

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 0.001M 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

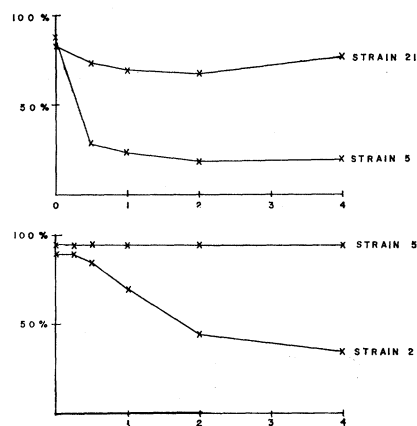


Fig. 1. (Top) Periodate oxidation, 0.001M sodium periodate at 37°C for times indicated on the abscissa (hours). (Bottom) Trypsin digestion, 100 µg of trypsin (1:250, Difco) per milliliter in 0.02M tris(hydroxymethyl)aminomethane buffer, pH 8.0, at 37°C, for times indicated (hours). Agglutinability was 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.

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).

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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-

tained consist chiefly of weekly Sedgwick-Rafter counts of phytoplankton and analyses of total phosphorus from water samples taken the same day. A number of estimates of nannoplankton and analyses of soluble phosphorus were also made during the study, which covered three summer months.

While the phytoplankton populations were relatively low, there was considerable variation in numbers from week to week, and several minor blooms were observed. As was expected, total phosphorus also varied considerably, increasing in all ponds after heavy rains.

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## Artificial Neuron

**Abstract.** An electronic model is described for simulating many of the gross operational functions which are believed to hold for living nerve cells. Synaptic growth is not included. Despite difficulties in drawing very rigorous analogies between the biological cell and its model, a sufficient number of rough similarities exist to make systemic experimentation interesting. Several approaches are mentioned.

Although the complete transmission properties of living nerve cells are not known, many gross behavioral aspects are reasonably well understood. The logical properties of neurons are thought

to be generally similar throughout a wide variety of organisms; major differences between different types of cells seem to involve time and level parameters. (A neuron is defined here to include the cell body plus all of its dendritic and axonal appendages.) Using models to simulate the functions of nervous tissue may be useful in understanding or in predicting neurological behavior. Because of the incomplete state of our knowledge of neurophysiology and neuroanatomy, such simulations can be at best only vague and approximate. Nevertheless, these models may be useful as research tools. Several neuron simulations have been previously specified (1). This report describes an electronic simulation of a neuron and indicates some of the research which is made possible by such modeling.

To put it in the simplest terms, a neuron may be considered to be an electrochemical black box, essentially a binary-output transducer, having two kinds of input and one output. It is binary only in the sense that, for a given internal state and set of input conditions, it either fires (transmits an output signal) or it does not. It is a transducer in the sense that, independent of the nature of the input signal (it may be electrical or chemical, for instance), a unique standardized electrical output is produced if any output occurs at all. The two types of input are excitatory and inhibitory. Because of complex interacting properties internal to the element, it cannot be considered to be a simple binary switch. It is in fact these very properties which give rise to the complicated behavior we wish ultimately to understand.

The gross properties of a neuron, vastly oversimplified, are described below. These are the functions incorporated in the electronic model.

**Input.** (i) Inhibition: A particular input connection to a neuron can, while

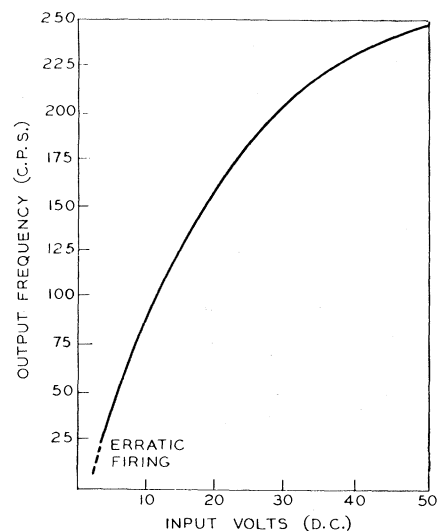


Fig. 2. Typical output firing frequency as a function of input excitatory voltage.

energized, inhibit firing of the neuron by other inputs. (ii) Excitation: Other input connections to a neuron will, if sufficiently energized, always fire the neuron if certain conditions are met. These conditions are described below. (iii) Threshold: A neuron may be fired if the triggering energy supplied to it exceeds a certain threshold value within a time limit. There are input pulses which have insufficient amplitude to cause firing no matter how long they last. This threshold is variable, being a function of the previous history of firing of the neuron. (iv) Refractory period: Immediately after firing, a neuron's threshold rises effectively to infinity and for a period on the order of a few milliseconds, no input signal can fire the neuron again. This absolutely refractory period is followed by a relatively refractory phase. During this second phase a decreasing threshold is observed, approaching the pre-firing threshold and reaching it after a few tens of milliseconds. (v) Summation: Two or more input pulses, each of insufficient energy to excite a neuron, can be integrated by the cell so that firing occurs. To be successful, this summation must occur within a maximum time, typically on the order of a millisecond or so. Since these inputs may arrive via different pathways, there can be both spatial and temporal summation.

**Output.** The output of a neuron is "all-or-none." If firing occurs, then a pulse of standard amplitude and duration is produced. There are exceptions, but as a first approximation we may consider the energy per output pulse to be constant.

A model which realizes these functions can be easily made with electronic circuits. One version is shown in Fig. 1 (2). This four-transistor device exhibits the properties of excitation, inhibition,

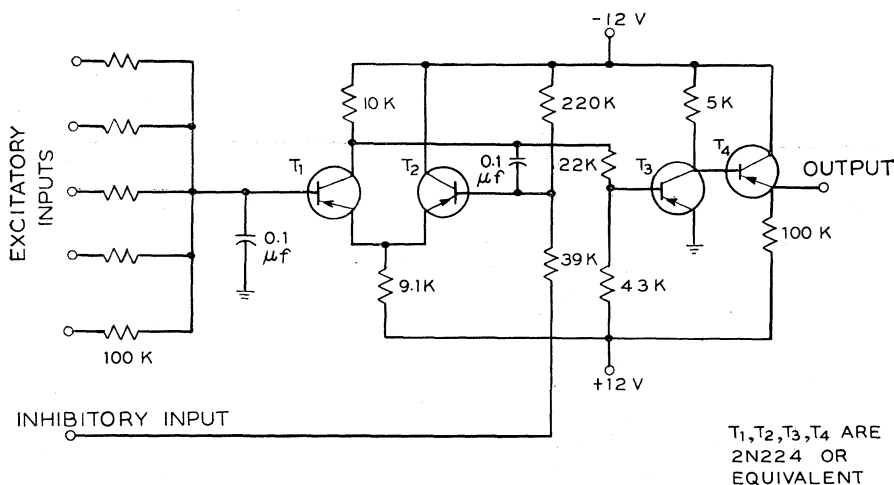


Fig. 1. Transistorized version of artificial neuron having excitation (threshold, summation, refractory) functions, inhibition, and all-or-none output, as described in text.