

of dogs of the  $\beta$  group possess all of the antigenic groupings present in the cells of animals of the  $\alpha$  group and in addition contain some other complex which may act as a foreign body when injected into dogs only of the  $\alpha$  group. Therefore, if it is true that 50% of the dogs belong to either the  $\alpha$  or  $\beta$  group and if these are the only 2 groups present in that species, it follows that there is only one chance out of 4 that an incompatibility will occur when a dog is subjected to repeated infusions of red blood cells from a donor animal. This is obvious when reference is made to the table which summarizes our findings. It may be for this reason that in Whipple's laboratory,<sup>4</sup> where animals were subjected to repeated injections of erythrocytes from a group of donor animals, hemoglobinuria and shock were observed with some of the dogs whereas others received similar exchanges of red cells from the same group of donors without untoward effects.

TABLE I.  
The Results of Repeated Injections of Red Blood Cells into Dogs.

Blood Group of		Results
Donors	Recipients	
$\beta$	$\alpha$	Blood incompatibilities develop in recipients
$\alpha$	$\alpha$	Dogs remain compatible.
$\beta$	$\beta$	" " "
$\alpha$	$\beta$	" " "

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#### Respiratory Effects of Substituted Phenols at Varying Carbon Dioxide Tensions.

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During a study of the reversible inhibition of cell division which is produced in fertilized marine eggs by certain nitro and halo-phenols<sup>1, 2</sup> the CO<sub>2</sub> tension was found to be a significant factor in the degree of inhibition attainable.<sup>3</sup> The respiratory effects of substituted phenols were then measured on cells which were exposed to a gas phase containing oxygen and CO<sub>2</sub> in various ratios.

<sup>1</sup> Clowes, G. H. A., and Krahl, M. E., *Science*, 1934, **80**, 384.

<sup>2</sup> Clowes, G. H. A., and Krahl, M. E., *J. Gen. Physiol.*, 1936, **20**, 145.

<sup>3</sup> Krahl, M. E., Clowes, G. H. A., and Taylor, J. F., *Biol. Bull.*, 1936, **71**, 400.

The manometric techniques of Warburg (direct method) and of Dickens and Simer (second method) were employed.<sup>4</sup> Fertilized eggs of the sea urchin (*Arbacia punctulata*) and a baker's yeast (Anheuser-Busch) served as material. The egg experiments were made at 20°C., using sea water as the suspension medium. This was 0.002 molar in bicarbonate. The yeast experiments were made at 25°C., using a suspension medium 0.1 molar in glucose and 0.004 molar in bicarbonate.

When the oxygen consumption of either type of cell is plotted against the logarithm of the concentration of a stimulating substituted phenol, the curve rises nearly linearly until an optimum is reached; beyond this optimum concentration the oxygen consumption falls rapidly, nearly to zero. Cell division in the fertilized eggs is inhibited by those concentrations of the reagent which give the falling portion of the oxygen consumption curve.<sup>1, 5</sup>

With fertilized sea urchin eggs in 4,6-dinitro-*o*-cresol a change in the partial pressure of CO<sub>2</sub> from the usual atmospheric level, about 0.0005 atmospheres, over a range of tensions up to 0.02 atmospheres, has little effect on the position of the rising portion of the oxygen consumption curve. The falling portion of the oxygen consumption curve is displaced to a lower range of concentrations of the 4,6-dinitro-*o*-cresol; the respiratory optimum concentration decreases from  $8 \times 10^{-6}$  to  $10^{-6}$  molar; and the number of units of excess oxygen consumed at this optimum decreases with rising CO<sub>2</sub> partial pressure. In 0.02 atmospheres CO<sub>2</sub> the oxygen consumption of the eggs, without the substituted phenol, is 0.68 cmm. per hour per mg. eggs (dry weight) as compared with the value of 1.7 in the absence of CO<sub>2</sub>. Hence, while the rate of oxygen consumption of eggs in normal sea water is sensitive to increase in CO<sub>2</sub> partial pressure, the oxygen consumption of eggs in concentrations up to  $10^{-6}$  molar 4,6-dinitro-*o*-cresol is, in great measure, independent of the changes in CO<sub>2</sub> partial pressures up to 0.02 atmospheres.

With yeast in 4,6-dinitro-*o*-cresol, increase in CO<sub>2</sub> partial pressure from 0.05 to 0.15 atmospheres causes little or no shift in the rising portion of the oxygen consumption curve, a marked displacement of the falling portion to lower concentrations, and a decrease in the optimum value of  $Q_{O_2}$ .

With yeast in 2,4-dichlorophenol, increase in CO<sub>2</sub> partial pressure from 0.05 to 0.15 atmospheres causes a considerable shift of the rising portion of the oxygen consumption curve to a higher range

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<sup>4</sup> Dixon, M., *Manometric Methods*, Cambridge University Press, 1934.

<sup>5</sup> Krahl, M. E., and Clowes, G. H. A., *J. Gen. Physiol.*, 1936, **20**, 173.

of concentrations and only a very small shift in the falling portion of the curve.

Increasing  $\text{CO}_2$  tension produces a tendency toward increase in intracellular hydrogen ion concentration.<sup>6</sup> The pH effects dealt with here are primarily on the cell interior and therefore to be distinguished from those obtained by Field,<sup>7</sup> who was chiefly concerned with changes in extracellular pH.

At the ionic strengths used here 4,6-dinitro-*o*-cresol has a  $\text{pK}'$  of about 4.4;<sup>8</sup> 2,4-dichlorophenol a  $\text{pK}'$  of about 7.7.<sup>9</sup> Thus at the normal intracellular cytoplasmic pH of about 6.5 to 7.0, 4,6-dinitro-*o*-cresol is almost completely in the dissociated form, while 2,4-dichlorophenol is chiefly in the undissociated form. In the first case, rising intracellular acidity produces a small relative change in the concentration of phenol anion and a large relative change in the concentration of undissociated phenol. In the latter case the situation is reversed. The probable effects of increased  $\text{CO}_2$  tension on intracellular ionization of these substituted phenols in an interior aqueous phase thus appear to parallel the effects of  $\text{CO}_2$  on the oxidative stimulating properties of these substances.

2,4,5-trichlorophenol occupies a middle position between 2,4-dichlorophenol and 4,6-dinitro-*o*-cresol in respect to the influence of  $\text{CO}_2$  on its respiratory effect. The  $\text{pK}'$  of 2,4,5-trichlorophenol is also intermediate between those of 2,4-dichlorophenol and 4,6-dinitro-*o*-cresol, being about 6.9.<sup>8</sup>

These experiments appear to provide some indication that oxidative stimulation may be favored by high intracellular concentrations of the dissociated form, while inhibition of oxidation and reversible block to cell division may be favored by high intracellular concentrations of the undissociated form of the substituted phenols.

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<sup>6</sup> Jacobs, M. H., p. 97 in Cowdry, E. V., *General Cytology*, Chicago, University of Chicago Press, 1924.

<sup>7</sup> Field, J., 2nd., *PROC. SOC. EXP. BIOL. AND MED.*, 1935, **32**, 1342.

<sup>8</sup> Krahl, M. E., and Clowes, G. H. A., unpublished experiments. See also Field, reference 7, for a slightly higher value for 4,6-dinitro-*o*-cresol.

<sup>9</sup> *International Critical Tables*, New York, McGraw-Hill, 1929, Vol. VI, p. 271.