

A final experiment was performed in order to show that removal of  $\text{NH}_3$  by the  $\text{H}_2\text{SO}_4$  wash bottles, included up to this point in both aeration trains, was not involved in the  $\text{CO}_2$  effects noted. To this end,  $\text{H}_2\text{SO}_4$  was omitted from both trains; all other purification units were left as before. Five cultures were started simultaneously. Three subjected at once to  $\text{CO}_2$ -free aeration,  $\text{CO}_2$ -containing aeration, and no aeration, yielded curves similar in all respects to the corresponding ones of Fig. 1. The other two cultures were also, as in Fig. 2, subjected to inhibition by  $\text{CO}_2$ -free aeration for one day. Then one was transferred to the  $\text{CO}_2$ -containing air train and one had aeration discontinued; both grew thereafter as in Fig. 2. Likewise, one continued on  $\text{CO}_2$ -free aeration for the second day prolonged its lag accordingly, but grew on the third day, after aeration was shut off. Thus it appears that presence or absence of an  $\text{H}_2\text{SO}_4$  wash bottle for absorption of  $\text{NH}_3$  from the air current has no effect on the  $\text{CO}_2$  results obtained. In short, removal or non-removal of  $\text{CO}_2$  appears responsible for the phenomena noted.

In concluding, it may be pointed out that by the method of introducing aliquots of a common inoculating suspension into batches of the same medium subjected to the three chosen conditions— $\text{CO}_2$ -free aeration,  $\text{CO}_2$ -containing aeration and no aeration—most of the factors claimed by earlier workers to be influential upon lag phenomena have been controlled so far as discernment of  $\text{CO}_2$  effects requires. Items so controlled include age of mother culture, transfer of inhibitory substances therefrom, size of inoculating dose, nature of both old and new medium, prior injury to cells, temperature shock, variable cell capacity for environmental adaptation, and even particularly favored biological state effects such as the need for a germination period or restoration of postulated intermediate bodies in protoplasmic synthesis. Removal of  $\text{CO}_2$ , independent from the effect of aeration itself, is therefore the variable studied. Accordingly, we conclude that:

(1) In a synthetic medium of low nutrient value, the lag period of (coli) cultures can be prolonged at will by aeration with an air current purified by passage through a washing train which includes an alkaline,  $\text{CO}_2$ -absorbent unit.

(2) When parallel cultures are aerated at a comparable rate with air washed through a train similar in all respects, save for the replacement of the alkali by water, normal growth occurs to an even higher degree than is the case in unaerated cotton-plugged test-tube cultures in the same medium.

(3) Cultures inhibited from growth by  $\text{CO}_2$ -free aeration for a day or more will, when aeration is

stopped or is resumed after replacement of the alkaline wash bottle by water, undergo a normal lag of a few hours and then grow in the manner characteristic of newly inoculated cultures in unaerated media or in media aerated with air from which  $\text{CO}_2$  has not been removed.

(4) Presence or absence of an acid  $\text{NH}_3$  absorbent in the air train does not alter the above phenomena.

(5) There are no differences in nutrient values of the substrate or in its reaction to account for the observed results.

(6) Aeration itself, either through mechanical agitation or through increased oxygenation of the medium, or by the washing out from the medium of metabolic products (other than  $\text{CO}_2$ ), does not account for the results noted.

(7) Other factors being similar, the phenomenon must be due to the substance removed from the aeration current, hence from the medium, by the caustic wash bottle,— $\text{CO}_2$ .

(8) Our results, then, tend to (a) Confirm in a fluid medium the necessity of  $\text{CO}_2$  for cell growth, which Valley and Rettger demonstrated in solid media; (b) Suggest that the phenomenon of lag may be due largely, if not entirely, to the time it takes the culture to build up the  $\text{CO}_2$  content of the medium or of the cells themselves to a value essential for growth; (c) Simplify the philosophy of the lag period by apparently rendering unnecessary the postulation of an essential formation of intermediate bodies in protein synthesis required for growth, other than  $\text{CO}_2$ .

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