CARBON DIOXIDE AND CELL DIVISION

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By definition, the discussion on the effects of carbon dioxide on cell division must be limited to the specific effects of carbon dioxide on this process. Growth and cell division are competitive, interdependent, and consecutive processes[1]. Cell division occurs after a certain mass of protoplasm has been accumulated. Thus, cell division depends on growth. An unequivocal demonstration of the specific favourable effect of carbon dioxide on cell division would require proof that this seemingly beneficial effect is not actually due to the promoting effect of carbon dioxide on growth. If carbon dioxide is utilized by the cells in growth processes, this proof seems to be an arduous task.

In an attempt to prove a favourable effect of carbon dioxide on cell division, Mer and Causton (preceding article) resorted to references on the effect of carbon dioxide on growth[2–6] which are largely irrelevant to the present discussion. In Geisler's work[2], the favourable effect of carbon dioxide was demonstrated on root growth of pea seedlings. Though growth of multicellular organisms involves also cell division, Geisler[2] did not attempt to discriminate between these two processes. He was interested in the morphogenic effects of carbon dioxide and the characteristics he studied were: dry weight of the main and lateral roots, length of the main root, and number of lateral roots.

Bach and Fellig[5] recorded a promoting effect of ethanol on the growth of *Chlorella vulgaris*, measured as increase in optical density. Observations on cell division were not attempted in those investigations and the term "cell division" has not been used in the title or text even once. Stimulation of growth was observed only under growth-limiting conditions, indicating that ethanol acted as a factor promoting heterotrophic growth.

A similar effect of ethanol on heterotrophic growth was reported by Street *et al.*[6], who observed a promotion of growth in *Chlorella vulgaris* cells by ethanol only when the culture contained limiting concentrations of glucose or was maintained in inorganic medium under light-limiting conditions. The nutritional value of ethanol, as an agent promoting mesocotyl growth in oat seedlings[3,4], has been paralleled in Mer's work[7] by that of sucrose, glucose and mannitol.

To account for the similarity in stimulation of growth by carbon dioxide and ethanol, Mer[8] offered two alternatives: either ethanol is formed from carbon dioxide and acts as an

intermediate metabolite, or ethanol is used by cells in respiration and an increased carbon dioxide production acts as a promoting agent. It is easy to see that the first alternative clearly suggests the nutritive value of both ethanol and carbon dioxide and the second alternative fails to exclude it.

In their reference to Loomis's work[9] on the effect of carbon dioxide on sexuality in *Hydra*, Mer and Causton (preceding article) name carbon dioxide "as the factor actuating those divisions which result in gametogenesis in *Hydra*". Loomis[10] observed that in water free from carbon dioxide, a *Hydra* bud grew into normal *Hydra* with six tentacles in two days. At pCO_2 of 4 per cent of an atmosphere, the bud grew slowly and produced only three tentacles. Higher levels of carbon dioxide inhibited growth of buds even more. With the inhibition of vegetative growth, the development of sexual organs was induced. Within a week, testa and ovaries began to form on the treated *Hydra*. Thus, inhibition of growth (and of cell divisions involved in vegetative growth) came first and, quantitatively, was probably expressed much more than the later promotion of the growth of sexual organs. Clearly, Loomis's observations on the effect of carbon dioxide on sexuality in *Hydra* involve a complex problem of interrelations between the development of different parts and organs in a multicellular organism. Morphogenic effects of carbon dioxide have been discussed by many investigators (see refs. 9 and 11). The whole problem is, however, outside the discussion on the specific effect of carbon dioxide on cell division.

Carbon dioxide in Mer and Causton's[12] investigations was administered as a pretreatment during the first three days of the experiments. Observations on growth and cell numbers in mesocotyls and coleoptiles were continued until the seventh day. If growth was favourably affected by carbon dioxide, then the cell proceeded quickly through its developmental stages and entered cell division sooner than in the absence of the growth-promoting agent. However, this indirect favourable effect of carbon dioxide on cell division did not prove the specific nature of this effect separate from that of carbon dioxide on growth.

It can be argued that, on the basis of observations on multicellular tissues, the favourable effect of carbon dioxide on cell division cannot be altogether discarded. However, experimentation with systems which permit much more rigorous control of cell microenvironment and much more precise delineation of developmental stages indicates that the possibility of such a favourable effect of carbon dioxide on cell division is improbable.

In synchronized microbial cells, growth processes can be, to some extent, separated in time from cell division.

By subjecting unicellular green algae to alternating periods of light and darkness, the great majority of cells present in the originally non-synchronized population can be brought into phase, that is, into more or less the same developmental stage. Then, by maintaining a suitable regimen, these cells can be grown in such a way that the majority of cells proceed through developmental stages and enter cell division more or less uniformly and simultaneously. In green algae, growth is then largely confined to light periods and cell division to dark periods.

A complete separation of cell division from growth is not possible in algae even during the dark period. As was shown by Meffert[13], nitrogen assimilation and the increase in dry weight continue in synchronized *Scenedesmus* cells during the dark period. Under these conditions, uptake of nitrogen and growth are favoured by carbon dioxide, indicating that carbon dioxide is used by these cells in growth processes. However, despite the usage of carbon dioxide during growth in darkness and an expected indirect promoting effect of the increased growth rate on cell division, the direct inhibitory effect of carbon dioxide on division in *Chlorella*[14–16] is so strong that the net effect of carbon dioxide results in suppression of cell division. Thus, the direct inhibitory effect of carbon dioxide on cell division in synchronized algal cells during dark periods must be quantitatively of a larger magnitude than can be detected experimentally, since the observable effect is actually a balance between the favourable effect of carbon dioxide on growth and its unfavourable effect on cell division.

Synchronized *Chlorella* cells, brought by means of autotrophic growth to the stage of readiness to cell division, divide in the course of time both in light and in darkness. In darkness they readily divide if suspended in a fluid buffered at a suitable *p*H. In unbuffered suspending fluids, as, for example, in distilled water, cell division proceeds in darkness to its completion in atmospheric and free from carbon dioxide air, but not in air supplemented with one per cent or more of carbon dioxide[14,16].

Studies on the detrimental effect of carbon dioxide on cell division in unbuffered suspending fluids have been substantiated by observations on the inhibitory effect of carbon dioxide on cell division in algal cells also in buffered media[17,18]. Earlier extensive work on cell division in marine eggs indicated that in these cells also, carbon dioxide had a clear-cut detrimental effect on cell division[19,20].

The specific inhibitory effect of carbon dioxide on cell division was thus demonstrated on unicellular algae and marine eggs in the absence of, or despite, heterotrophic growth. The inhibitory effect was most pronounced in unbuffered suspending fluids[14,15]. In unbuffered media, an increase in carbon dioxide concentration coincides with the decline in *p*H of the suspending fluid.

Several observations indicated that a low *p*H of the suspending fluid may drastically suppress cell division[14-16,21-24]. The mechanism of action of low *p*H has been a subject of speculation. The capacity of a cell to maintain its inner *p*H in media with wide differences in their *p*H has been well documented in the literature[25-27], and other evidence[28] brought by Mer and Causton (preceding article) to that effect adds nothing to the well-established fact. However, the effect of carbon dioxide present in the medium on lowering the intracellular *p*H may be different from that of other acids. Cell membranes are highly permeable to carbon dioxide and it has been reported[26] that externally supplied carbon dioxide may lower the internal *p*H of the cell.

The main point, however, is that H-ions do not need to penetrate the cell wall to affect cell activity[27]. Several processes essential for cell activity occur within the cell wall or at the cell surface. Of particular importance is the secretory activity of cells which has been recently shown to be essential for cell division[16,22,29]. It has been demonstrated that low *pH* interferes with the secretory activity of cells[22,29]. Thus, if the *p*H of the surrounding medium changes due to the changes in the concentration of carbon dioxide, it is most reasonable to expect that the decrease in *p*H beyond a certain point will adversely affect cell division.

It must also be emphasized that "carbon dioxide may affect cell metabolism directly and/or through its effect on *p*H"[16]. Thus, the effect on *p*H is only one of several functions of carbon dioxide as a factor of biological importance. In certain circumstances, "the *p*H effect is obscured by the dramatic action on cell division of the dissolved undissociated carbon dioxide"[15]. My work[15] actually demonstrated that there is an effect of carbon dioxide other than that exerted through changes in *p*H. Several investigators[19,30] emphasized this role of carbon dioxide in cell division and other biological processes. Mer and Causton (preceding article) would seem to be trying to force an open door in their attempt to prove that carbon dioxide *per se* may act as an agent affecting cell growth and, in general, cell metabolism.

Buffering a suspending fluid removes that portion of carbon dioxide action which is exerted by the dissociated carbonic acid on the *p*H of the medium. Several buffers act in a more or less similar way, and bicarbonate buffers are as effective as others. Mer and Causton (preceding article) are being somewhat fanciful in attempting to deny a 'favourable' effect of bicarbonate, as such, on cell growth or cell division. I actually never expressed myself on the subject, and, to limit the discussion, I am still trying to avoid the broad problem of the utilization and of the effect of bicarbonate ions on metabolic processes. As has

been stated, "an investigation of simultaneous effects of carbon dioxide and of bicarbonate indicated that bicarbonate counteracts the adverse effect of carbon dioxide on cell division . . ."[16]. Therefore, carbon dioxide "as a source of bicarbonate can, within proper concentration range, favourably affect cell division"[16]. Thus, only the buffering properties of bicarbonate are involved. The biological importance of bicarbonate is bound with its formation in the external medium as the result of vital activities of cells. Both carbon dioxide and cations can be supplied by the cells in the process of respiration and cell secretion.

To account for the discrepancies in the views on the effect of carbon dioxide on cell division expressed by Mer and Causton (previous article and ref. 12) on one side and by myself[14-16] on the other side, I have proposed two hypotheses. One of these hypotheses was offered in the previous communication[16]; the other is elaborated in this article. These two hypotheses are not necessarily exclusive of each other. In one hypothesis[16], the role of *p*H and its changes, as affected by carbon dioxide, bicarbonate, and cell secretions, was brought forth as possibly responsible for the differences in the observations made in these two laboratories. Due to the choice of experimental material and technique, the control of *p*H and of its effect on cell division was not feasible in Mer's and Causton's experiments.

In another hypothesis, the effect of carbon dioxide on growth, and the dependence of cell division on the amassment of cell material and therefore on growth, was emphasized. This became particularly necessary because several investigators, and among them Mer and Causton (previous article), fail to dissociate these two processes and to consider only the specific effects of carbon dioxide on cell division. The effect of carbon dioxide on growth, and, through it, on cell division, is not a subject of this discussion. The complexity of conditions for the development of individual cells in multicellular tissues is such that other hypotheses could also be proposed to account for the favourable effect of carbon dioxide observed by Mer and Causton[12].

Mer and Causton[12] explained differences in the effects of carbon dioxide on cell division in oat mesocotyls (positive effect) and in coleoptiles (no effect) by assuming that a high concentration of carbon dioxide is required for cell division in the meristem, and that carbon dioxide "will influence mitosis only in a compact meristem such as that found at the node of the mesocotyl"[12].

I made no attempt to evaluate the last hypothesis, since no theoretical considerations or comparative observations were laid at its basis except for the reference to the possible difference in reaction to the same environmental

factor (carbon dioxide) on the part of different plants[12] and of different organs of the same plant (previous article).

The universally recognized fact of genetic and physiological individuality makes comparative investigations a difficult task. However, biological investigations would turn into piling of unidentified and unrelated observations if investigators, in defending their views, take refuge every time in the specificity of their experimental material and techniques. An attempt must be made to relate the diversified observations and to understand them from broad theoretical principles.

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