The Effect of *cis*-Vaccenic Acid on Respiration and Growth of *Bacillus subtilis*

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It is well established that long-chain unsaturated fatty acids are essential for the growth of certain micro-organisms (see recent reviews by Pollock, 1949; Kodicek, 1949). With some strains of lactobacilli (Williams & Fieger, 1946, 1947; Williams, Broquist & Snell, 1947) and with *Clostridium sporogenes* (Shull & Peterson, 1948; Shull, Thoma & Peterson, 1949) oleic and similar acids are growth promoting only in absence of biotin, which they effectively replace, while with other organisms absence of biotin is not a necessary condition. However, the same acid which at a certain concentration is growth promoting becomes inhibitory at only a slightly higher concentration. This is the so-called 'double action' (Williams *et al.* 1947). The basis for the growth promotion by fatty acids and their mode of action are not yet entirely understood. Experimental evidence seems to indicate that fatty acids are metabolized 'involving an enzyme system' (Williams & Fieger, 1946), or that they 'form an essential constituent of the cell protoplasm' (Pollock, Howard & Broughton, 1949). Opposed to this view is the physico-chemical theory, that fatty acids act by being adsorbed on or incorporated into the cell surface. They are assumed thereby to change the permeability of the cell membrane towards substrates and to cause either stimulation or inhibition of growth, according to the quantitative relationship between fatty acids and preformed inhibitors of fatty acids, which are present in the cell wall (Kodicek & Worden, 1945; Kodicek, 1949). The two theories may not be mutually exclusive.

In a recent series of papers (Laser & Friedmann, 1945; Laser, 1948, 1949) a number of normal and pathological tissues have been shown to contain an unsaturated fatty acid possessing very high haemolytic activity *in vitro*. Its relation to haemolysis and to certain aspects of malaria has previously been examined (Laser, 1946, 1948, 1949). The acid has been identified by Morton & Todd (1950) as *cis*-vaccenic acid, $\text{CH}_3 \cdot (\text{CH}_2)_5 \cdot \text{CH} = \text{CH} \cdot (\text{CH}_2)_9 \cdot \text{COOH}$, and this has been confirmed by synthesis. In the present paper the effect of *cis*-vaccenic acid on growth and respiration of *Bacillus subtilis* has been examined with a view to studying its mode of action and gaining some insight into the nature of the double effect of unsaturated fatty acids on bacteria.

MATERIAL AND METHODS

The strain of *B. subtilis* used was No. 2586 of the National Collection of Type Cultures. It was grown in Roux flasks upon the following medium: 10 g. peptone, 5 g. Lemco, 5 g. NaCl, 25 g. agar and tap water to 1 l., adjusted to pH 7.6 with NaOH and sterilized for 20 min. at 110°. The flasks were inoculated from a stock spore culture preserved on the same medium. To obtain vegetative forms, the flasks were incubated at 30° for 16 hr., when the organisms were carefully removed and washed three times with distilled water in a centrifuge.

Yeast extract was prepared by heating baker's yeast with an equal amount (w/v) of distilled water for 10 min. at 90° and filtering hot through kieselguhr. The extract was filled into ampoules, which were sterilized by steam for 30 min. and sealed, the same extract being used for all experiments described in this paper.

The medium in which the washed cells were suspended was 0.15 M- K_2HPO_4 , which was obtained by adjusting the different components of the medium (cell suspension, vaccenic acid, glucose and yeast extract) in such a way as to establish a final ratio of $\text{KH}_2\text{PO}_4:\text{K}_2\text{PO}_4=1:1$, containing 1% glucose $\pm$ 10% yeast extract (v/v).

The fatty acids tested were synthetic *cis*- and *trans*-vaccenic acids (Morton & Todd, 1950). They were dissolved with heating in 0.3 M- K_2PO_4 (0.64 mg./ml.) and were pipetted into the reaction vessel immediately before adding the organisms, following a procedure previously described (Laser, 1949). A $1:10^6$ concentration of acid = 35.5 μM .

Growth determinations were made turbidimetrically by means of the Spekker absorptiometer with a combination of two filters (Ilford neutral H 503 and red 608). A calibration curve was prepared from serial dilutions of a washed cell suspension, the dry weight of which had previously been determined.

Growth in a liquid medium in presence of vaccenic acid was obtained in test tubes in a water bath at 30° with fairly vigorous aeration of the medium. Each tube contained a narrow capillary reaching to the bottom of the tube and connected at the upper end to a glass manifold through which water-saturated air was passed by means of a pump. A trace of tri-*n*-butyl citrate (1 drop of a fine emulsion in water) was added to each tube to prevent frothing. The initial level of liquid in the tubes was marked in order to note evaporation, and was readjusted before making density determinations.

Treatment of *B. subtilis* with vaccenic acid prior to determination of growth or O_2 uptake of treated cells was done in the same way as for the measurement of growth in the liquid medium.

Respiration was measured manometrically in Barcroft-Warburg manometers at 37°. As a rule all components of the

medium (total volume 3 ml.) were filled into the main compartment of the flasks before the beginning of the experiment, vaccenic acid being added immediately before the organisms. The initial amount of cells in all experiments was 2.4 mg. dry weight, contained in 0.1–0.2 ml. of a suitably diluted washed suspension, the density of which had been determined. In a few experiments in which the acid was added to the cell suspension at $t=0$, or in which a second amount of acid was added during the course of the experiment, flasks having two side bulbs were used. One of these contained vaccenic acid dissolved in 0.15 M- K_3PO_4 and the other an equivalent amount of 0.15 M- KH_2PO_4 . The contents of the side bulbs were run into the main compartment in quick succession, KH_2PO_4 being run in first. The gaseous phase contained air or O_2 . Q_{O_2} values of growing cells were calculated on the basis of the mean increase of density with time obtained in four growth experiments.

Symbols and abbreviations. $a:c$ = ratio of weight of vaccenic acid in the medium: dry wt. of cells; treated and untreated cells = cells which prior to the experiment had been incubated in presence or absence of vaccenic acid; treated and untreated supernatant = supernatant fluid from treated or untreated cells.

RESULTS

The effect of pH. The potassium salt of vaccenic acid, like that of any other fatty acid, is water-soluble at alkaline pH. It was not desirable to add the acid to the organisms in ethanolic solution, as a lipid solvent might produce, or in combination with the acid accentuate, phenomena unrelated to the acid alone. The highest pH was therefore determined at which O_2 uptake and growth of the organisms did not differ from that obtained in 0.15 M-phosphate buffer solution at pH 7.3. This was done by measuring the O_2 uptake of the organisms in presence of glucose and yeast extract in media having different ratios of $KH_2PO_4:K_3PO_4$, i.e. ratios of 1.6 (pH 7.3), 1.4, 1.2, 1.0 (this constituting dibasic phosphate, pH 9.2) and 0.8:1.0. No difference in O_2 uptake and logarithmic increase in O_2 uptake with time, corresponding to growth, was found down to the ratio $KH_2PO_4:K_3PO_4 = 1:1$. This ratio was therefore employed throughout the present experiments.

Effect of *cis*-vaccenic acid on growth. Fig. 1 shows the relation between different amounts of acid and growth based on opacity measurements. It will be seen: (1) that the acid, except for the lowest concentration in which it was used, inhibits growth; (2) that the inhibition increases with increasing amounts of acid; and (3) that the organisms recover from this inhibition and eventually attain a rate of growth at least equal to that of the control. The proportionality of the duration of inhibition of growth of the same amount of cells to the concentration of acid in the medium indicates that the governing factor is the ratio of acid:cells. That this is the case is borne out in the experiment summarized in Fig. 2, which records the relative growth rate of different amounts of organisms suspended in the same volume

of medium having the same concentration of acid, i.e. varying ratios $a:c$. Here the inhibition was found to be directly related to the ratio $a:c$.

Fig. 1. Relation between varying concentrations of *cis*-vaccenic acid and growth. Initial amount of cells 2.4 mg. dry wt./3 ml. 0.15 M- K_3HPO_4 , containing 1% glucose + 10% yeast extract.

Fig. 2. Effect of varying ratios of *cis*-vaccenic acid:cells on growth. 0.15 M- K_3HPO_4 , 1% glucose, 20% yeast extract. Concentration of acid in medium 142 μM, with 1.2, 4.8 and 9.6 mg. dry wt. cells/3 ml. medium; ratios of acid:cells, 1:10, 1:40 and 1:80.

Effect of *cis*-vaccenic acid on the respiration of growing cells. The O_2 uptake of *B. subtilis* was found to be independent of the O_2 tension between 20 and

100% O_2 . Experiments in which the rate of O_2 uptake was comparatively small, as with washed cells, were therefore done in air, while those using growing cells and of longer duration were done in O_2 , in order that the decreasing O_2 tension should not eventually become a limiting factor. The concentrations of acid previously tested for their effect on growth were used (Fig. 3). The decrease in total O_2

Fig. 3. Effect of *cis*-vaccenic acid on O_2 uptake with varying concentrations of acid acting on the same amount of cells (initial dry wt. = 2.4 mg.). Conditions as in Fig. 1. Acid added at $t=0$.

uptake was similar to that of growth, but less marked: only at the highest concentration of acid used was there a pronounced effect. However, the inhibition is greater than the additive curve of O_2 uptake suggests, and becomes apparent if the rate of O_2 uptake/mg. (Q_{O_2}) is plotted against time, i.e. if the different growth rates in presence of varying amounts of acid are taken into consideration (Fig. 4). The inhibition of respiration is not instantaneous, as there is no significant difference in the curves for the first 20 min. After that a fairly strong inhibition occurred with all concentrations of acid, the degree of inhibition being greater with increasing amounts of acid. This lasted for 20 min. only with the three lower concentrations of acid, after which gradual recovery took place, the organisms with the smallest amount of acid (35.5 μM) attaining the rate of the control after about 150 min. and remaining at that level, while the cells with an acid concentration of 71.0 and 142 μM exceeded the rate of the control by between 40 and 50% after about 180 min. The inhi-

bition by 284 μM , on the other hand, increased after the first 20 min. and continued for 180 min. when recovery set in.

Fig. 4. Effect of *cis*-vaccenic acid on O_2 uptake, plotted as percentage of Q_{O_2} of the control in absence of acid. Acid added at $t=0$.

Fig. 5. Comparison of effect of *cis*-vaccenic acid on O_2 uptake of washed and growing cells, plotted as percentage of Q_{O_2} of the corresponding controls in absence of acid. 0.15 M- K_2HPO_4 , containing 1% glucose $\pm$ yeast extract; 142 μM -vaccenic acid.

Effect of cis-vaccenic acid on the respiration of washed cells. The O_2 uptake of washed cell suspensions of *B. subtilis* in presence of glucose was found to be inhibited in a manner similar to that of growing cells and recovery from the inhibition occurred after various periods of time, according to the concentration of acid. In comparison with growing cells, the onset of recovery was usually more delayed. However, the delay was not always so marked as shown in Fig. 5, which compares the recovery from the same concentration of acid (142 μM) in presence and

absence of yeast extract. There was, however, a difference in the rate of O_2 uptake attained by either growing or washed cells after recovery from the respiratory inhibition. The Q_{O_2} of growing cells was usually stabilized at between 40 and 50 % in excess of the untreated control, while that of washed cells showed an increase of between 100 and 300 % over that of untreated washed cells. No growth occurred in the washed cell suspensions. The increase in O_2 uptake cannot be caused by the oxidation of the acid itself, because it was many times greater than could be accounted for by the oxidation of the amount of acid, which had been added.

Late effect of *cis*-vaccenic acid. The increased rate of O_2 uptake of *B. subtilis* following upon an inhibition of respiration by vaccenic acid continues for a considerable time. Thus cells which had been incubated

Fig. 6. Effect of incubation with *cis*-vaccenic acid on subsequent growth. Pre-incubation 16 hr., after which turbidity determinations were made and amounts equivalent to 2.4 mg. (dry wt.) transferred into fresh media.

Media: Curve A, pre-incubation: 1 % glucose, 10 % yeast extract; then 1 % glucose, 10 % yeast extract. Curve B, pre-incubation: 1 % glucose, 10 % yeast extract, 533 μ M-vaccenic acid; $a:c=1:20$; then 1 % glucose, and 10 % yeast extract. Curve C, pre-incubation: 1 % glucose, 10 % yeast extract; then 1 % glucose, 10 % yeast extract, 142 μ M-vaccenic acid. $a:c=1:20$.

with *cis*-vaccenic acid (532.5 μ M, $a:c=1:20$) in presence of glucose $\pm$ yeast extract had as high a Q_{O_2} after 16 hr. as immediately after recovery from the inhibition. This applied to both washed and growing cells.

A stimulating effect on the growth rate of treated cells, comparable to that on the Q_{O_2} , has not been established with certainty under the present experimental conditions, i.e. in a medium not deficient in biotin, although in about half the experiments a small increase of the growth rate has been observed following 16 hr. incubation with *cis*-vaccenic acid

(Fig. 6). However, the fact that the slope of the growth curves after the second hour is practically the same for treated and untreated cells suggests that the observed increase is probably the result of a shorter time lag before the onset of appreciable growth. The figure shows, furthermore, that incubation and growth of cells in alkaline phosphate does not by itself produce resistance to the acid.

Repeated addition of *cis*-vaccenic acid. Since the inhibition of growth and respiration of the organisms by *cis*-vaccenic acid is transient, it was of interest to study the effect of a second addition of acid to cells which had recovered. Fig. 7 shows that when *cis*-vaccenic acid was added to cells 4.25 hr. after the beginning of the experiment, when the treated cells had re-attained a respiratory rate equal to that of the untreated control, the second addition of acid had no effect on the steady rate of O_2 consumption nor, by implication, on the growth rate, as shown by the logarithmic increase of the O_2 uptake.

Fig. 7. Effect of second addition of vaccenic acid on O_2 uptake. Initial amount of cells 2.4 mg. dry wt./3 ml. 0.15 M- K_2HPO_4 , containing 1 % glucose + 10 % yeast extract. Initial concentration of acid in medium 142 μ M and ratio of acid: cells = 1:20. At the times indicated by vertical arrows 142 μ M-*trans*- and *cis*-vaccenic acid respectively were added; $a:c=1:14$ for *trans*-vaccenic and 1:10.5 for *cis*-vaccenic acids (calculated on the increase of cells at the moment of second addition of acid.)

The resistance of treated cells to further addition of acid was also found not to be restricted to the period immediately following recovery. Thus washed and growing cells which had been incubated for 16 hr. in presence of vaccenic acid (532.5 μ M; $a:c=1:20$) and glucose $\pm$ yeast extract, and had then been transferred into fresh media $\pm$ yeast extract and $\pm$ vaccenic acid (142 μ M; $a:c=1:20$), fully maintained their increased rate of respiration on renewed exposure to vaccenic acid. The growth of cells, which after recovery from the first addition of vaccenic acid (532.5 μ M; $a:c=1:20$) were transferred into a new medium containing yeast extract $\pm$

vaccenic acid, was similarly unaffected by renewed contact with acid in the ratio $a:c = 1:20$.

Effect of supernatant fluid from treated cells. It seemed of interest to examine whether supernatant fluid of treated cells affects the oxidation rate of untreated cells. It was found that under the experimental conditions ($a:c = 1:20$) practically the total added acid is adsorbed by the bacteria. After short incubation at 37° or even standing at room temperature the added acid could almost quantitatively be recovered from the spun down bacteria, according to a method of extraction previously described (Laser, 1949) and determined by the haemolytic activity of the extracted acid, while the supernatant contained

Fig. 8. Effect of supernatant fluid from treated cells on O_2 -uptake of fresh cells. 'Treated' and 'untreated' supernatant obtained by centrifugation after 16 hr. incubation of the organisms in $0.15\text{ M-K}_2\text{HPO}_4$ in presence of glucose $\pm$ *cis*-vaccenic acid ($532.5\text{ }\mu\text{M}$, $a:c = 1:20$); glucose added to 0.5% . Fresh cells (2.4 mg. dry wt.) were suspended in fresh medium ($0.15\text{ M-K}_2\text{HPO}_4 + 1\%$ glucose) and in the supernatant fluids. Q_{O_2} as percentage of control. A: in untreated supernatant; B: in treated supernatant.

only traces of the added acid. The same applies to dead (freeze-dried) bacteria. However, the supernatant fluid of treated cells affects the O_2 uptake of fresh cells in a manner shown in Fig. 8, where the data have been plotted as percentage of the Q_{O_2} of control cells against time, as in Fig. 4. There is a small (25%) increase of the respiratory rate of cells when suspended in the supernatant fluid derived from untreated cells (Fig. 8, curve A) compared with cells suspended in a fresh medium. This is probably due to the diffusion of stimulating substances out of the bacteria during incubation. However, the increase of the oxidation rate of untreated cells in the supernatant from treated cells is considerably greater, amounting to 125% (curve B). The rise of curve B for the first 45 min. seems to indicate that the optimum concentration inside the cells of a metabolite contained in the treated supernatant, and

responsible for the rise in oxidation rate, is only gradually reached or that this substance required further metabolism by the bacteria.

The same experiment was carried out with the supernatant fluid of dead (freeze-dried) cells after incubation $\pm$ *cis*-vaccenic acid. Both supernatant fluids caused an increase of O_2 uptake by 80–90% compared with cells in a fresh medium, which is due to substances extracted from the dead bacteria during incubation. There was, however, no difference between the oxidation rates obtained in either of the supernatant fluids. The effect shown in Fig. 8 therefore requires the activity of the living cell.

Aggregation. During incubation with *cis*-vaccenic acid ($a:c = 1:20$) marked aggregation of cells, visible macroscopically, occurred after about 3–4 hr. The aggregation can temporarily be broken by vigorous shaking of the suspension; this must be done immediately before the measurement when turbidity determinations are made, as the aggregates tend to reform quickly. A similar aggregation has been described by Saz (1949) for tubercle bacilli in presence of long-chain carbon compounds some of which inhibit the O_2 uptake of the bacilli. While Saz assumes that the inhibition of respiration of aggregating cells may be due to a decrease or change in the total free surface of the bacilli, no inhibition of the growth or respiratory rate of aggregated cells has been found in these experiments.

Effects of trans-vaccenic acid. A few experiments only have been carried out with *trans*-vaccenic acid in order to compare its activity with that of the *cis*-form. These concerned the effect on growth with the ratio $a:c = 1:20$, on O_2 uptake (Fig. 7) and on the rate of respiration after 16 hr. incubation with $1:25,000$ acid ($a:c = 1:20$). In all three cases *trans*-vaccenic had about 40–50% of the activity of the *cis*-form.

DISCUSSION

The 'double action' of fatty acids refers, in the first instance, to the very narrow margin between inhibiting and stimulating doses. The term might also be applied to the phenomenon, that the same concentration of acid, acting on a given amount of cells, produces at first an inhibition and later an increase of growth. Most workers have concentrated on one or other of the two aspects. Thus Williams & Fieger (1946), dealing with the growth-promoting effect of oleic acid on lactobacilli, state that the growth in presence of oleic acid exceeds that of the controls only after 72 hr., and have chosen the 72 hr. period for their acidimetric titration. They noted, however, that oleate, up to 48 hr., produced a marked inhibition both in presence and in absence of added biotin. Kodicek (1949), on the other hand, in studying the bacteriostatic effect of fatty acids, has mainly concentrated on the 48 hr. period, stating that even

with the most efficient inhibitor (linolenic acid) 'growth and acid production were depressed over a period of at least 72 hr.', according to the concentration of the acid. In his experiments the period of inhibition (48 hr.) seems also to have been followed by one of normal and, in the case of oleic acid, of increased growth. The two properties of unsaturated fatty acids, therefore, namely stimulation or inhibition either by varying concentrations of acid at a given time or by the same concentration of acid at different times, must be considered as closely related and, most probably, as having the same basis. Both effects have been observed in the present experiments, taking the rate of respiration as a criterion (see Fig. 4 at 180 min. and Fig. 5).

These experiments also show that inhibition of growth and of respiration are simultaneous. Below a certain concentration of acid the respiratory rates eventually regained or exceeded the initial level. When the respiratory rate returned to the initial value a lag period set in, except with the lowest concentration of acid, which was followed by normal growth. It seems, therefore, justifiable to conclude that inhibition of growth is due to a temporary blocking of the respiratory system, as would be expected to be the case, at least with a strictly aerobic organism, like *Bacillus subtilis*. A similar parallelism between bacteriostasis and inhibition of respiration has previously been described for linoleic acid acting on *Mycobacterium tuberculosis* (Bergström, Theorell & Davide, 1946). In their experiments the margin between non-effective and inhibitory doses of linoleic acid was very narrow and, with higher doses, bacteriostasis led, after about 18 hr., to the death of the cells. Two factors seem, therefore, to be involved in recovery from inhibition of growth and of respiration, especially with higher concentrations of acid, namely, the ability of the cell to survive protracted respiratory inhibition, and also the ability to overcome or reverse it. The former condition is probably fulfilled with the majority of aerobic bacteria, but may not be with *M. tuberculosis*, especially if toxic products of anaerobiosis accumulate.

The mechanism by which respiration is temporarily inhibited has not been established. However, a purely physico-chemical mechanism, acting by surface adsorption (for theory and literature see Kodicek, 1949) does not seem adequate for some of the observed effects, especially the reversibility of the respiratory inhibition, for this would necessitate, besides an active incorporation of fatty acids into the cell wall, a specific rearrangement with time of the adsorbed molecules coupled with a gradual binding by preformed inhibitors in the cell wall leading to innocuous complexes.

On the other hand, combination of the fatty acid with substances such as albumin, cholesterol and

lecithin, which suppress the effect, is instantaneous, as has been shown in experiments on haemolysis by *cis*-vaccenic acid (Laser, 1949). The observed effects, therefore, especially the reversibility of respiratory inhibition, seem to point to reactions caused by acid which has penetrated into the cell. Slater (1949) has shown that detergents, especially *cis*-vaccenic acid, are non-specific inhibitors of the succinic-oxidase system, by affecting the mutual accessibility of the components of the system. It is therefore suggested that the inhibition of oxygen uptake is due to the effect of the acid on intracellular oxidizing catalysts, and the reversibility to the ability of the organisms to metabolize the acid which has entered.

It is not assumed that, under the experimental conditions, the whole of the acid added enters the cells because (1) the marked aggregation of the organisms on incubation with vaccenic acid indicates its persistent adsorption on the cell surface accompanied by physical changes in the cell membrane, (2) recovery of *cis*-vaccenic acid from the organisms immediately after its addition and after 16 hr. incubation ($a:c=1:20$), as tested by the haemolytic activity of serial dilutions of the recovered acid (Laser, 1949), showed no significant difference between the two samples. Unless the metabolic product of the acid has the same haemolytic activity as the original acid, the amount which, it is assumed, penetrates the cell and is metabolized is only a small fraction ($<1\%$) of the added amount, too small to be detected by difference in the haemolysis test. Nevertheless, since the ratio $a:c$ in these experiments was 1:20, and complete inhibition of the succinic oxidase system was shown by Slater to occur at a concentration of 1:4000, such an amount, though undetectable, might be effective.

It has been commented upon by several workers (Bergström *et al.* 1946; Pollock, 1949) that commonly occurring acids, like oleic acid, to which parasitic bacteria are normally exposed in their natural environment, produce a marked bacteriostatic effect *in vitro*. The experiments dealing with repeated addition of acid offer an explanation for this discrepancy, as growth and respiration were unaffected by a second addition of acids to cells which had recovered from the primary inhibition. Here too it is improbable that the effect is due solely to surface phenomena, such as the formation of a protective surface film around the cells making them inaccessible to a second amount of added acid, partly for the reasons already stated, but mainly because resistance to renewed exposure to acid persisted even after prolonged (16 hr.) incubation with and growth in presence of acid. By this time the original cell population had increased several fold, i.e. the ratio $a:c$ had changed from 1:20 to $<1:100$. Even assuming that each new cell had a protective film of vaccenic acid molecules, it seems difficult to visualize

that it should protect cells from a further addition of acid leading to a ratio $a:c=1:11.5$ in view of the fact that initial addition of acid at ratios $a:c=1:20$ or $1:100$ produced quantitatively a very marked difference.

It is suggested, therefore, that on first addition of acid a penetration gradient is established, and that, within limits, at each concentration of acid a fraction which varies with the concentration enters the cell; once this process has come to a steady state, thereby inhibiting the respiration, the entered acid is gradually metabolized, and the end product causes an increase in the oxygen uptake and, under suitable conditions, stimulates the growth rate. The resistance of the organisms to further addition of acid may be due to a process resembling the acquired drug resistance of bacteria. The processes may not, however, be entirely analogous, as in the present case the cells were grown throughout in presence of vaccenic acid and had not been subcultured in its absence after recovery from the initial growth inhibition. Resistance in these experiments, furthermore, also developed in washed cell suspensions, which is not the case in acquired drug resistance, except in the case of the acridines. It is hoped to examine this point in further experiments. However, the conditions with regard to the continuous presence of, and exposure to, the acid are similar to those obtaining in the body.

The greater efficiency of the *cis*- than of the *trans*-form of the acid has also been observed with other acids for either growth inhibition (Kodicek, 1949), or for growth promotion (Shull *et al.* 1949).

SUMMARY

1. *cis*-Vaccenic acid, within the range of the concentrations used, produces a concomitant inhibition of growth and respiration of *Bacillus subtilis*.

2. The respiratory inhibition is transient, its degree and duration being dependent on the ratio of acid to cells.

3. The organisms, after recovering from the inhibition of oxygen uptake, respire at a considerably increased rate and grow, in presence of yeast extract, at a normal or slightly increased rate.

4. Growth and respiration of acid-treated recovered cells are unaffected by a second addition of acid.

5. The supernatant fluid from treated recovered cells causes an increase in respiration of fresh cells.

6. It is concluded that physico-chemical surface phenomena cannot by themselves account for all the observed facts, which can be more readily explained on the basis of a penetration of acid into the cell, an inhibition of the oxidative system inside the cell, an ability of the organisms to metabolize penetrated acid, and the development in treated cells of a mechanism resembling that in acquired drug resistance.

7. *trans*-Vaccenic acid has about half the activity of the *cis*-form.

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