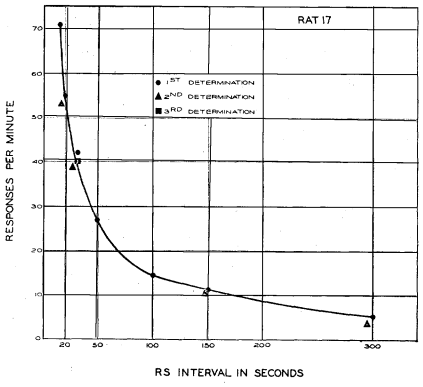

Fig. 1. Rate of avoidance responding as a function of the RS interval.

shock, thus delaying its occurrence. If, for example, each depression of the lever reset a 30-sec timer, a minimum interval of 30 sec was insured between avoidance behavior and shock. Following Sidman's usage, the time interval between a response and shock will be labelled the RS interval and the time interval between successive shocks, when no responding occurs, the SS interval.

The technique permits use of the rate of responding as a continuous and direct indicator of the effects of experimental manipulations. Its usefulness for the study of more complex behavioral phenomena, however, is limited by the fact that at RS > 90 sec the rate of responding drops to a very low level (*2*). In some animals a stable rate of responding cannot be maintained at such low response rates (*3*). At RS = 20 sec no rats were ever observed to emit a sustained rate of more than 20 responses per minute. This is true for any value of the SS interval or the shock level so far employed (*2–4*). The present report demonstrates that a simple modification of Sidman's original procedure can generate extremely high rates of responding. Instead of requiring only a single response on the part of the animal to reset the RS interval, a number of responses had to be made before a new RS interval was started. These responses had to be emitted within the time of the RS interval to avoid shock. The introduction of this additional contingency in the avoidance schedule made the procedure analogous to the "differential reinforcement of high rate" (DRH) schedules described by Ferster and Skinner in the case of positive reinforcement (*5*). In the present case, the lowest reinforced rate was equal to the number requirement divided by the RS interval.

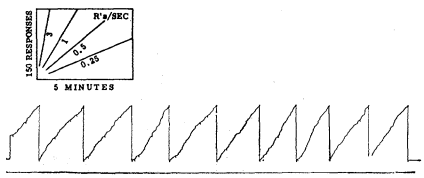

Fig. 2. Portion of the cumulative response record obtained during a session at SS = RS = 30 sec and RR = 8. An oblique pip on the record indicates the occurrence of a shock.

The data reported here were obtained from a single male hooded rat approximately 6 months old at the start of the experiment. The animal was initially conditioned at SS = 3 sec and RS = 30 sec. The shock was provided by a constant-current generator passing half-wave 60-cy current at 1.5 ma. The shock duration was 0.2 sec. The experimental space was 4½ in. wide, 10 in. long, and 7 in. high and was provided with a stainless-steel grid floor. A grid-scrambler, which alternated the polarity of each grid rod, ensured that the animal would receive a pulsating shock regardless of which rod it was standing on. A modified Switchcraft No. 3002 switch was used as a lever (*6*).

After the rate had become reasonably stable, the avoidance schedule was changed to SS = RS = 30 sec and, later, to a requirement of two depressions of the lever to reset the RS timer. During subsequent sessions the animal was gradually shifted to a higher number requirement. Each shock reset the stepper relay which programmed the number of responses required to restart the RS interval. Once the animal gave a stable performance at SS = 30 sec, RS = 30 sec and RR = 8, the RS interval was used as the independent variable of the experiment. The animal was successively run with the following sequence of RS intervals: 30, 300, 30, 150, 100, 50, 30, 20, 15, 150, 20, and 300 sec. Four 6-hr sessions were given at each value. Eight sessions totalling 48 hr were given at the final value of 300 sec. The animal was run every other day.

Figure 1 shows the rate of responding as a function of the RS interval. Each value represents the mean rate of responding during the last 4 hr of the last two successive sessions at each RS value.

As with Sidman's original schedule, where the number requirement was equal to 1, the rate of responding is a logarithmic function of the RS interval (*2*). The present data can best be described by the following equation, which was fitted by the method of least squares

$$y = 157.38 + 0.1588x - 79.84 \log x$$

in which y represents the rate of responding in minutes and x the duration of the RS interval in seconds. A cumulative record of a segment of a daily performance at SS = RS = 30 is shown in Fig. 2. The curve shows that, in spite of the number requirement's being 8, the performance is characterized by a low shock rate when compared with previously obtained data (*2, 4*). It also gives an impression of the stability of the rate of responding during a session. An important feature of the present data is that at RS = 300 sec a substantial rate of 5.6 responses per minute was still maintained. At SS = RS = 30 sec the present schedule generated a rate of 54.9 responses per minute, which is more than twice the highest rate ever observed with the original avoidance schedule. The general features of these data have been confirmed with several other animals with different number requirements.

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15 October 1958

Biochemical Basis of Mating in Yeast

Abstract. One mating type of the yeast *Hansenula wingei* possesses a specific protein on its cell surface which is complementary to a specific polysaccharide on the cell surface of the opposite mating type. The initial phase of mating in which cells of opposite types combine is therefore analogous to a reaction between an antibody and a polysaccharide antigen.

The heterothallic microorganisms probably possess the simplest type of sexual differentiation known. In the haploid stage there are two mating types, identical morphologically and metabolically, but different in genetic composition and in their mating behavior. Mating types in heterothallic organisms are characterized by the fact that cells of one type will mate only with cells of the other type, but not with themselves. In this way they differ from homothallic organisms, in which individuals of the same genetic constitution are able to mate. There are a number of heterothallic species of yeast. These species are especially valuable for studies of the basis of heterothallism, for the haploid cells are the vegetative phase of the organism and are as well the gametes which mate. Two haploid cells of opposite mating type will fuse when they are brought into contact under appropriate conditions, and a diploid cell is formed. No good evidence

has been presented to explain the behavior of mating types on a biochemical basis, although the phenomenon of heterothallism was first discovered in 1904.

Recently Wickerham (*1*) discovered a new species of yeast, *Hansenula wingei,* which is especially suited for studies on the nature of heterothallism. When suspensions of vegetative cells of the two mating types (strains 5 and 21) are brought together under appropriate conditions, a mass agglutination of the cells takes place, indicating a strong attractive force between the two mating types. Once the cells are in intimate contact, cell fusion and diploid formation can promptly proceed. The efficiency of conjugation is probably considerably higher in this yeast than in other species.

Since the agglutination reaction is visible macroscopically, it has been possible to develop an assay for this phenomenon and to study this initial phase of the mating process in a quantitative way (*2*). Previous work has shown that the components responsible for the agglutination are present on isolated cell walls (*3*), indicating the strictly surface location of the mating components.

The mating component of one of the mating types (strain 21) has been shown to be removable by trypsin, and is probably a protein (*3*). The mating component of the other mating type (strain 5) has been shown to be probably *not* a protein, both by its insensitivity to proteolytic enzymes and by its insensitivity to protein extractants such as 80-percent phenol. It was hypothesized that the mating component of strain 5 might be a polysaccharide, so that the agglutination reaction would then be due to a combination between a protein structure in one cell type with a complementary polysaccharide structure in the other cell type. The chemical forces holding the cells together would then be hydrogen bonds, and it has been shown that substances which break hydrogen bonds, such as urea, are able to prevent agglutination or bring about deagglutination (*4*).

It has now been possible to adduce evidence for the necessity of a polysaccharide for agglutination of strain 5, by employing the technique of periodate oxidation first used to demonstrate the carbohydrate nature of the influenza virus receptor of the red blood cell (*5*). Highly agglutinable cells of strains 5 and 21 were treated separately with 0.001*M* sodium periodate for varying periods of time. The cells were then washed and tested for agglutination against untreated cells of the opposite type. Figure 1 (top) shows the results of this experiment. It can be seen that the agglutinability of strain 5 drops rapidly, while that of strain 21 is relatively unaffected.

Since periodate functions by breaking bonds between carbon atoms containing adjacent hydroxyl, or hydroxyl and amino groups (*6*), this means that the mating component of strain 5 probably contains such groups. Hotchkiss (*7*) has tested a large number of biological substances and has shown that polysaccharides are the only common substances sensitive to periodate oxidation which would be present on the yeast surface. Figure 1 (bottom) presents data on trypsin action on strains 5 and 21 and clearly reveals that these two strains show exactly the opposite behavior with trypsin as with periodate. The parts of Fig. 1 together provide good evidence that there are biochemical differences in the cell surfaces of the two mating types.

The hypothesis that mating agglutination is due to configurations of specific macromolecules, polysaccharide and protein, seems to be quite tenable. Such a hypothesis would explain the highly specific nature of the mating agglutination, since diploid hybrids of strains 5 and 21 show no agglutinating characteristics with either haploid strain. Chemical procedures for the extraction of these components have been developed, but as yet it has not been possible to demonstrate the agglutination reaction in extracts, possibly because the amount of material of specific configuration per cell may be very small. The present results reveal for the first time biochemical differences between mating types of a heterothallic organism which seem to explain its mating behavior, and they show that there is a possibility of studying, at the molecular level, one of the results of gene action (*8*).

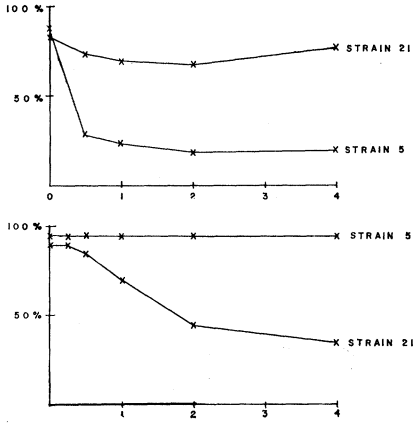

Fig. 1. (Top) Periodate oxidation, 0.001*M* sodium periodate at 37°C for times indicated on the abscissa (hours). (Bottom) Trypsin digestion, 100 μg of trypsin (1:250, Difco) per milliliter in 0.02*M* tris(hydroxymethyl)aminomethane buffer, *p*H 8.0, at 37°C, for times indicated (hours). Agglutinability was tested against untreated cells of opposite type in 1 percent $MgSO_4$, by a quantitative method previously described (*2*). Values are percentage reduction in turbidity of agglutinated over unagglutinated controls; greater reduction in turbidity means stronger agglutination.

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Bound Phosphorus and Growth of Phytoplankton

Abstract. No correlation was found between phytoplankton pulses in four North Carolina ponds and variations in bound phosphorus. It is concluded that the interaction of a complex of chemical and physical factors produces both seasonal fluctuations and sporadic blooms of phytoplankton.

The problem of the causes for the sometimes sudden and enormous increases in phytoplankton populations, as well as the more regular seasonal variations, has intrigued limnologists and phycologists almost since the discovery of plankton. Pearsall (*1*) suggested that phosphorus is a limiting factor. While some laboratory work such as that of Rodhe (*2*) seems to support this theory, field investigations have shown no correlation between variations in dissolved phosphorus and phytoplankton pulses. In a critical examination of this problem, Hutchinson (*3*) concluded that periodicity in phytoplankton is the result of the interaction of a complex of chemical and physical factors. Recently Abbott (*4*) has suggested that phytoplankton derive their phosphorus directly from complex polyphosphates or organic phosphorus compounds in colloidal matter. He found, however, "an apparent high negative correlation between non-phosphate phosphorus and plankton algae counts."

We have attempted to prove whether there is a correlation between variations in numbers of phytoplankton organisms and variations in total phosphorus in four central North Carolina ponds. We chose two ponds in the lower Piedmont having a high colloidal clay content and two ponds in the upper Coastal Plain only 15 to 20 miles away which have a much lower concentration of colloids in the water. The data ob-