sion are cited. A convincing analysis of repressed enzyme formation in the pathway leading to pyrimidine compounds has been carried out by R. A. Yates and A. B. Pardee (*J. Biol. Chem.* [in press] and personal communication).

[2] Induction and repression may respectively be brought about by inducers and repressers acting separately. When, however, an inducer and a represser of one and the same enzyme are present simultaneously, an antagonistic effect involving the two regulators may be expected to result. More generally, it is possible that inducer or represser action is antagonizable by substances that, in themselves, may or may not be regulators.

[3] S. Spiegelman and A. M. Campbell, in D. E. Green (ed.), *Currents in Biochemical Research, 1956* (New York: Interscience Publishers, Inc., 1956), p. 115.

[4] M. R. Pollock, in E. F. Gale and R. Davies (eds.), *Adaptation in Micro-organisms* (Cambridge: At the University Press, 1953), p. 150.

[5] C. E. Dalgliesh, *Science*, **125**, 271, 1957.

[6] F. Haurowitz, *Chemistry and Biology of Proteins* (New York: Academic Press, Inc., 1950).

[7] M. Cohn and J. Monod, in E. F. Gale and R. Davies (eds.), *Adaptation in Micro-organisms* (Cambridge: At the University Press, 1953), p. 132.

[8] Final shaping may include one or more of the following: (*a*) the folding or other intramolecular arrangement of a nascent template product; (*b*) the aggregation of products from the same or from different templates; (*c*) the combination of a template product with another type of substance; (*d*) the breaking of one or more bonds within a template product; and (*e*) the removal of a portion of a template product. The term "dynamic site" refers to that region of an enzyme which is primarily associated with the enzyme's characteristic catalytic action.

[9] J. Monod, *Growth*, **11**, 223, 1947.

[10] F. Lipmann, in D. E. Green (ed.), *Currents in Biochemical Research, 1956* (New York: Interscience Publishers, Inc., 1956), p. 241.

[11] Clearly, regulator action may depend either on both these effects or primarily on one or the other.

[12] J. Monod and M. Cohn, *Advances in Enzymol.*, **13**, 67, 1952.

[13] J. Monod, G. Cohen-Bazire, and M. Cohn, *Biochim. et Biophys. Acta*, **7**, 585, 1951. This paper includes an example of the specific inhibition of induced enzyme formation by an analogue of the inducer. Such inhibition would fall under the definition of enzyme repression given previously (Vogel, *op. cit.*).

[14] M. Cohn and A. Torriani, *Biochim. et Biophys. Acta*, **10**, 280, 1953. See also M. Cohn, Discussion of paper by Cohn and Monod, *op. cit.*, p. 149.

[15] J. Mandlestam, *Internatl. Rev. Cytol.*, **5**, 51, 1956.

[16] J. Yudkin, *Biol. Revs.*, **13**, 93, 1938.

[17] J. Mandlestam, *Biochem. J.*, **51**, 674, 1952.

[18] S. Spiegelman, *Cold Spring Harbor Symposia Quant. Biol.*, **11**, 256, 1946.

[19] J. Monod, in O. H. Gaebler (ed.), *Enzymes: Units of Biological Structure and Function* (New York: Academic Press, 1956), p. 7.

[20] The inducer would thus act as the master model or prototype of the (structurally complementary) dynamic site.

THE SITES OF ORTHOPHOSPHATE UPTAKE BY BARLEY ROOTS

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Kinetic analysis of measurements of steady-state uptake has established the extent to which the $HPO_4^{=}$ and $H_2PO_4^{-}$ ions contribute to the total uptake of orthophosphate by excised barley roots.[1] The binding compounds involved in the rate-limiting step of orthophosphate uptake are shown here to be components of the

respiratory chain. Further, the point where each phosphate species is coupled to the electron-transfer system is shown to be the site of energy conservation during oxidative phosphorylation by mammalian mitochondria.[2-4] The bearing of these results on theories of ion uptake is not developed. Discussion rather awaits results on the pathways of uptake of ions other than phosphate.

KINETICS OF PHOSPHATE UPTAKE

Uptake of ions by plant roots arises from an initial combination between an ion and a specific binding compound, as written in equation (1). Breakdown of this labile ion complex results in the uptake of ions into the system, as expressed by equation (2).

$$R + M_{\text{outside}} \underset{k_2}{\overset{k_1}{\rightleftarrows}} MR, \tag{1}$$

$$MR \underset{k_4}{\overset{k_3}{\rightleftarrows}} R' + M_{\text{inside}}, \tag{2}$$

where R represents a binding site or compound; M, the ion; MR, the labile intermediate; k, the rate constant for each reaction. The rate constants of association and dissociation, k_1 and k_2, of the intermediate, MR, are large with respect to the rate constant, k_3, of the breakdown of the intermediate; k_4 is negligible.

Uptake of the ion into the root may be described by the velocity, v, in the linear equation (3), when the concentration of the external ion is held constant and the concentration of R is constant during the period of absorption. Equation (3) is analogous to the steady-state expression describing the kinetics of enzyme reactions.[5, 6]

$$v = -\frac{v}{[M]} K_m + V_{\max}. \tag{3}$$

The maximum uptake at infinite ion concentration is $V_{\max}$, and K_m denotes the apparent dissociation constant of the intermediate ion complex, MR. When the steady-state uptake, v, by excised barley roots from single-salt solutions of alkali cations,[7] as well as halides,[8] is plotted against $v/[M]$, a straight line results over a wide range of concentrations. Thus a single first-order reaction is involved in the rate-limiting step of uptake for each of these monovalent ions, with the maximum uptake, $V_{\max}$, given directly by the ordinate intercept and the apparent K_m by the slope, according to equation (3). The steady-state uptake measurements of orthophosphate, when plotted in this manner, are curvilinear, because two first-order reactions are acting simultaneously, but independently on different substrates which are in a non-rate-limiting equilibrium.

Steady-state analysis of the measurements of orthophosphate uptake from solutions containing various concentrations of orthophosphate (10^{-6}–10^{-3} M) and hydrogen ion (pH 4–8) leads to the quantitative interpretation that excised barley roots absorb the ion species $H_2PO_4^-$ through one site and $HPO_4^=$ through another.[1] The system is described by the following reactions:

$$H_3PO_4 \rightleftarrows H^+ + H_2PO_4^- \rightleftarrows H^+ + HPO_4^= \rightleftarrows H^+ + PO_4^{\equiv}, \tag{4}$$

$$R_a^+ + H_2PO_4^- \rightleftarrows R_aH_2PO_4, \quad (5)$$

$$R_b^+ + HPO_4^= \rightleftarrows R_bHPO_4^-. \quad (6)$$

Further, the hydroxyl ion, OH^-, competitively inhibits the uptake of both ion species without affecting the concentration of either of the binding sites:

$$R_a^+ + OH^- \rightleftarrows R_aOH, \quad (7)$$

$$R_b^+ + OH^- \rightleftarrows R_bOH. \quad (8)$$

The concentration of all the intermediate ion complexes, expressed in equations (5)–(8), approximate equilibrium values, since their rate constants of association and discussion are large in comparison with the rate constant of breakdown, k_3. Thus the observed velocity of steady-state uptake, v, is directly proportional to the concentration of the ion-complex intermediates at all values of orthophosphate concentration. However, the total phosphate associated with the root, as measured in uptake studies, also includes the equilibrium value of the phosphate in combination with both sites. The observed uptake where these values contribute a significant amount to the total is denoted as v', as expressed in equation (9):

$$v' = \{[R_aH_2PO_4]k_{3a} + [R_bHPO_4^-]k_{3b}\}t + [R_aH_2PO_4] + [R_bHPO_4^-]. \quad (9)$$

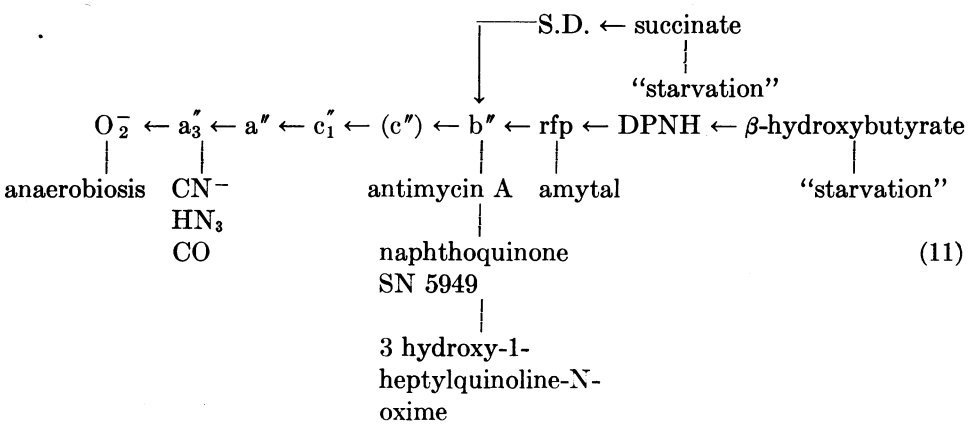
Sorption periods measured in seconds show a significant amount of phosphate in association with the roots because of the equilibrium value of the intermediate phosphate-site complexes (Fig. 1). Sixty-second absorption measurements must, therefore, be expressed by equation (9), where a plot of v' against $v'/[\Sigma P]$ (Fig. 2) results in a numerical value of the ordinate intercept which describes $V_{max\ a}$ and $V_{max\ b}$ for each reaction. The numerical values of $[R_aH_2PO_4]$ and $[R_bHPO_4^-]$ are equal to $[R_a]$ and $[R_b]$ at infinite substrate concentration ($v'/[\Sigma P] = 0$), since the sites exist completely in the combined form. Therefore, the maximum uptake for 1-minute absorption periods is expressed as follows:

$$V'_{max\ a} + V'_{max\ b} = \{[R_a]k_{3a} + [R_b]k_{3b}\}t + [R_a] + [R_b]. \quad (10)$$

Inspection of equation (9) shows that the phosphate in combination with the sites becomes negligible with respect to the total phosphate absorbed with longer periods of uptake, t. The 3-hour sorption periods used in previous studies[1] were of sufficient duration to express uptake solely as a velocity.

RELATIONSHIP OF PHOSPHATE UPTAKE TO RESPIRATION

The hypothesis for electron transport in the oxidative phosphorylation system has been extended by the accurate measurement and rigorous interpretations of the effects of substrates and inhibitors on the oxidation-reduction levels of the components of the respiratory chain.[9, 10] Certain inhibitors intercept electron transport at different points along the respiratory chain. These inhibitors cause those components on the oxygen side of the interaction to become more oxidized, while those on the substrate side become more reduced. Other types of inhibitors restrict or block electron flow and cause a difference in the steady-state oxidation-reduction level of the components of the respiratory chain. Each type of inhibitor has been studied with respect to the point of interruption of electron transport,[9] as expressed by the following scheme:



(11)

The sites at which energy-conservation reactions occur in isolated mammalian liver mitochondria[2-4] and in intact baker's yeast[4, 9] have been shown to be reduced cytochrome c, reduced cytochrome b, and DPNH.

A kinetic interpretation of the effect of similar respiratory chain inhibitors on the absorption of orthophosphate shows that reduced cytochrome b and DPNH are the components of the respiratory chain involved in the rate-limiting steps of orthophosphate uptake by excised barley roots.

The method for determining the components of the respiratory chain taking part in the rate-limiting steps of orthophosphate uptake is based on the following applications of first-order reaction kinetics:

a) A separation of the component reactions involved in both the presence and absence of an inhibitor, according to Figure 2, determines whether the effect is on one or both of the first-order reactions involved in orthophosphate uptake.

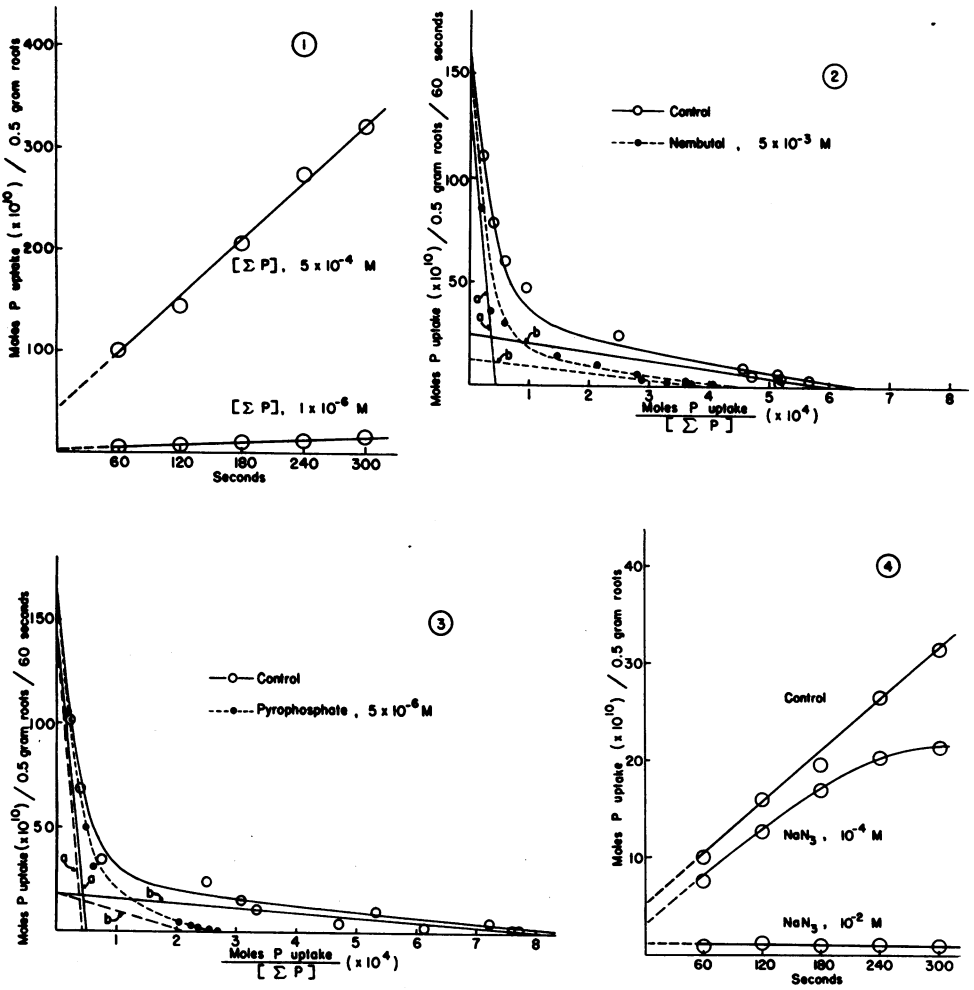
b) A change in slope of either reaction in the presence of an inhibitor (Fig. 3) reflects an apparent change in K_m. A strictly competitive type of inhibitor is denoted by a change in K_m without change in V_{max}'.

c) An inhibitor which changes the intercept with the ordinate, V_{max}', of either reaction reflects an effect on either the respective concentration of the site, $[R]$, k_3, or both, according to equation (10).

d) Measurements of uptake with time permit a further separation of the effect of an inhibitor which changes the V_{max} of either reaction. When absorption is plotted against time in the presence of an inhibitor, a change in the slope of the resulting curve, with no change in the ordinate intercept, reflects an effect solely on k_3, according to equation (10). An effect on $[R]$ is reflected by both the slope and the intercept changing by the same ratio in the presence of an inhibitor.

EXPERIMENTAL METHODS

Excised roots from 6-day-old seedlings of *Hordeum vulgare*, variety Atlas 46, were used as the experimental material. The seedlings were grown essentially as described by Epstein and Hagen.[7] The seeds were soaked for 24 hours in continuously aerated demineralized water and then planted on a layer of cheesecloth supported by a stainless-steel screen suspended over a solution of 2×10^{-4} *M* $CaSO_4$. Roots were excised from the seedlings, which were grown for 5 days in a dark chamber maintained at 24° C.



FIGS. 1–4.—The uptake of orthophosphate by 0.5 gm. of excised barley roots. Fig. 1, from 1×10^{-6} and 5×10^{-4} *M* orthophosphate as a function of time. Fig. 2, as a function of orthophosphate uptake divided by total orthophosphate concentration with separation of the component reactions, in the presence and absence of nembutal. Fig. 3, as a function of orthophosphate uptake divided by total orthophosphate concentration with separation of the component reactions, in the presence and absence of pyrophosphate. Fig. 4, from 2×10^{-6} *M* orthophosphate in the presence and absence of azide.

The concentration of orthophosphate remained essentially constant over an absorption period of 5 minutes, using 25-ml. quantities of solution with each 0.5-gm. portion of excised roots. The roots, which were placed in test tubes, were rinsed three times with demineralized water. Single-salt solutions, at pH 4.0, of orthophosphate containing P^{32}, both in the absence and in the presence of various concentrations of substrate or inhibitor, were added to the roots at zero time. The pH value remained constant to within 0.1 unit during the absorption periods. At pH 4, the substrates and inhibitors were predominantly in the undissociated form, which has been shown to penetrate cells more readily than the charged ions.[11–13] At the end of the absorption period, the roots were rinsed four times with deminer-

alized water, which was sufficient to remove the phosphate not associated with active uptake. The absorption of phosphate was determined from the P^{32} associated with the roots.[1]

EXPERIMENTAL RESULTS

Location of Phosphate-binding Sites.—Amytal intercepts electron transport between DPNH and flavoprotein.[9] The presence of this inhibitor causes all the components in the respiratory chain on the substrate side of flavoprotein to become reduced, while those on the oxygen side become oxidized. A decrease of orthophosphate uptake by barley roots was observed on testing with either amytal or nembutal. Separation of the component reactions showed a decrease solely of $V'_{\max b}$ (Fig. 2). Both the slope and the intercept decreased by the same ratio when the phosphate uptake was measured as a function of time; therefore, the decreased uptake of the $HPO_4^{=}$ ion was due to a decrease solely of $[R_b]$. The action of amytal and nembutal establishes that the rate-limiting step of $HPO_4^{=}$ uptake could be either an oxidized component of the respiratory chain on the substrate side of flavoprotein or a reduced component on the oxygen side.

Paraphenylenediamine has been demonstrated to be a reductant of cytochrome c. All components in the respiratory chain toward the substrate side of cytochrome c become reduced in its presence. An increase in uptake occurred when the effect of this inhibitor was tested on orthophosphate uptake. Similar results were obtained with naphthoquinones, methylene blue, and 2,3-dimercaptopropanol. Separation of the component reactions showed an increase in $V'_{\max b}$, with no effect on $V'_{\max a}$. Measurement of the phosphate uptake as a function of time established that $[R_b]$ had been increased, since both the intercept and the slope increased by the same ratio. The deduction is that the rate-limiting step of $HPO_4^{=}$ uptake is coupled to reduced cytochrome b, since only changes in $[R_b]$ are involved both in the increase of $HPO_4^{=}$ uptake in the presence of this type of inhibitor and in the decrease of $HPO_4^{=}$ uptake in the presence of nembutal and amytal. Further, the uptake of $H_2PO_4^{-}$ is coupled to a reduced component of the electron-transfer system toward the substrate side of flavoprotein.

Addition of acetoacetate, the product of β-hydroxybutyrate oxidation, inhibited both $HPO_4^{=}$ and $H_2PO_4^{-}$ uptake. The inhibition was a function of a decrease in $V'_{\max b}$ and $V'_{\max a}$. The inhibition was further resolved by time studies to show that a decrease solely in $[R_a]$ and $[R_b]$ had occurred. Thus $H_2PO_4^{-}$ uptake is coupled to a reduced component of the respiratory chain which lies between the substrate and flavoprotein: the DPNH.

Systems in the steady state of aerobic metabolism generally maintain cytochrome b in a considerably less reduced state than DPNH, which is largely reduced. A measurable effect on $HPO_4^{=}$ uptake could occur with a negligible change in $H_2PO_4^{-}$ uptake in the presence of inhibitors which cause both cytochrome b and DPNH to become more reduced. This is the case, apparently, in the nonmeasurable effect of *p*-phenylenediamine, nembutal, and amytal on the DPNH which is coupled to $H_2PO_4^{-}$.

Site Concentrations.—Chance and Williams[14] have shown that the concentration of DPNH exceeds many fold that of cytochrome b in intact mammalian mitochondria. The relative concentrations of the two sites involved in the rate-limiting

step of orthophosphate uptake by barley roots may be approximated without reference to the chemical identity of the sites. The ratio of the concentrations provides a criterion for corroboration of cytochrome b and DPNH as the two sites of uptake. Most of the total phosphate associated with the roots from solutions of 1×10^{-6} M orthophosphate (where $v'_{a+b}/[\Sigma P] = 5 \times 10^{-4}$) is contributed by the $HPO_4^{=}$ ion (Fig. 2). Thus an approximation of $[R_bHPO_4^{-}]$ can be determined from the value of the intercept at zero time obtained from extrapolation of uptake measurements with time (Fig. 1). A value of k_{3b} can be calculated by substitution of a value of v_b' and $[R_bHPO_4^{-}]$ at 1×10^{-6} $M[\Sigma P]$, obtained from Figure 1, into the expression for $HPO_4^{=}$ uptake, equation (12):

$$v_b' = [R_bHPO_4^{-}]k_{3b}t + [R_bHPO_4^{-}]. \quad (12)$$

Solution of the equation gave a value for k_{3b} of 2.3×10^{-2} moles P/mole $R_b \times$ sec. The steady-state uptake of $HPO_4^{=}$ is directly proportional to the concentration of the $R_bHPO_4^{=}$ at all values of orthophosphate concentration. Thus a value for $[R_b]$ of 11.4×10^{-10} moles R_b/0.5 gm roots is obtained when the value for k_{3b} is substituted in equation (13), which expresses the maximum absorption where $[R_b]$ is completely saturated with $HPO_4^{=}$; the $[R_b]$ is equal to $[R_bHPO_4^{-}]$:

$$V'_{\max\ b} = [R_b]k_{3b}\,t + [R_b]. \quad (13)$$

Essentially total saturation of R_b is obtained at 5×10^{-4} M orthophosphate concentrations (where $V'_{a+b}/[\Sigma P] = 0.2 \times 10^{-4}$) (Fig. 2) and $[R_bHPO_4^{-}]$ is approximately equal to $[R_b]$. The value of $[R_aH_2PO_4]$ is obtained by subtraction of $[R_b]$, since the intercept of the time curve for concentrations of orthophosphate of 5×10^{-4} M is a measure of phosphate in combination with both sites (Fig. 1). Total uptake at 5×10^{-4} M $[\Sigma P]$ may therefore be expressed as

$$v'_{a+b} = \{[R_aH_2PO_4]k_{3a} + [R_b]k_{3b}\}t + [R_aH_2PO_4] + [R_b], \quad (14)$$

where numerical values of $[R_aH_2PO_4]$, $[R_b]$, k_{3b} are known approximately and the value of v'_{a+b} is obtained from Figure 1. Solution of equation (14) gave a value of k_{3a} of 1.4×10^{-6} moles P/mole $R_a \times$ sec. Substitution of this value into an expression for $V'_{\max\ a}$ results in a value for $[R_a]$ of 78×10^{-10} moles R_a/0.5 gm roots. The relative concentration of R_b to R_a must be at least 1:7, a ratio which is of the same magnitude as the ratio of cytochrome b to DPNH in mammalian mitochrondria.

Actions of Competitive Inhibitors.—Adenosine diphosphate and pyrophosphate similarly inhibited orthophosphate uptake by barley roots. In the presence of a 5×10^{-6} M concentration of either inhibitor, a separation of the component reactions showed a change in K_{mb} with no change in $V'_{\max\ b}$ (Fig. 3), with no measurable effect on the $H_2PO_4^{-}$ uptake at this concentration. Within the range of inhibitor concentrations from 1×10^{-4} to 1×10^{-1} M, ADP and pyrophosphate decreased the uptake of both $HPO_4^{=}$ and $H_2PO_4^{-}$. The inhibition was shown to be a function solely of a change in apparent K_{ma} and K_{mb}. The inhibition of $H_2PO_4^{=}$ uptake became apparent only at the high concentrations of the inhibitor, because of a lesser affinity for the $H_2PO_4^{-}$ site than for the $HPO_4^{=}$ site. The effect of either ADP or pyrophosphate was competitive to the uptake of both $H_2PO_4^{-}$ and $HPO_4^{=}$, since the inhibition was abolished by increasing concentrations of orthophosphate.

Several schemes describing oxidative phosphorylation implicate an acceptor which forms an inorganic phosphate–ADP complex. Since R_a and R_b exist completely in the forms $R_aH_2PO_4$ and $R_bHPO_4^-$ at infinite orthophosphate concentrations, additions of ADP would be expected to increase the maximum uptake, $V'_{\max\ a+b}$, if such a complex were essential to uptake. However, $V'_{\max\ a}$ and $V'_{\max\ b}$ are unchanged by the additions of ADP. Either the endogenous ADP differs from the ADP in solution, or a phosphate-ADP complex is not required in the rate-limiting step of orthophosphate uptake by barley roots.

The competitive inhibition by hydroxyl ion,[1] DNP,[15] ADP, and pyrophosphate implies that these competitive inhibitors combine with the same sites involved in the rate-limiting steps of orthophosphate uptake: the reduced cytochrome b and DPNH. The relatively high affinity of the hydroxyl ion[1] suggests that the sites of phosphorylation may exist primarily in combination with the hydroxyl ion. Competitive inhibition of orthophosphate uptake implies a direct reaction of the orthophosphate species and the competitive inhibitors with the sites involved. First-order reaction kinetics cannot exclude the possibility that the actual competition occurs at some intermediates which are coupled to cytochrome b and DPNH.

Inhibitors Which Block Electron Flow.—Azide is believed to block electron flow by combining with the oxidized form of the terminal oxidase, cytochrome a_3, which results in the components of the respiratory chain becoming more reduced.[3] The presence of a low concentration of azide (10^{-6}–10^{-5} M) caused a small increase in uptake from 10^{-6} M orthophosphate. The increased phosphate uptake was a function primarily of an increased $[R_b]$, with no measurable change in the value of k_{3b}. A decrease in the apparent rate of breakdown of the phosphate-site complexes, k_{3a+b}, is obligatory upon an electron restriction, since oxidative phosphorylation is a direct function of electron flow. The apparent absence of a change in the value of k_{3b} is in accordance with Chance's observation that large changes in the oxidation-reduction levels of components involved in electron transfer are observed with only a small inhibition of respiration over a considerable range of azide concentrations. With greater azide concentrations (10^{-4} M), the rate of phosphate uptake decreased with increasing times of sorption (Fig. 4). This deviation from steady-state uptake was peculiar to this type of inhibitor. Inspection of the initial slope of the time curve, where steady-state uptake was approximated (Fig. 4), showed that a decrease in $[\Sigma R]$ had occurred. Further, a separation of the component reactions after 1-minute sorption periods ascribed the inhibition to a decrease of $[R_b]$. Increasing concentrations of orthophosphate did not overcome the inhibition. Thus, to be consistent with the previous interpretations, an oxidation of cytochrome b, R_b, must be assumed at these concentrations of azide. In the presence of even greater concentrations of azide (10^{-2} M), a drastic decrease in the apparent k_{3a+b} was evident, as well as an even further decrease in $[\Sigma R]$. Separation of the component reactions showed that the phosphorylations coupled to both R_a and R_b are decreased. Increasing orthophosphate concentrations did not overcome the inhibition in either case, and an oxidation of both cytochrome b and DPNH must have occurred. The contribution of $HPO_4^=$ uptake is small in comparison with total uptake in the presence of 5×10^{-4} M orthophosphate. At this phosphate level, uptake is inhibited over the whole range of azide concentrations, with a significant decrease in both $[R_a]$ and k_{3a}.

The substrate-inhibitor method for the examination of a respiratory chain is based on the premise that components of the respiratory chain on the oxygen side of the point of action of an inhibitor become more oxidized and those on the substrate side more reduced. The increased uptake of orthophosphate at low azide and phosphate concentrations is in accordance with the reduction of the components of the respiratory chain induced by an azide block of electron flow at cytochrome a_3. However, higher concentrations of azide inhibit uptake by decreasing both the k_{3a+b} and $[R_{a+b}]$. A secondary site of action of azide with a respiratory component toward the substrate side of DPNH must be assumed on the basis of the substrate-inhibitor method. Further, the deviation from steady-state uptake with time suggests that azide combines with a state of the respiratory component which is a product arising from electron transfer. The conclusion is that azide combines with the oxidized form, DPN, as well as with cytochrome a_3, on the basis that the current hypothesis adequately describes the components of the respiratory chain involved in oxidative phosphorylation (eq. [11]).

The recent finding that cyanide combines with the oxidized form, DPN,[16] suggested a study of the effect of cyanide on orthophosphate uptake. The characteristics of the cyanide inhibition were identical with those of azide, although slightly greater effective concentrations of cyanide were required to effect the same degree of inhibition as azide.

Action of Substrates.—The respiratory chain responds differentially to the type of substrate which is dehydrogenated. The respiration rates of intact mammalian mitochondria, for instance, have been shown to be about three times larger with succinate as a substrate than with β-hydroxybutyrate.[17] Further, a greater reduction of components of the respiratory chain occurred with the greater rate of electron flow effected by succinate.[18]

Mitochondrial preparations, after starvation periods of relatively short duration, essentially are depleted of endogenous substrates. However, excised barley roots are not amenable to a quantitative depletion of endogenous substrates, and addition of a high concentration of substrate is required for assurance that the measured response is a function primarily of the prevailing substrate.

Succinate addition to an effective concentration of 1×10^{-1} M decreased the uptake of orthophosphate by excised barley roots. Separation of the component reactions showed the inhibition to be essentially a function of a decrease in $V_{\max\ a}$. Measurement of phosphate uptake as a function of time showed that the slope decreased to a lesser extent than the intercept (Fig. 5). Thus the effects of a high concentration of succinate on phosphate uptake were resolved as a decrease in $[R_a]$ and an increase in the apparent k_{3a+b}. The prevailing concentration of succinate severely restricts electron flow through the competing pathway of β-hydroxybutyrate. The decrease in $[R_a]$, therefore, results from the DPNH becoming more oxidized with the slower rate of electron flow through the β-hydroxybutyrate pathway.

A significantly larger decrease in uptake was effected by a 1×10^{-1} M concentration of β-hydroxybutyrate. An analysis of the effect, identical with that used for succinate, resulted in the conclusion that β-hydroxybutyrate had decreased significantly both $[R_b]$ and the apparent k_{3a+b}. A severe restriction of electron flow through the succinate pathway is effected by the competing pathway of β-hydroxy

butyrate. This effect alone would not necessarily decrease $[R_b]$, since both cytochrome b and DPNH are involved in the pathway of the reducing reaction. However, the decrease in k_{3a+b} showed that a slower rate of electron flow was effected by this substrate than by succinate. The slower electron flux results in cytochrome b becoming more oxidized.

These experimental observations are in qualitative agreement with the current hypothesis of oxidative phosphorylation in the electron transport system.[9] Inspection of the chemical representation of the respiratory chain (eq. [11]) shows that the electrons from both the succinate and the β-hydroxybutyrate pathways of reducing reactions converge on cytochrome b. The dehydrogenation of a substrate such as β-hydroxybutyrate, which requires DPN in the pathways of reducing reactions, results in the uptake of both the $H_2PO_4^-$ and $HPO_4^=$ ions which are coupled to DPNH and reduced cytochrome b, respectively. The values of k_{3a} and k_{3b} must be equal, since the rate of breakdown of both $R_aH_2PO_4$ and $R_bHPO_4^=$ is a direct function of electron flow, and the breakdown of both occurs with the transfer of an electron pair. The magnitude of their numerical value is a measure of the rate of electron flow effected by the β-hydroxybutyrate pathway.

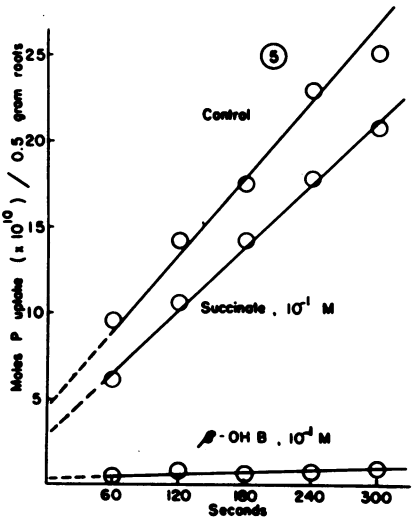


FIG. 5.—The uptake of orthophosphate from 0.5 gm. of excised barley roots from 1×10^{-6} *M* orthophosphate in the presence and absence of succinate or β-hydroxybutyrate.

The succinate pathway does not involve DPNH but is linked to cytochrome b. In this instance, however, the numerical value of k_{3b} is a measure of the rate of electron flow effected by the succinate pathway. The value of k_{3a+b} was shown to be increased by succinate and decreased by β-hydroxybutyrate. A greater value of k_{3b} must have occurred in the presence of succinate than with β-hydroxybutyrate, since k_{3a} is a function of β-hydroxybutyrate dehydrogenation. Therefore, a more concise description must be accorded k_{3b}—namely, $k_{3b\ \text{succinate}}$ and $k_{3b\ \beta\text{-OHB}}$. The apparent k_{3b} of control roots, utilizing an endogenous level of substrate, is a numerical value dependent on the Michaelis affinities, concentration, and ratio of the substrates.

Approximate values for $k_{3b\ \text{succinate}}$, $k_{3b\ \beta\text{-OHB}}$, and k_{3a} may be calculated on the supposition that a high concentration of an added substrate effects a single pathway of electron flow. Values of $[R_b]$ and v_b', obtained from the succinate curve of Figure 5, are substituted in equation (12), which expresses the uptake of the $HPO_4^=$ ion. Solution of the equation resulted in a value of 2.1×10^{-2} moles P/mole $R_b \times$ sec. Similar treatment of the measurements of uptake from 1×10^{-1} *M* β-hydroxybutyrate resulted in a value of 8.3×10^{-3} moles P/mole $R \times$ sec. This value expresses either k_{3a} or $k_{3b\ \beta\text{-OHB}}$, since both are equal in the β-hydroxybutyrate pathway.

The 2.5 times larger uptake of the $HPO_4^=$ ion with succinate than with β-hydroxybutyrate as a substrate is in close agreement with the 3 times larger respiration rate

shown with the same substrates by Chance and Williams for mammalian mitochondria.

Also, the value of 2.1×10^{-2} moles P/mole $R \times$ sec for $k_{3b\ \text{succinate}}$ is in essential agreement with the value of 2.3×10^{-2} moles P/mole $R \times$ sec for the k_{3a} calculated from kinetic analysis of uptake measurements of control roots utilizing an endogenous level of substrate. However, the value of 8.3×10^{-3} moles P/mole $R \times$ sec for either k_{3a} or $k_{3b\ \beta\text{-OHB}}$ is much lower than the value for k_{3a} of 1.4×10^{-2} moles P/mole $R \times$ sec which was calculated from uptake measurements of control roots. A study of the effect on orthophosphate uptake of numerous substrates which require DPN in the pathways of reducing reactions has resulted in the conclusion that the k_{3a} of glutamate corresponds most closely to the k_{3a} calculated from uptake measurements of excised barley roots utilizing an endogenous level of substrate.

CONCLUSIONS

Orthophosphate uptake by barley roots is coupled to sites identical with those for oxidative phosphorylation during electron transport in mammalian mitochondria.

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*THE NORM FORM OF A RATIONAL DIVISION ALGEBRA**

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Let $\mathfrak{D}$ be a finite-dimensional associative division algebra over the field $\mathfrak{R}$ of all rational numbers. Then the center $\mathfrak{F}$ of $\mathfrak{D}$ is an algebraic extension of finite degree over $\mathfrak{R}$, and $\mathfrak{D}$ is a cyclic algebra of degree n and dimension n^2 over $\mathfrak{F}$. We may then write $\mathfrak{D} = (\mathfrak{Z}, S, \gamma)$, where $\mathfrak{Z}$ is a cyclic field of degree n over $\mathfrak{F}$, γ is a nonzero