



Using peroxide-free 'Analar' ether, thrice distilled in all-glass apparatus, we have obtained from the water-extract of beet seeds an unsaturated yellow oil, which acts as a powerful germination inhibitor when tested on cress (*Lepidium sativum*) and other seeds. The inhibitor seems to exert a specific influence on seedlings, its effect being most marked on the radicle (see table).

Ether extract (p.p.m.)	Hypocotyl fresh wt. per 100 seeds (gm.)	Radicle fresh wt. per 100 seeds (gm.)	Total wt. of seedlings (gm.)	% Germination after 6 days	Ratio radicle/hypocotyl
100	0.396	0.126	0.522	82	0.31
50	0.619	0.170	0.789	90	0.26
10	0.735	0.277	1.012	100	0.37
Control	0.793	0.391	1.184	100	0.49

In attempting to elucidate the physiological action of the inhibitor, respiration studies were carried out on hand-cut sections of sugar beet in a Warburg apparatus. Using fourteen disks of 1 cm. diameter per vessel suspended in 2.5 ml. pH 5.5 Sorensen's phosphate buffer and potassium hydroxide in the centre cup, results were obtained as expressed in the accompanying graph.

The inhibitor markedly affects the rate of oxidation of catechol by the polyphenolase enzyme of sugar beet. It apparently does not affect other enzyme systems. Inhibition of the cress seedlings can be reversed by washing, and they then germinate very rapidly. It is thought, therefore, that glycolytic reactions continue in the inhibited seed, so that, when the inhibitor is removed by washing, abundant energy becomes available for growth.

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<sup>1</sup> Tolman, B., and Stout, M., *J. Agric. Res.*, **61**, 817 (1940).

<sup>2</sup> Stout, M., and Tolman, B., *J. Agric. Res.*, **63**, 687 (1941).

<sup>3</sup> Dymy, C. P. A., Komen, J. G., Ultee, A. J., and van der Weide, B. M., *Proc. Kon. Ned. Akad. Wet.*, **50**, 527 (1947).

### Effect of D-Tubocurarine Chloride and Carbaminoylcholine on the Respiration of *Daphnia*

MEASUREMENTS of the respiration of *Daphnia* were made by a modification of the Cartesian diver micro-manometer<sup>1</sup>. With D-tubocurarine chloride, decrease of respiration and movement begins in a concentration of 2 mgm./100 ml. At 25 mgm./100 ml. paralysis is complete and respiration diminished by 32 per cent; increasing the concentration to 150 mgm./100 ml. produces no further inhibition of respiration. Thus it appears that the diminution of respiration is due to the loss of muscular activity, which accounts for 32 per cent of the total respiration. This proportion is found to be the same in *Daphnia* with normal respiratory-rates ranging from 0.23 to 0.56 c.mm. oxygen/individual/hr.

Carbaminoylcholine increases respiration and movement in a concentration of 0.01 mgm./100 ml. The increase of respiration reaches a maximum of 190 per cent normal between the concentrations of 1 and 10 mgm./100 ml., when movement is considerably increased. Higher concentrations produce smaller increases of respiration and partial paralysis.

The results with D-tubocurarine show that, in normal *Daphnia*, the oxygen consumption associated with muscular activity bears the proportion 32 : 68 to oxygen otherwise consumed. If the maximum increase to 190 per cent normal, produced by carbaminoylcholine, is due to increased muscular activity, this proportion becomes 122 : 68. It is therefore concluded that, when exerting its maximum effect on respiration, carbaminoylcholine increases by approximately four times the oxygen consumed in association with muscular activity.

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<sup>1</sup> O'Connor, R. J., *J. Exp. Biol.*, **25**, 313 (1948).

### Distribution of the Protective Factors for Vitamin C in Fractions of Liver Homogenates of the Rat

It has been reported by de Caro and Giani<sup>1</sup>, Mawson<sup>2</sup> and Giri and Shouri<sup>3</sup> that animal tissue extracts contain factors which protect vitamin C against oxidation. These protective factors were found to be of the nature of thiols<sup>1</sup>. Mawson observed that liver extracts from which glutathione was removed by prolonged dialysis retained the protective properties of fresh extract and concluded that glutathione is not the only constituent responsible for the protective mechanism existing in animal tissues. Later, Giri and co-workers<sup>4</sup> reported that certain purines such as xanthine, uric acid, adenine, guanine, theophylline and nucleic acids exert a protective action on vitamin C against oxidation. Since purines and nucleic acids are widely distributed in cells, it seemed of interest to find out the distribution of the protective factors for vitamin C in various fractions of liver homogenates, in the hope of gaining an insight into the nature of the factors responsible for the protective action.

Rat liver was homogenized with about two volumes of ice-cold hypertonic sucrose solution by means of a Potter-Elvehjem homogenizer, and the fractiona-