no greater than 0.1 responses/min appearing between the average rate for each session.

The behavior generated by this procedure can be explained by a model which holds that avoidance responding increases in rate at the expense of other behavior that is depressed by shock. An equivalent statement, in reinforcement terms, is that the avoidance response is strengthened when it terminates incompatible behavior that has been paired with shock (*3, 4*). Several lines of evidence indicate that the avoidance rate is not simply some form of temporal conditioning in which the responses are triggered-off by the passage of a time interval. Once the response has developed, each occurrence automatically varies the interval between shocks. Furthermore, the relatively stable rates that finally emerge are considerably higher than are required by the shock schedule. If a temporal discrimination were to develop, it should, according to previous findings with the white rat in other situations, produce much more efficient responding than is displayed here (*5, 6*). Mitigating against a time discrimination, once the avoidance behavior appears, is the fact that the animal is provided with no indication that the end of an interval is approaching. While the shock can mark the start of a time interval of, i.e., 20 sec, once a response occurs this interval increases. It is, in fact, possible to vary the delay produced by each response, so that the amount of increase is completely unpredictable, even if the organism were to be provided with an internal resetting timer.

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An Apparatus for Centrifugation and Washing of Particulate Matter in a Controlled Atmosphere

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In the course of an investigation of the ascorbic acid oxidase of the fungus *Myrothecium verrucaria* it was desired to treat a suspension of spores with isoascorbic acid in the absence of oxygen and subsequently to wash the spores while still excluding oxygen. The apparatus described herein was devised and employed successfully for this purpose. A device of this type could find more widespread application and no comparable device has been encountered in the literature. The method can be used equally well for any gas mixture, and cells could be grown under pure culture conditions in the unit A-B. The apparatus is shown in Fig. 1. All joints are standard taper 24/40 with the exception of the male joint on B, which is shortened to a length of *ca.* 15 mm so as to clear the centrifuge head. An aliquot of spore suspension is placed in tube A. The adapter B is then seated and the whole freed from oxygen by repeated evacuation and flushing with nitrogen through adapter C. With nitrogen in A, the stopcock on B is closed, adapter C is removed, and 1 ml of isoascorbate added to the well above the stopcock on B. Adapter C is then replaced and the isoascorbate freed of oxygen as above. While the isoascorbate is under vacuum, stopcock B is opened slowly, allowing the nitrogen in tube A to bubble slowly through the liquid to remove final traces of oxygen. Nitrogen is now re-admitted to the system, forcing the isoascorbate into tube A and the stopcock on B is closed. The centrifuge unit A + B is removed, incubated at 30° for 1 hr, placed in a 100-ml Cornell-style metal centrifuge tube, which is then filled with water to cushion the unit, and centrifuged 15 min at about 1000 rpm. Upon removal of the tube from the centrifuge, it is inverted and inserted in the open female joint in D of the washing apparatus D-E, the sedimented material remaining at the top of the tube. The chamber in D is freed

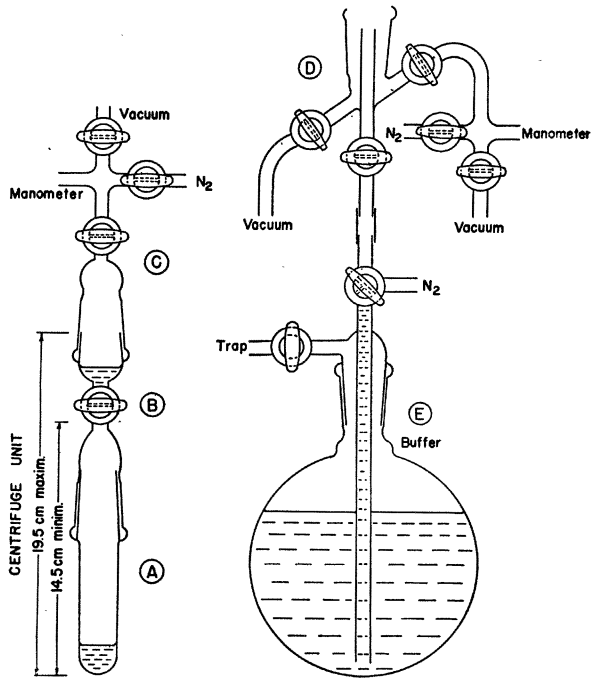

FIG. 1. Apparatus for washing cells in a controlled atmosphere by centrifugation; A–B, Centrifuge unit; C, Adapter for gassing centrifuge unit; D, Adapter for withdrawal of supernatant and admission of buffer; E, Reservoir of oxygen-free buffer.

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of oxygen. The supernatant, now being in B, is drawn off through the vacuum line after opening stopcock B. After flushing B and D with several changes of buffer, buffer is drawn into tube A and B, nitrogen is admitted to equalize the pressure, stopcock C is closed, and the centrifuge unit is removed. After shaking gently to stir up the sediment, the preparation is centrifuged and washed the required number of times as described previously. The centrifuge unit has been spun at 2200 rpm without breaking. Under these conditions the average relative centrifugal force was 900, and the maximum at the bottom of the tube was about 1500 times gravity.

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The Inhibitory Action of Hydroxylamine on Biological Oxidations

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Hydroxylamine is used as a reagent for acylphosphates (*1*) and acylmercaptans (*2*) and, in the concentration of 0.02 *M*, as a detecting agent for these compounds in enzymatic systems (*3*).

The oxidation of various substrates by rat liver mitochondria, isolated in isotonic sucrose, is strongly inhibited by 0.02 *M* of hydroxylamine.

TABLE 1

INHIBITION OF OXIDATION*

Expt	Substrate	O_2 uptake: Without	O_2 uptake: With NH_2OH	Time min	Inhibition %
1	*l*-Malic	99.5	50.3	30	49.5
2	Fumaric	31.5	12.6	15	60.0
3	Succinic	62.5	49.5	20	20.8
4	*l*-Glutamic	54.0	14.2	22	73.6
5	Succinic†	107.5	77.1	20	28.7
6	*l*-Glutamic‡	105.0	4.0	22	96.2

* Frozen Warburg vessels contained $MgSO_4$ 0.05 *M*, NaF 0.005 *M*, glycylglycine 0.02 *M*, adenosine-5-phosphate 0.004 *M*, orthophosphate 0.006 *M* pH 7.4, substrate 0.025 *M*, $NH_2 \cdot OH$ 0.02 *M*. Final pH 7.4. At 0 time 0.5 ml of mitochondria suspension in isotonic KCl. Gas phase air, temperature 20–25° C. Results are means of three or more flasks. Different substrates used with different mitochondria preparations.

† Enzyme aged for 15 min at 37° C.

‡ With 2,4-dinitrophenol 2.10^{-4} *M*.

Expts. 5 and 6 show that the inhibition remains even when oxidative phosphorylation is uncoupled by 2,4-dinitrophenol (DNP) or by aging at 37° C.

The inhibition is, at least, partly due to the combination with iron enzymes (*4*). In our conditions 0.02 *M* of hydroxylamine inhibited 100% the oxidation of added reduced cytochrome c, to mitochondria preparations.

Another point of inhibition exists, and it is shown by inhibition of the reduction of methylene blue in Thunberg tubes.

TABLE 2

INHIBITION OF DEHYDROGENASE SYSTEMS*

Substrate	Other conditions	ΔD, 660 mμ	$NH_2 \cdot OH$
l-Malic	—	34	4
Succinic	—	44	5
l-Glutamic	2.10^{-4} *M* DNP	44	2
l-Glutamic	15.10^{-3} *M* arsenate	36	2
l-Glutamic	Previous incubation with 1 mg riboflavin	47	3
Choline chloride†		90	5

* Thunberg tubes contain 2 ml orthophosphate buffer 0.125 *M* pH 7.4; 0.2 ml methylene blue 1/5000; 0.3 ml mitochondria suspension. In the sidearm 0.4 ml substrate 0.4 *M*. After evacuation and tipping, reaction was followed by direct reading at 660 mμ in Klett photocolorimeter.

† K_2HPO_4 0.01 *M* extract of guinea pig liver, instead of mitochondria.

No inhibition was found with crystalline alcohol dehydrogenase from yeast (*5*), nor with crude glutamic dehydrogenase from rat liver (*6*) measured by spectrophotometric determination of DPN reduction.

Diaphorase was prepared by the method of Edelhoch *et al.* (*7*), and its activity was measured in Thunberg tubes with methylene blue, diphosphopyridine nucleotide (DPN), and alcohol dehydrogenase. 2,6-Dichlorophenolindophenol cannot be used because it is reduced by hydroxylamine. Neotetrazolium confirms the results obtained by methylene blue, and it is not directly reduced by dehydrogenase-DPN, in absence of diaphorase. Hydroxylamine, in the same concentration, inhibited 90–100% diaphorase preparation before the alcohol step, but not after. The inhibition can be restored by the addition of alcohol insoluble protein. Trichloroacetic filtrate or boiled extract does not have this activity.

TABLE 3

INHIBITION OF DIAPHORASE*

Enzyme	Add	Direct reading 660 mμ: $NH_2 \cdot OH$ 0.02 *M*	Direct reading 660 mμ: —
Crude	—	9	79
Alcohol purified	—	86	85
"	0.2 ml alcohol insol. protein	8	84
"	Same & trichloroacetic filtrate	86	85
"	Same boiled 2 min	89	82

* In Thunberg tubes, 2 ml orthophosphate buffer 0.125 *M* pH 7.4; 0.1 ml alcohol dehydrogenase (100 units); 0.2 ml methylene blue 1/5000 0.1 ml diaphorase. 0.1 ml alcohol in sidearm. After evacuation and tipping reaction was followed by direct reading at 660 mμ in Klett photocolorimeter.

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