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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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

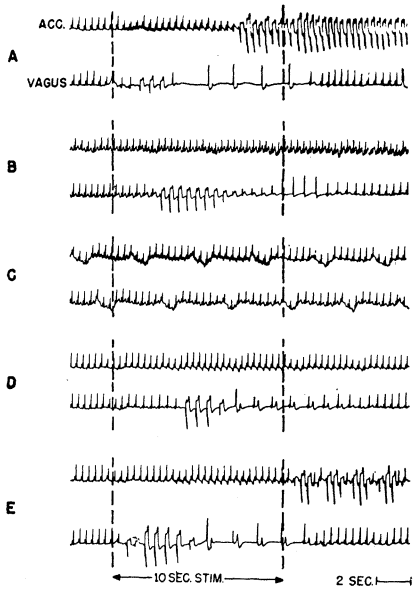

Fig. 1. Reversible cardiac synaptic blockade induced by succinylcholine in anesthetized cat. Upper trace of each set shows effects of stimulating inferior cardia nerve; lower shows results of stimulating the vagus nerve. (*A*) Prior to intravenous injection of succinylcholine; (*B*) 5 min after 8 mg/kg 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.

of succinylcholine caused marked changes in the neural effects on the heart, largest when the stimuli were submaximal, but also evident with maximal stimulation (Fig. 2*B*). However, total blockade of the effects of maximal neural stimuli required larger doses, ranging from 8 to 50 mg/kg (Figs. 1*C*, 2*C*). This total blockade was reversible (Fig. 1*D*, *E*). In all the Nembutalized preparations, sympathetic effects were eliminated before vagal blockade developed fully (Fig. 1*B*, *C*). In all five experiments with spinal, unanesthetized animals, on the other hand, vagal blockade developed somewhat earlier than did the sympathetic (Fig. 2*B*, *C*). There was also an increase in the basal cardiac rate with successive injections of the drug into the spinal animals (Fig. 2), whereas no marked changes of this occurred in the Nembutalized animals (Fig. 1).

Because of the different experimental conditions obtaining in the different tissues, the relative dosages of succinylcholine required for neuromuscular and cardiac blockade cannot be directly compared on the basis of the available data. In the cat, neuromuscular transmission is blocked for about 10 min by 0.25 mg/kg of the drug (*2*). On the other hand, 50 mg/kg is required to cause paralysis lasting 1 hr in the rabbit (*1*). As may be seen in Fig. 1*D*, *E*, 23 mg/kg of succinylcholine caused vagal blockade lasting ½ hr and sympathetic for 1½ hr. The cardiac actions of the drug described here therefore appear to be within the range of its pharmacological effects on other tissues.

Succinylcholine is a cholinomimetic agent that acts like acetylcholine or decamethonium (*6*) to depolarize muscle endplates prior to blocking neuromuscular transmission. Its blockade of vagal cardiac effects resembles the cardiac action of another "depolarizing" drug, nicotine, when the latter is applied slowly and in low concentration (*7*). In neither case is a slowing of the heart observed such as occurs with injections of acetylcholine or larger concentrations of nicotine. The vagal blockade caused by succinylcholine is therefore ascribable to depolarizing blockade of transmission from the preganglionic vagus to the intracardiac postganglionic parasympathetic fibers. Succinylcholine appears to lack the direct, "muscarinic" action of acetylcholine upon the cholinergic myocardial effector junctions, since large quantities of succinylcholine do not decrease, but rather increase, the rate.

Blockade of the cardiac effects of stimulating the postganglionic sympathetic inferior cardiac nerve can be instituted only at the appropriate myocardial effector junctions. Therefore, although the latter are predominantly adrenergic, the generally cholinomimetic agent succinylcholine also acts on these. Since the excitatory cardiac junctions probably develop depolarizing postjunctional (or postsynaptic) potentials, succinylcholine blockade of myocardial effects might come about through a depolarization of these junctions such as succinylcholine also produces at the cholinergic neuromuscular synapses (*8*).

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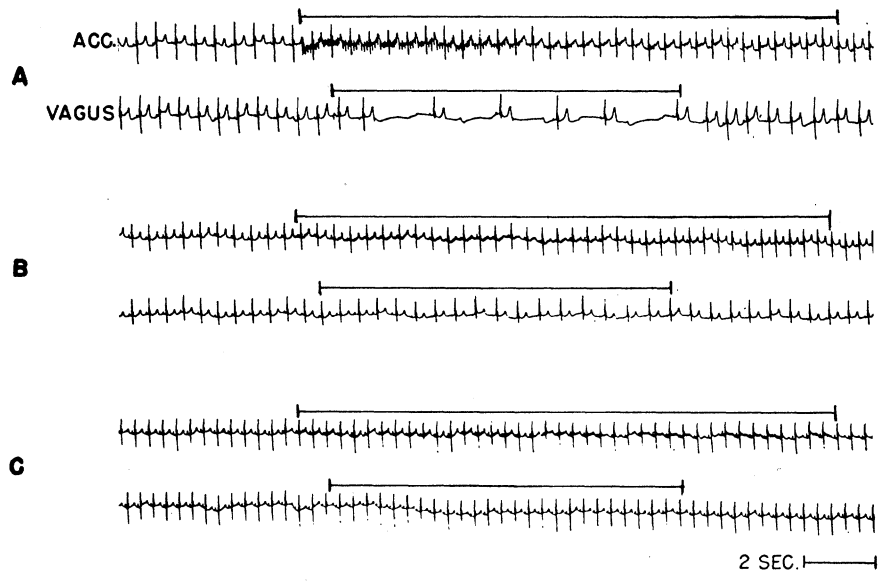

Fig. 2. Vagal blockade developing earlier than that of sympathetic stimulation in a spinal cat. Sequence as in Fig. 1. Bars indicate periods of stimulation. (*A*) Stimulating cardiac nerve produced a relatively small effect, an increase of 20 percent in rate; (*B*) 2 min after injection of 3 mg/kg succinylcholine; basal rate increased about 10 percent, but sympathetic stimulation again increased this by 20 percent; vagal stimulation became much less effective than before; (*C*) 2 min after complete blockade was instituted by an additional injection (15 mg/kg); basal cardiac rate, now 40 percent higher than originally, is not affected by stimulating either nerve.

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Biological Decontamination of Fission Products

It is well known that phyto- and zooplankton are contaminated by radioactivity in fairly high concentrations. L. A. Krumholz (*1*) reported that *Volvox*, *Pandoria*, and *Euglena* acquired radioactivity that was 100 times greater than that of water containing fission products at White Oak Creek, Tenn. According to the report (*2*) of the research vessel *Shunkotsu Maru*, around Bikini Atoll in 1954, the extent of radioactivity detected in such zooplankton as copepods was 1000 times greater than that of the sea water that they inhabited. Although the mechanism of the accumulation of radioisotopes in plankton and the action of the radioisotopes in the organism are still vague, it is certain that plankton selectively accumulate specific radioactive elements from the water into their bodies. For example, Boss (*3*) announced that most phytoplankton had high selectivity of Y^{90} and *Carteria* of only $Sr^{89,90}$ from a culture medium containing $Sr^{89,90}$ and its daughter-product Y^{90}.

It has been reported (*4*) that *Aphanocapsa koordersii*, abundant in brackish lake water, are consumed by *Brachionus plicatlis* during the latter's breeding season, and the latter begin to perish with the decrease in the abundance of the former