilted.

The distribution of hemagglutination titers (reciprocal of the highest dilutions showing clear agglutination) among controls and medical patients, including those with clinical jaundice of nonviral origin, was homogeneous and ranged from less than 4 to 64. Neither blood type nor jaundice caused any detectable variation from the normal range. Saline-typing sera of the major groups and Rh types (anti-C, anti-D and anti-E) failed to produce any agglutination of the monkey cells.

Among the sera from hepatitis cases, both infectious hepatitis (IH) and serum hepatitis (SH) sera gave titers ranging into the thousands, usually 1024 or 2048, and occasionally higher. When serial samples were taken, the SH sera retained the higher titers both initially and throughout the recovery period. Some continued to show high readings even when no further clinical or biochemical evidence of hepatitis could be detected. On the other hand, the titer of the IH cases dropped off at approximately the same rate as the other criteria of the disease.

Of the 51 sera from clinically active cases of viral hepatitis tested, all showed titers exceeding 128. More than half of these were 1024 or over. In 22 sera from convalescents showing no clinical symptoms or biochemical abnormalities, about half were in the normal range, while the others ranged between 128 and 2048. There appeared to be no correlation between any of the clinical or biochemical tests and the level of hemagglutination.

Among convalescent SH cases, two with no detectable agglutination titers were mixed (in equal volumes) with both active IH and SH material and incubated at 37°C. The agglutination titers of all SH sera tested were rapidly and uniformly reduced (Fig. 1); but no decrease of effect was seen with any IH serum. However, none of the sera from convalescent IH cases tested had any effect on either IH or SH, under the above conditions. Commercial gamma globulin, which has a reproducible titer of 32, also failed to reverse the effect of either IH or SH material. Of ten normal sera tested, none had ability to reduce the hemagglutinating activity of any serum studied.

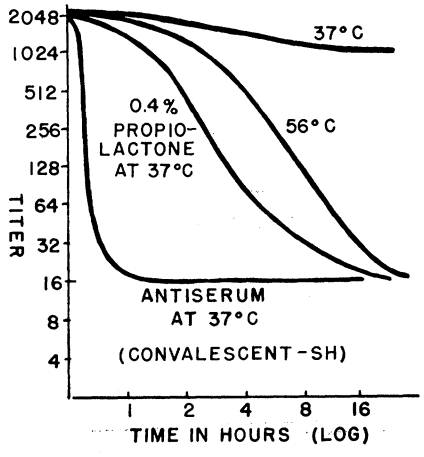

Fig. 1. Effect of various treatments on the agglutination of *Macaca rhesus* erythrocytes by the serum of an acute case of serum hepatitis (SH).

High-titer IH and SH sera were incubated at 37°C and at 56°C for up to 30 hours. Periodically, samples were tested for hemagglutination. Typical results are seen in Fig. 1. The lower temperature had only a slight effect, while the higher temperature effectively reduced the titer. The lower the initial titer, the more rapid was the reduction by heat.

A number of active serum samples were incubated under various conditions with β-propiolactone, a compound known to be strongly viricidal (*2*). The rate of disappearance of the hemagglutination titer depended on the concentration of the chemical and the temperature of incubation. A typical result is shown in Fig. 1. Higher concentrations at elevated temperatures were more rapidly effective. Refrigeration of serum containing 0.4 percent β-propiolactone caused only a slight loss of activity during the test period.

All the mixtures that were studied contained penicillin and streptomycin, and all were tested for sterility at the end of the experiment. No contaminations were observed, nor did the antibiotics alone interfere with the hemagglutination reaction.

In some cases treatment with heat, β-propiolactone, or serum from convalescents reduced the hemagglutination titers of active sera to less than four (the minimum test level). Usually, however, as shown in Fig. 1, there was an irreducible minimum below which the titer would not fall. In control runs, in which the three test treatments were compared in nonhepatitis sera that showed appreciable titers (32 to 64), no diminution of hemagglutination could be achieved. These methods also failed to lower the agglutinating ability of antisera experimentally prepared against the same monkey erythrocytes.

Our data could be explained by the presence of two independent mechanisms for the agglutination of rhesus erythrocytes by human sera. Clear low levels of effect are found in most human serum, regardless of the source and these seem to be of a nonspecific nature. But superimposed upon these levels there exists a