of the deterioration seems to be directly proportional to that of the cause by which it is elicited. In spite of this basic similarity, flaplessness and the various other manifestations of resistance can be shown to be distinct in their causation as well as in their effects.

J. F. MICHEL

Central Veterinary Laboratory,
Weybridge, Surrey.

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Inhibition of Respiration of *Chlorella vulgaris* by Simultaneous Application of Cupric and Fluoride Ions

IN experiments of short duration, the respiration of many micro-organisms is diminished only a little, if at all, by cyanide or by fluoride at concentrations which inhibit the main respiratory system of higher animals. I have used a strain of *Chlorella vulgaris* in which the respiration is insensitive to fluoride, cyanide and (in aerobic conditions) copper ions, but when suitable amounts of copper sulphate and sodium fluoride are added simultaneously to a cell suspension, oxygen uptake almost ceases. No similar effect has been seen when other metals are substituted for copper, or when most other anions are substituted for fluoride. Cyanide and iodide are exceptions, but both of these interact with cupric copper. The addition of citrate, to avoid possible precipitation of sparingly soluble basic copper fluoride, does not make copper sulphate or sodium fluoride more toxic when these are added to cells singly, nor does it reduce the inhibition of respiration which occurs when they are applied simultaneously (Fig. 1).

In mixtures containing less copper sulphate or sodium fluoride, the respiratory inhibition is less and apparently can disappear at appropriate concentrations. Nevertheless, interaction is still taking place, for cell suspensions treated with even smaller concentrations absorb considerably more oxygen than do untreated suspensions. The figures in Table 1 represent the respiration of standard cell suspensions expressed as percentages of the respiration of untreated controls, the data being based on the oxygen uptake for the 60 min period after addition of the poisons. The experiments were carried out in aerobic conditions

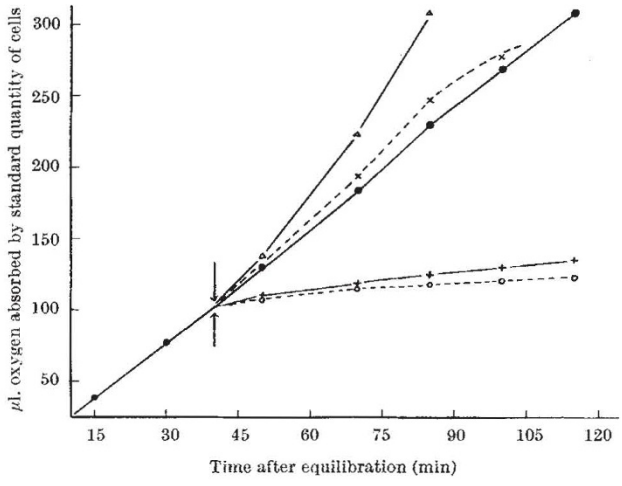

Fig. 1. Respiration of *Chlorella vulgaris* after applying sodium fluoride and copper sulphate singly or together. Side-arm contents were added at time indicated by the arrow. ●—●, Untreated cells (with or without citrate); △—△, sodium fluoride alone, 4×10^{-2} molar after addition to cells. × - - - ×, copper sulphate alone, 8×10^{-3} molar after addition; +—+, mixture of sodium fluoride and copper sulphate at the concentrations above; ○ - - - ○, same mixture, but 3×10^{-3} molar citrate also present.

Table 1. RESPIRATION, AS A PERCENTAGE OF THAT OF UNTREATED CONTROLS, AFTER ADDING MIXTURES OF COPPER SULPHATE AND SODIUM FLUORIDE TO A SUSPENSION OF CELLS OF *Chlorella vulgaris*

Copper sulphate (moles/l.)	Sodium fluoride (moles/l.) 0	8×10^{-3}	$1{\cdot}7 \times 10^{-2}$	4×10^{-2}
$6{\cdot}6 \times 10^{-4}$	110	226	43	9
$1{\cdot}3 \times 10^{-3}$	111	256	82	10
$4{\cdot}0 \times 10^{-3}$	112	237	175	18
$2{\cdot}5 \times 10^{-3}$	101	140	160	53
$3{\cdot}1 \times 10^{-4}$	100	—	144	322
0	100	121	146	193

in a Warburg respirometer provided with a light-excluding canopy and at a temperature of 30° C.

Insensitivity of respiration to fluoride has often been attributed to the presence of a respiratory pathway which is not dependent on enolase. *Escherichia coli*[1], *Pseudomonas aeruginosa*[2], *Bacillus subtilis*[3] and *Chlorella pyrenoidosa*[4] are among organisms, strains of which have been demonstrated to be fluoride-insensitive, though the techniques used have sometimes measured gas exchange and sometimes substrate utilization. The alternative pathway has been characterized tentatively as the pentose phosphate system[5,6].

Perhaps the most striking respiratory peculiarity of *Chlorella* when it is treated with a respiratory poison is its ability to switch from a main to an alternative pathway capable of assuming the full respiratory load; in contrast, tissues of higher organisms in similar circumstances respond by undergoing a reduction in respiration to a "cyanide-resistant" or "fluoride-resistant" level. If fluoride blocks a main pathway dependent on enolase, the foregoing observations suggest that (*a*) an alternative respiratory pathway is disturbed or inhibited by concentrations of copper ions exceeding approximately 2×10^{-4} molar, and (*b*) both respiratory pathways are blocked when the two poisons are applied together. I am investigating the possibility that the pentose phosphate system is the alternative copper-sensitive system.

K. A. HASSALL

Department of Physiology and Biochemistry,
University of Reading.

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BIOCHEMISTRY

Evidence for binding of Cytoplasmic Creatine Kinase to Structural Elements in Heart Muscle

THERE have been suggestions[1,2] that cytoplasmic creatine kinase is not always free in solution in the cell, but that it is in part bound to structural elements, for example myofibrils. Some evidence for this is provided *inter alia* by localization studies[3–5]. I have obtained direct evidence for the binding of some cytoplasmic creatine kinase by isolation and crystallization of the enzyme from the myofibrillar fraction of a heart muscle homogenate. The enzyme was differentiated from the mitochondrial creatine kinase, which forms about 50 per cent of the total creatine kinase activity in heart muscle[6].

Beef heart (800 g) was homogenized in 4 l. of 0·25 mmolar sucrose, containing 20 mmolar triethanolamine buffer, *p*H 7·2, and 2 mmolar EDTA, according to the recommendations of Green and Ziegler[7]. The myofibrillar fraction was centrifuged down at 1,000*g* for 15 min and the supernatant, containing the mitochondria as well as the sap, was removed. The precipitate was suspended in