summation, variable threshold, and all-or-none output, as described.

The model has an integrating time constant of 2 msec and a refractory time constant of about 10 msec, approximating corresponding values in the biological neuron. Quiescent threshold is from 1 to 5 volts (depending on the number of inputs connected), while the output pulse level is 10 volts. These levels are many times greater than those found in nerve tissue (there thresholds are typically 5 to 10 mv; output spike potentials are approximately 50 mv), but the *ratios* between threshold and output levels are commensurate. These ratios in part determine input summation characteristics when several cell outputs combine. The output pulse duration is approximately 4 msec; this is considerably greater than the action spike length found in biological nerve, but it can be shortened at will by use of a suitable differentiating network. The output characteristics are compatible with the input (excitatory and inhibitory) requirements such that a chain or network can be readily assembled. One unit will drive up to 100 others without serious deterioration of output wave form or output level.

This circuit can be used to give either single pulse outputs or variable frequency pulse trains, depending on the nature of the input. A typical direct-current input versus frequency output characteristic is shown in Fig. 2. This mode of operation is useful for simulating peripheral receptors, such as retinal elements, when used in conjunction with suitable transducers.

Photoresistive cells (for example, cadmium selenide) and these neuron models have been used to simulate some of the simple structures and functions of the retina. "On," "off," and "during" receptors are easily produced, as are flicker-fusion phenomena. Mutual inhibition of cells in an array, resulting in spatial differentiating of optical images, is also readily arranged. Similar experiments in audition are contemplated.

The relative simplicity and low unit cost (less than $10) of this model makes feasible network experiments in which large numbers of cells are used. Simple circuit changes to obtain other input and refractory time constants or excitation and inhibition thresholds can be easily made if desired.

L. D. HARMON

Bell Telephone Laboratories, Murray Hill, New Jersey

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Isotope Dilution Method for Assay of Inagglutinable Erythrocytes

Abstract. The number of cells that remain free in the presence of agglutinin is usually much larger than the number of inagglutinable cells. The true inagglutinable proportion can be found by successive agglutinations of a labeled population in the presence of unlabeled carrier cells. By this means it is shown that group A persons possess non-A erythrocytes in proportions of the order of 10^{-3}.

Estimation of small proportions of inagglutinable cells has hitherto presented insurmountable difficulties. Ashby (*1*) found a range of 0.03 to 3.5 percent of free cells in human anti-A or anti-B agglutinations. It was uncertain whether the cells remained free because they were truly inagglutinable or because of some other limitation of the reaction. McKerns and Denstedt (*2*) reasoned that if free cells were inagglutinable they would accumulate with successive additions of fresh cells to the reaction mixture. Since successive additions did not increase the free-cell count, the free cells are mainly agglutinable. In these circumstances the count does not reveal the proportion of inagglutinable cells or even if any such cells are present.

If cells are radioactive, they can be distinguished from unlabeled cells added later. Thus initial cells can be traced through many agglutinations with unlabeled "carrier" cells. This eventually removes the labeled agglutinable cells, and the remaining activity represents inagglutinable cells.

An experiment with known mixtures of agglutinable and inagglutinable cells labeled in vitro with sodium Cr^{51}-chromate (*3*) illustrates the feasibility of the method. About 0.03 ml of labeled O cells and 2 ml of unlabeled AB cells were mixed with 25 ml of saline and 10 ml of lima bean extract (*4*) having an anti-A titer of 1:128. A 1-ml sample was removed for Cr^{51} counting. The remainder was agglutinated at 4°C in a 7- by 9-in. pan. The mixture was transferred to a separatory funnel and allowed to settle 5 minutes, and the agglutinated mass separated from the supernatant. One milliliter of supernatant was removed for counting. The remaining volume was noted and returned to a pan with 3 ml of added agglutinin and 3 ml of 66 percent suspension of unlabeled AB carrier cells. This was repeated six times, then 0.5 ml of labeled AB cells was added with the usual carrier and agglutinin. This reaction mixture was sampled for counting, and the experiment continued through five more stages. The supernatant sample at each stage was immediately centrifuged, and the pellet was washed once with saline and counted in a well-type scintillation counter while it was in the centrifuge tube. Preliminary dilutions of reaction mixture and supernatant samples of stage 7 were required to bring them within counting range. In Fig. 1, corrected sample activity is plotted against stage. The inagglutinable cells remain in the system while agglutinable cells added midway in the experiment are swept out.

The dilution owing to additions of agglutinin and carrier was noted at each stage, and the activity was corrected by the cumulative product of prior individual dilution factors. An individual dilution factor does not include the packed-cell volume of carrier which is, in effect, both added and removed between samplings. One might suppose the proper factor to be the ratio of reaction mixture volumes after and before the fluid additions. Reconstruction experiments corrected in this manner, however, showed a paradoxical increase of inagglutinable cells. If the data for Fig. 1 had been so treated, for example, the proportion of labeled cells would have seemed to double within eight stages. Evidently agglutination does not entrap inagglutinable cells but excludes them locally, thus tending to concentrate such cells in the supernatant. We infer that agglutinated cells possess an extracellular volume inaccessible to free cells. Under our experimental conditions this associated volume was about one-third the total volume of the agglutinated mass, since omission of *all* the 66 percent carrier from the calculations for Fig. 1, rather than just its packed-cell volume, nearly compensated for the concentration effect. This unexpected exclu-

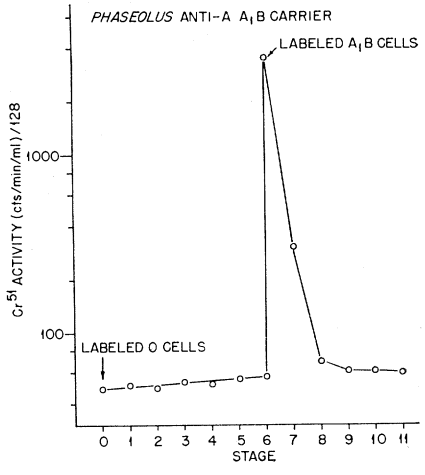

Fig. 1. Reconstruction experiment with inagglutinable O cells and agglutinable AB cells. The point on the left is the initial reaction mixture in which only the O cells are labeled. The following six points are supernatants of successive agglutinations with unlabeled AB carrier cells. The high point is the reaction mixture of stage 7 after the addition of labeled AB cells. The remaining five points are supernatants as before.

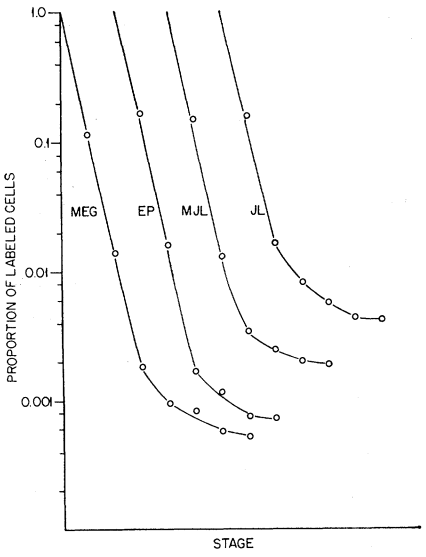

Fig. 2. Determination of the proportion of inagglutinable cells in four normal donors, with respect to lima bean anti-A lectin. The corrected proportion of labeled cells in the supernatant is plotted against the stage. M.E.G. belongs to group A_1B; the others to A_1. The phenotypes of the remaining labeled cells are accordingly B or O (*5*).

sion of free cells is in contrast to the entrapment seen when mixtures are centrifuged (*5*). Clearly, the dilution series is a likely source of error unless it is checked in reconstruction experiments.

To determine the proportion of naturally occurring inagglutinable cells with respect to a given agglutinin, 5 ml of washed cells were labeled and then washed six to eight times in 40-ml aliquots of saline to remove unbound chromate. Labeled cells release small amounts of Cr^{51}, partly by invisible hemolysis and partly by elution with a half-time of 75 days (*6*). Washing was considered sufficient when the supernatant activity was less than 10^{-4} that of an equal volume of cells. The initial reaction mixture (brought to a volume of about 40 ml) contained sufficient agglutinin to give massive agglutination within a few minutes. The components were mixed and sampled quickly before agglutination. The first sample usually required a dilution of 1:1000 for counting, a step facilitated by lysis in water. Inclusion of suspending fluid in the sample introduces no error early in the experiment since the activity in solution at this time is negligible compared with that in cells. Subsequent stages followed the protocol described for the reconstruction experiment (except, of course, that no further labeled cells were added). The total volume decreased 1 to 3 ml per stage but rarely became a limiting factor. Hemolysis during the experiment did not significantly decrease the number of labeled cells but often released enough activity during the first stage to exceed that of the few labeled cells remaining in the last stages. From the third stage on, therefore, the cells must be separated from suspending fluid before samples are counted. Chromium released from cells does not contaminate the carrier. Sedimentable activity other than that in erythrocytes was checked by lysis of pellets with 1 percent acetic acid. As in fresh cells, 80 percent or more of the label was released regardless of the stage tested, indicating that all the sedimentable label is equivalent to erythrocytes.

Progress of the experiment may be visualized in a semilogarithmic plot of stage versus ratio of the corrected activity to initial activity. Figure 2 shows experiments on three AB and one A blood with lima bean anti-A lectin. During the first two or three stages, the proportion of labeled cells removed per stage is remarkably constant; then it decreases as the inagglutinable fraction is approached. After the cells that react with a given antibody have been exhausted, the addition of an antibody of different specificity may result in a further removal of cells (*7*).

Activities of the initial reaction mixtures were in the neighborhood of 2×10^6 count/min ml. These were achieved with Abbot Rachromate containing about 0.03 mg of Cr per milliliter (specific activity about 30 mc/mg of Cr), mixed in 1:5 ratio to cell volume in concentrated saline suspensions and held at room temperature for about 30 minutes. Chromium uptake was less than one-tenth that necessary to increase the mechanical fragility of the erythrocytes (*6*). The inagglutinable cell levels indicated (0.0005 to 0.004) far exceed levels to which the method may be adapted with confidence. With maximum uptake, larger amounts of blood, higher specific activity of Cr, last-stage sampling of all cells, and counter operation at near-background levels, measurement of inagglutinable cells in the proportion of 10^{-6} should be entirely feasible.

K. C. Atwood*
S. L. Scheinberg†
Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee

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* Present address: Department of Obstetrics, University of Chicago, Chicago, Ill.
† Present address: Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Md.

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Carbon Monoxide in Green Plants

Abstract. Green plants grown in a closed, illuminated system liberate small quantities of carbon monoxide. Similarly, finely divided powder and chlorophyll extracts of green plants, when illuminated in an environment of oxygen and water, will yield small quantities of carbon monoxide as well as certain aldehydes. The component of the light spectrum which is absorbed in photosynthesis (480 to 680 mμ) was found to be responsible for the CO and aldehyde phenomena.

The literature contains numerous reports concerning the effects of carbon monoxide on the growth and development of plants subjected to varying concentrations of this gas. It is generally agreed that CO in very high concentrations inhibits growth and development, whereas with respect to low concentrations of this gas, the reports are somewhat controversial.

Concerning the natural occurrence of carbon monoxide in green plants, very little work has been reported. In a short report Langdon (*1*) observed CO in the floater of Pacific Coast kelp, *Heterocystis lulkeanea,* and considered it a by-product of respiration. He states that no gas was formed when the plant was killed, and that none was formed when the plant was macerated and allowed to undergo autolysis or decomposition. Rigg (*2*) repeated Langdon's observation on the bladder of sea kelp and also interpreted the CO as a by-product of respiration. Metz and Sjöstrand (*3*) and Sjöstrand (*4*) have reported the endogenous formation of CO in mammals and have attributed the presence of the gas to the oxidation of some methane group in the hemoglobin molecule by the addition of ascorbic acid, with formation of choleglobin and release of CO.

This report concerns the observation and measurement of carbon monoxide in a number of green plants and the possible significance of its presence. The investigation stemmed from observation of unusually high carbon monoxide content in a specimen of human muscle tissue into which a considerable quantity of green vegetation had been impacted during an aircraft accident.

A method for CO determination by means of carbon monoxide sensitive indicator tubes has been developed (*5, 6*) which can be used to determine CO concentrations as low as 2.5 ppm. This sensitivity was also obtained with the Liston-Becker model 15-A infrared CO analyzer. The method is based on the homogenization of a few grams of tissue in a gastight, nitrogen-filled homogenizer in the presence of 30 percent potassium ferricyanide $[K_3Fe(CN)_6]$, sodium acetate (*p*H 6), and caprylic alcohol. This is a standard procedure for releasing carbon monoxide from blood. Aliquots of the gas from the homogenizer were removed