equilibrium value throughout the resin which can, however, be different from the concentration c in the supernatant solution, as has been pointed out by Davies and .Thomas.[1] We define a distribution coefficient λ between pore liquid and supernatant solution

$$\lambda = \bar{c}/c \qquad (3)$$

which can be larger or smaller than unity, depending on the respective strength of the interactions reactant–resin and reactant–solvent. Substituting $\bar{c}$ by $c\lambda$, and considering that the reaction occurs in the pores only, we obtain for the over-all rate in the heterogeneous system consisting of pore liquid and supernatant solution

$$-\left(\frac{dn}{dt}\right)_{het} = -\frac{d\bar{n}}{dt} = -\bar{V}\bar{k}c\lambda \qquad (4)$$

where n is the number of moles and $\bar{V}$ is the pore volume. Evaluating this equation $\bar{k}$ can be calculated. (Equation (4) implies that $d\bar{V}/dt = 0$, *i.e.*, that no swelling or shrinking occurs with the reaction.)

Experimental results of other authors[1-6] show that the over-all rate in heterogeneous systems obeys first order kinetics

$$-\left(\frac{dc}{dt}\right)_{het} = \frac{1}{V}\left(\frac{dn}{dt}\right)_{het} = k^*c \qquad (5)$$

where the formal constant k^* does not depend on c. Comparison of (5) and (4) show that therefore $\bar{k}\lambda$, and very likely both $\bar{k}$ and λ, should be independent of c.

This approach can be extended to systems with reverse reactions and with other than first order kinetics.

The complex mathematics of diffusion controlled catalysis has been dealt with for spherical beads by Smith and Amundson.[7] Their concept can be extended by introducing the distribution coefficient λ which was not included in their treatment.

Other investigators[1-4] have compared homogeneous and heterogeneous systems containing equivalent amounts of catalyst (*i.e.*, $(\bar{V}\bar{c}_{cat})_{het} = (\bar{V}c_{cat})_{hom}$), the homogeneous in a constant concentration throughout the system, the heterogeneous in a higher concentration in a part of the system. The efficiency q of a resin, as defined by Hammett,[2] is, in terms of this approach

$$q = \frac{k^*}{k} = \frac{\bar{k}\bar{V}}{k\bar{V}}\lambda = \frac{\bar{k}'}{k'}\lambda \qquad (6)$$

when the relations $k = k'c_{cat}$ (2) are assumed to hold for both pore liquid and homogeneous solution. Hitherto published results of other authors[1-6,8] can be accounted for qualitatively in terms of λ, though, of course, $\bar{k}'/k'$ might differ from unity.

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LIGHT INDUCED PHOSPHORYLATION BY CELL-FREE PREPARATIONS OF PHOTOSYNTHETIC BACTERIA[1]

Sir:

In the course of a study of phosphorylation with cell-free preparations from *Rhodospirillum rubrum* (strain S-1)[2] it was observed that light induced anaerobically a pronounced disappearance of orthophosphate. This could be demonstrated with added yeast hexokinase, mannose[3] and catalytic amounts of adenosine polyphosphates, or with substrate amounts of ADP.[4] In the latter case the disappearance of orthophosphate could be stoichiometrically accounted for by the increase in P_7[4] (Table I).

TABLE I

Experimental conditions	Time of incubation in minutes	ΔP/reaction vessel in μM. ΔP_i	ΔP_7
Dark, + ADP	10	+ 1.6	− 1.6
(10.3 μM.)	35	+ 2.3	− 2.5
Light, alone	10	− 0.3	+ 0.7
	35	− 0.5	+ 0.7
Light, + ADP	10	− 4.7	+ 4.3
(10.3 μM.)	35	−10.9	+10.7

The above crude sonic preparation was obtained by disintegrating washed cells in a 10 KC. Raytheon magnetostrictive oscillator for 4 minutes at 5°. The resulting suspension was centrifuged for 5 minutes at 10,000 × gravity. The sediment was discarded and the supernatant filtered through a fine porous glass filter to remove large cell fragments. The filtrate was used as indicated. The experimental suspension contained 13.4 mg. of protein and the following additions: 35 μM. $MgCl_2$, 30 μM. KF, 20 μM. P_i, 10.3 μM. ADP, 1.5 μM. DPN; total volume, 3 ml.; suspending medium: 0.2 *M* potassium salt of glycylglycine, *p*H 7.4. All experiments performed at 25°, under anaerobic conditions (helium); illumination by incandescent lamps; intensity, 1200 foot candles.

The ATP formed by the light induced reaction was further identified through the hexokinase catalyzed phosphorylation of glucose.

On centrifuging the preparation at 135,000 × gravity for one hour (*cf.*[5]), all the phosphorylating activity was found in the sediment. Repeated washing of the sediment produced a slight increase in the specific activity. α-Ketoglutarate increased

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(2) Cell-free suspensions prepared with modifications according to the method described by L. P. Vernon and M. D. Kamen, *Arch. Biochem. Biophys.*, **44**, 298 (1953).

(3) Mannose was employed because glucose-6-phosphate was rapidly metabolized by the preparation.

(4) Abbreviations used: P_i for orthophosphate, P_7 for orthophosphate liberated by 7 minute hydrolysis in *N* HCl at 100°, ADP for adenosinediphosphate, ATP for adenosinetriphosphate, DPN for diphosphopyridine nucleotide, cyt. c for cytochrome c, KGA for potassium salt of α-ketoglutaric acid, and mersalyl for sodium salt of *o*-[(3-hydroxymercuri-2-methoxypropyl)-carbamyl]-phenoxyacetic acid.

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markedly the activity of the washed sediment but not of the crude preparations (Table II).

TABLE II

Preparation	Additions	Initial rates of P_i uptake in the light: μM. P/hr./mg. protein	μM. P/hr./unit opt. d. at 800 mμ
Crude sonic		2.0	22.6
	DPN	1.9	21.5
	DPN, KGA	1.9	21.5
		2.1	9.1
Resuspended sediment	DPN	2.1	9.1
	KGA	4.3	18.7
	DPN, KGA	5.1	22.1
	DPN, KGA, cyt. c	5.1	22.1
	TPN, KGA	4.4	19.1

All preparations contained the following additions per ml.: 12 μM. $MgCl_2$, 10 μM. KF, 7 μM. P_i, 10.3 μM. ADP. Other additions were present as indicated in the following concentration per ml.: 0.5 μM. DPN, 0.25 μM. TPN, 0.012 μM. cyt. c, 15 μM. KGA. Resuspended sediment consisted of top half of sediment obtained after centrifugation of crude sonic preparation for 1 hour at 135,000 $\times$ gravity. Other experimental conditions the same as indicated for Table I.

Light dependent phosphorylation of ADP did not require oxygen and was not affected by 10^{-4} M dinitrophenol or 10^{-3} M iodoacetamide, 10^{-3} M hydrogen cyanide and 10^{-3} M mersalyl[4] gave inhibitions between 25–50%, 10^{-3} M *o*-phenanthroline inhibited completely, and 2,6-dichlorophenolindophenol inhibited phosphorylation from 60 to 100%. The system was completely inactivated after incubation at 80° for two minutes. Slow freezing and thawing of the preparation also caused extensive inactivation. Preparations kept on ice in the dark for eight hours did not show an appreciable loss of activity.

Similar systems have been described in the case of chloroplast preparations from higher plants.[6,7] In these systems, however, labelled P_i was employed to demonstrate the light induced formation of ATP.

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INCORPORATION OF PHOSPHATE OXYGEN INTO CARBON DIOXIDE FORMED BY ENZYMIC DEGRADATION OF CITRULLINE COUPLED WITH ATP SYNTHESIS[1]

Sir:

The biosynthesis of citrulline from ornithine by animal tissues requires adenosine triphosphate (ATP)[2] while the bacterial degradation of the ureido group of citrulline is coupled to ATP synthesis according to the over-all equation

$$\text{Citrulline} + \text{Orthophosphate} + \text{ADP} \rightleftharpoons \text{Ornithine} + NH_3 + CO_2 + \text{ATP}^3$$

From knowledge of the fate of phosphate oxygen in the enzymic formation of 3-phosphoglycerate and in phosphate transfer reactions,[4] the intermediate formation of an anhydride compound between carbonic acid or a derivative thereof and orthophosphoric acid accompanying CO_2 release and ATP synthesis would be expected to result in incorporation of phosphate oxygen into the CO_2.

It appeared feasible to check this possibility with the use of bacterial preparations[5] which catalyze the above reaction provided that the exchange of oxygen of carbon dioxide with that of water could be sufficiently minimized to allow detection of excess O^{18} from O^{18}-labeled orthophosphate in the CO_2 formed.

Using conditions similar to those described in Table I, a small but definite incorporation of excess O^{18} of orthophosphate into the CO_2 formed was found (mass 46/44 ratio of CO_2 = 0.00431). Increased yield of O^{18} in the CO_2 was obtained with change of conditions to reduce the exchange of oxygen of CO_2 with that of water before escape of CO_2 into the evacuated reaction chamber; *i.e.*, decrease in total liquid volume from 30 to 17.5 ml., increase in flask size from 100 to 250 ml. and with the reaction mixture strongly swirled instead of occasionally mixed. Results of such experiments are shown in Table I; they give conclusive proof that oxygen from orthophosphate appears in the CO_2 formed. The CO_2 produced from citrulline in presence of unlabeled orthophosphate contained, within experimental error, the same amount of O^{18} as tank CO_2. The amount of O^{18} in the CO_2 from the sample containing orthophosphate-O^{18} was approximately 28% of that expected if one oxygen of phosphate appeared in the CO_2 formed directly as a gas or 43% of that expected if one oxygen of phosphate appeared in H_2CO_3 which liberated gaseous CO_2. Under the reaction conditions at 37° with phosphate present (without consideration of any possible carbonic anhydrase activity of the bacterial preparation), the half-time for complete exchange of CO_2 oxygen with that of water was estimated from the data of Mills and Urey[6] to be about 19 seconds. That relatively rapid exchange was occurring was also shown by the increased yield of O^{18} in the CO_2 obtained as a result of reduction in liquid volume, increase of size of reaction flask, and vigorous swirling of the reaction mixture. These considerations together with the known specificity of enzyme reactions make it

(1) Supported by a grant from the National Science Foundation and grant 1033 from the National Institutes of Health, Public Health Service. Contribution No. 3226, Scientific Journal Series, Minnesota Agricultural Experiment Station.

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