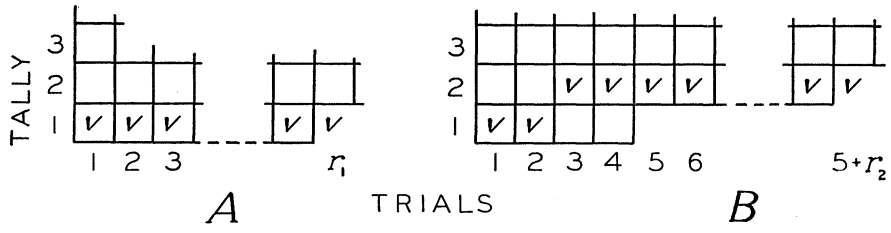

Fig. 1. Graphic representation of the results of sampling: (A) only one individual observed during sampling; (B) two individuals observed, the second occurring for the first time on the third trial.

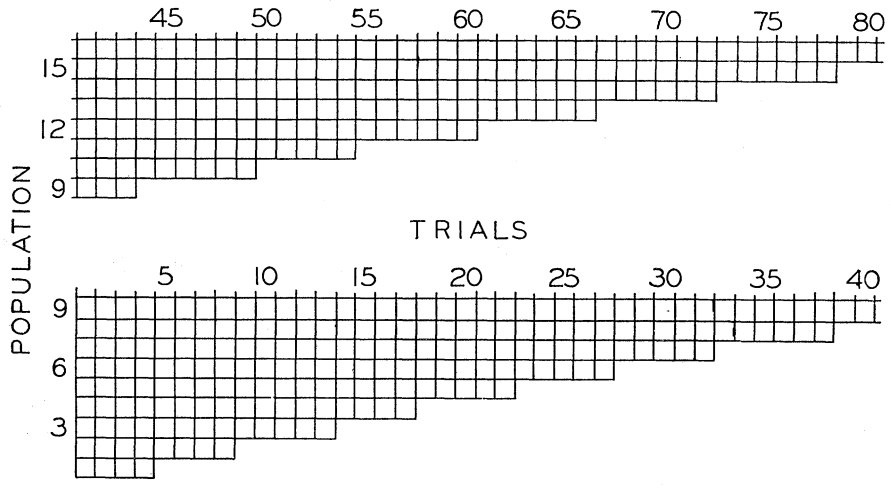

Fig. 2. Region that does not warrant acceptance of hypotheses regarding size of the population at 90-percent level of confidence. Any entry beyond the right boundary warrants such acceptance, with the estimate given by the ordinate at which the entry is made.

fore one may state with a 90-percent or higher degree of confidence that the population consists of a single member.

Figure 1B illustrates another hypothetical case in which unmarked individuals are observed on two of the first five drawings. In the diagram, observation of the second unmarked individual is located at the third trial, although, of course, the event may occur on any one of the four trials following the first. Should every individual drawn subsequently be found to have been marked, the observer is again confronted with the problem of ascertaining the minimal number of additional drawings that would permit him to state with 90-percent confidence that the population consists of only two members.

Assuming, for the moment, that the process is terminated after r_2 additional drawings, one considers the probability of type-II error in the rejection of an alternative hypothesis that the population is of size three. The error may be committed in either of the following ways: (i) by accepting, after five drawings, the hypothesis that the population is of size one, or (ii) by accepting after drawings of $5 + r_2$ the hypothesis that the population is of size two. Calculation of the sum of probabilities of the two events may be facilitated by the Markov process, after the manner suggested by Feller (*2*):

$$ {}_Np_{ab}{}^{(r)} = \binom{N-a}{N-b} \sum_{\nu=0}^{b-a} (-1)^{b-a-\nu} \binom{b-a}{\nu} \left(\frac{a+\nu}{N}\right)^r \quad (1) $$

The symbol on the left reads as the probability of a change in state from a to b in r trials, given N states. In application to the present problem, it is the probability that a population of size N, a of whom have been tallied and marked, will have a total of b different members tallied and marked after r additional drawings. Interpreted graphically, it is the probability of transition from (x, a) to $(x+r, b)$.

The sum of the two probabilities is now written in this notation and equated to 0.10, thus,

$$ {}^3p_{01}{}^{(5)} + {}^3p_{02}{}^{(5)} \cdot {}^3p_{22}{}^{(r_2)} = 0.10 \quad (2) $$

The only unknown here is r_2, and solution of the equation yields $r_2 = 3.5$. This implies that the observer must have at least four consecutive drawings of marked individuals beyond the fifth observation if he is to state with 90-percent confidence that the population is of size two.

By following the procedure illustrated in these two examples one may establish terminal points for higher values on the vertical axis. The calculations become somewhat complex, but the basic approach remains the same. Figure 2 shows the terminal boundary that I computed for 90-percent confidence which is adequate for a population not exceeding 15 members. Current work includes extension of the boundary for higher values of the population and construction of boundaries for other levels of confidence.

Problems in research to which the criterion is applicable arise frequently, one of the most pertinent being the enumeration of wild-life specimens in a circumscribed area. Another example is one in which size of the population has no importance of itself, except insofar as the experimenter wishes to test every available member in order to increase the statistical significance of his results. The method is also being examined for potentialities of application to research in psychology. One promising possibility is that of a criterion of mastery in conditioning, based on my premise that a conditioned response depends on recurrence of one of a finite number of specific vigilance reactions (*3*).

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References and Notes

1. Since the power of the test increases for alternative hypotheses of populations exceeding two members, such hypotheses are rejected without violating the criterion selected on the basis of the alternative of only two members.
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13 June 1956

Enzymatic Reduction of Disulfide Bonds in Cell Wall Protein of Baker's Yeast

The presence of a pseudokeratin-type protein, which contains 2.1 percent sulfur, has been demonstrated in isolated, clean cell walls of baker's yeast (*1*). The protein was solubilized and found to be firmly attached to a mannan component of the cell wall. Physical studies on this mannan-protein, which constitutes a major structural component of the yeast cell wall, indicate that it is monodisperse. It can now be reported that enzymatic reduction of disulfide linkages in the protein has been achieved by the use of cell-free particulate preparations from baker's yeast (*2*).

Portions of pound cakes of baker's yeast (Anheuser Busch, *3*), suspended in 8.5-percent (weight per volume) sucrose solution containing 5 percent (volume

Table 1. Enzymatic reduction of disulfide linkages in cell-wall protein of baker's yeast.

Reaction system*	Mercaptide formation† (Optical density at 255 mμ)	
Cell-wall protein, oxidized	0.137	0.656
Mitochondrial particulates	0.519	
Cell-wall protein + particulates	0.780	
Cell-wall protein, oxidized	0.137	0.381
Heated mitochondrial particulates	0.244	
Cell-wall protein + heated particulates	0.379	

* The components indicated were added to the following basal mixture and incubated at 37°C for 2 hr: sodium succinate, 10 mg; ethanol, 4.5 mg; liver coenzyme concentrate (Armour) 0.5 mg; and 0.02*M* phosphate buffer, *p*H 7.0. The reaction volume was 3.8 ml. Where indicated, 625 μg of cell-wall protein and 0.5 ml of mitochondrial particulate suspension (in 8.5-percent sucrose) were added.

† For determination of the sulfhydryl content of protein, the mixtures were centrifuged at 22,000*g* for 20 min to remove particulate matter, and 2.0-ml samples of the clear supernatant were added to 1.0 ml of 0.3*M* acetate buffer of *p*H 4.6, and 0.5 ml of $1.17 \times 10^{-4}M$ *p*-chloromercuribenzoate (assayed spectrophotometrically at 234 mμ in 0.1*M* acetate buffer of *p*H 4.6, according to the method of Boyer, *5*). Mercaptide formation was allowed to proceed for 2 hr at 37°C and then was determined at 255 mμ.

per volume) of redistilled thiodiglycol (2,2′-thiodiethanol), were broken by agitation with glass beads in a Waring Blendor according to the technique of Lamanna and Mallette (*4*). The particulate fraction was separated from cell-wall fragments and other debris by low-speed centrifugation, followed by repeated washing in 8.5-percent sucrose solution (without thiodiglycol) and centrifugation at 14,000*g*. The particulate fraction obtained was determined microscopically to be free of intact cells and of cell-wall fragments. These mitochondrial particulates were incubated together with a coenzyme concentrate, with succinate and ethanol as hydrogen donors, and with the mannan-protein (oxidized) isolated from clean cell-wall fragments (*1*) as a hydrogen acceptor. Sulfhydryl groups of the mannan-protein were oxidized with 0.001*M* ferricyanide as in the method of Anson (*5*).

After incubation for 2 hours at 37°C, the particulate matter was removed by high-speed centrifugation. The sulfhydryl content of the soluble cell-wall protein in the supernatant fraction was measured by spectrophotometric determination of mercaptide formation with *p*-chloromercuribenzoate according to the method of Boyer (*6*)

As is shown in Table 1, the complete system, containing oxidized cell-wall protein (equivalent to about $10.8 \times 10^{-5}M$ sulfur), mitochondrial particulates, and $11.7 \times 10^{-5}M$ *p*-mercuribenzoate, showed mercaptide formation equivalent to $2 \times 10^{-5}M$ *p*-mercuribenzoate after a reaction time of 2 hours. The same system with heated particulates had no greater absorbancy than the sum of its components. This analysis constitutes definitive evidence for the formation of sulfhydryl groups in the cell-wall protein on incubation with an active enzyme preparation. Disulfide reductase systems have been described that operate on oxidized glutathione (*7*) and on cystine (*8*), but this is the first time that enzymatic reduction of disulfide linkages of a protein has been demonstrated.

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2. Work supported in part by a grant (E-251) from the National Institutes of Health, U.S. Public Health Service.
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19 June 1956

Blockade of Cardiac Synapses by Succinylcholine

The pharmacology of succinylcholine has been intensively studied (*1, 2*), particularly since the cholinomimetic drug is widely used as a relaxant in surgery. Nevertheless, its action on cardiac synapses, which is the subject of the present report, has not been hitherto described. This action is the blockade of the cardiac effects normally produced in the cat by stimulating the preganglionic vagus or the postganglionic inferior cardiac nerve. These findings have theoretical importance (*3*) and may possibly have some clinical bearing as well. The present account is limited to the manifestation of total blockade of the effects caused in the heart by maximal stimulation of the nerves (*4, 5*).

Eight cats anesthetized with Nembutal (35 mg/kg) and five spinal preparations unanesthetized after transection of the cord at C_1 under ether were used for this series of experiments. Artificial ventilation was instituted as needed after the transection in the spinal preparations and as respiratory paralysis developed in the Nembutalized cats. Both vagi were cut to prevent cardiac reflexes, and their peripheral segments were placed on a pair of stimulating electrodes. The inferior cardiac nerve was cleared of connective tissue close to its origin in the left stellate ganglion and also placed on stimulating electrodes. The stimuli were square pulses, supramaximal in the present experiments and usually 0.5 msec in duration. They were repetitive at 20 or 30 per second, but in some experiments frequencies of stimulation as low as 10 per second were also used. The actions of the nerves on the heart were recorded as the electrocardiogram in one or several lead combinations on a standard multichannel inkwriter (Grass model III). The electrocardiographic effects of stimulating the inferior cardiac nerve varied in different preparations, the extremes being shown in Figs. 1*A* and 2*A*.

Intravenous injections of 2 to 3 mg/kg

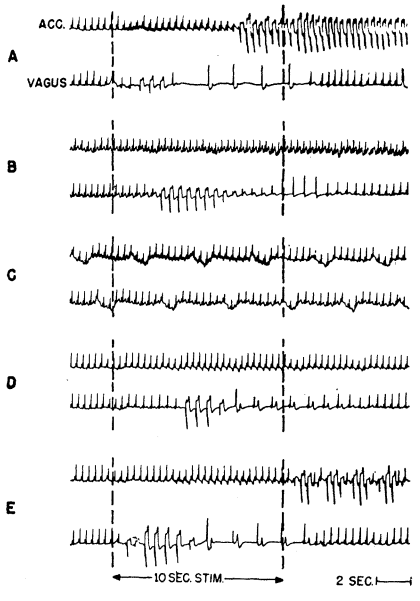

Fig. 1. Reversible cardiac synaptic blockade induced by succinylcholine in anesthetized cat. Upper trace of each set shows effects of stimulating inferior cardia nerve; lower shows results of stimulating the vagus nerve. (*A*) Prior to intravenous injection of succinylcholine; (*B*) 5 min after 8 mg/kg succinylcholine, sympathetic blockade was established, but not vagal; (*C*) 5 min after injecting 15 mg/kg additional, vagal blockade almost complete; (*D*) 30 min later, stimulation of vagus nerve again produced marked effects; (*E*) 1 hour later than *D*; blockade was almost completely reversed.