

clearly indicating the great importance of the vagus nerves in the control of breathing.

These findings show the dependence of vagal function on inflowing afferent nerve impulses and further strengthen the general principle that breathing may be largely a resultant of numerous and various afferent nerve impulses.

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Reduction of Selenites and Tellurites by the Sulphydryl Group.

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The reducing effect of compounds containing the sulphydryl group has been tested with sodium selenite and with sodium tellurite as indicators. The following test solutions were prepared: Reagent (1), an acid reagent made up of 100 cc. of 2% sodium selenite or sodium tellurite plus 20 cc. concentrated hydrochloric acid; reagent (2), 2% sodium selenite or sodium tellurite solution, which is slightly alkaline; reagent (3), a more alkaline reagent containing 2% sodium selenite or sodium tellurite in 10% sodium carbonate. The compounds studied were the thio compounds, ethyl mercaptan, thioacetic acid, thioglycollic acid, thiocresol, thiourea, glutathione, cysteine hydrochloride, and the sulphur containing compounds not possessing the SH group—cystine, and thiophene.

On reduction sodium selenite yields free selenium in the form of a brick red precipitate or a red to brick red colloidal solution depending upon the intensity of the reaction and the quantity of the free element liberated. Sodium tellurite under similar conditions yields a brownish black precipitate of free tellurium or a black brown colloidal solution of this element. Compounds that reduce in the cold give more profuse reduction under the application of heat. The test is carried out by allowing 3 cc. of the compound in aqueous solution to interact with 3 cc. of the selenite or tellurite reagent. The reduction of sodium selenite or tellurite by the compounds is of special value, since the reaction is irreversible and the reduced selenium or tellurium does not display a tendency to undergo re-oxidation.

All the thio compounds examined with the exception of thiourea have very marked reducing capacity. The limits of sensitivity are given in Table I.

TABLE I.

Compound	Acid Reagent (1)		Alkaline Reagent (2)		Alkaline Reagent (3)	
	Cold	Heat	Cold	Heat	Cold	Heat
Selenite Reagents.						
Ethyl mercaptan	negative	0.010%	0.30%	0.050%	0.30%	0.050%
Thioacetic acid	"	0.005	0.08	0.070	0.08	0.800
Thioglycollic acid	"	0.001	0.10	0.006	0.04	0.003
Thiocresol	"	0.003	0.03	0.005	0.30	0.010
Glutathione	"	0.008	0.10	0.020	0.07	0.010
Cysteine HCl	"	0.020	0.03	0.030	0.05	0.050
Tellurite Reagents.						
Ethyl mercaptan	0.09%	0.030%	0.30%	0.070%	0.30%	0.070%
Thioacetic acid	0.08	0.009	0.10	0.007	0.10	0.008
Thioglycollic acid	negative	0.001	0.07	0.003	0.09	0.003
Thiocresol	0.04	0.004	0.04	0.005	0.03	0.010
Glutathione	negative	0.009	0.25	0.080	0.04	0.040
Cysteine HCl	"	0.090	0.03	0.030	0.04	0.040

The acid selenite reagent is not reduced in the cold by any of the thio compounds. The acid tellurite reagent is reduced in the cold by ethyl mercaptan thioacetic acid and thiocresol, but not by thioglycollic acid, glutathione, and cysteine. On heating, however, all the thio compounds reduce the acid selenite and the acid tellurite reagents. All the thio compounds reduce at room temperature and at the temperature of the water-bath the slightly more alkaline selenite and tellurite reagents.

Reduction in the cold takes place after a slight lapse of time. Two minutes we found the maximum time necessary for a precipitate to form. As the concentration of the thio compound is lowered a reddish colloidal solution forms but the time of its appearance is prolonged. A series of reaction mixtures may be made with different concentrations of thio compound as a classroom demonstration of reaction time.

The thio compounds are the only organic compounds that we have thus far studied with the exception of pyruvic acid, glucoreductone, and ascorbic acid that have such marked reducing properties that they reduce even at room temperature. Pyruvic acid reduces in the cold the 2% sodium selenite or sodium tellurite reagent containing 10% sodium carbonate. On the application of heat pyruvic acid reduces all the other selenite reagents and the acidified tellurite reagent. Pyruvic acid may be differentiated from the thio compounds by failure to reduce in the cold the slightly alkaline or the acidified selenite solution.

Glucoreductone reduces the highly alkaline selenite and tellurite reagents in the cold, but the slightly alkaline and the acidified selenite and tellurite reagents only on heating. Glucoreductone was prepared by heating a 5% solution of glucose in 2% sodium hydroxide and neutralizing the mixture with hydrochloric acid. The term reductones has been given by von Euler and Klusmann¹ to the highly reducing materials obtained when alkaline sugar solutions are heated. Kertesz² has recently advised the use of glucoreductone for the standardization of 2,6-dichlorophenol-indophenol solutions used for the estimation of ascorbic acid.

Ascorbic acid* reduces in the cold only the acidified selenite and tellurite reagents, but the application of heat brings about reduction in the slightly alkaline and in the more highly alkaline selenite and tellurite reagents. Ascorbic acid may be readily differentiated from glucoreductone and from the thio compounds. Ascorbic acid reduces in the cold the acidified selenite reagent, while glucoreductone and the thio compounds do not.

The sulphur-containing compounds—cystine, thiophene, and thiourea do not reduce. Thiourea possesses the sulphydryl group. Old and slightly discolored samples of thiourea possess some reducing power, but not the fresh compound. Cystine can be differentiated from cysteine, since the latter has marked reducing properties while the former has none.

Sodium selenite and sodium tellurite are very sensitive reagents for the detection of thio compounds including cysteine and glutathione. Thio compounds with the exception of thiourea can be differentiated from all other reducing compounds we have thus far studied³ on the basis of the fact that the former react in the cold, that is at room temperature, with all the 3 selenite reagents. On the other hand, the thio compounds may be utilized in the detection of selenium in the form of selenite and of tellurium in the form of tellurite. They may also be employed in quantitative procedures where selenium or tellurium is estimated by weight in the element obtained by precipitation with a reducing agent. Cysteine HCl is especially adapted for this purpose, since it tends to give precipitates rather than colloidal solutions of selenium or tellurium.

¹ von Euler, H., and Klusmann, E., *Ark. Kemi, Mineral. o. Geol.*, 1933, **11B**, Nos. 8, 11, 12.

² Kertesz, Z. I., *J. Biol. Chem.*, 1934, **104**, 483.

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³ Levine, V. E., *Biochem. Bull.*, 1915, **4**, 217; *Science*, 1920, **52**, 391.