SUMMARY

1. The use of alumina paper for the chromatography of steroids is described. The elimination of displacement effects due to impurities demands tedious purification of most biological extracts.

2. Partition systems of the aqueous methanol/hydrocarbon type can be run very conveniently on paper if certain special procedures are adopted. Extracts sufficiently pure to be handled by these systems can be simply and rapidly prepared.

3. Special methods for quick concentration of these extracts and their even deposition on filter paper are described.

4. Some applications to pure substances, extracts

of adrenal glands, and adrenal venous blood are described.

5. A preliminary examination of the 'amorphous fraction' obtained from adrenal extracts by Wintersteiner & Pfiffner (1936) indicates the presence of at least three very polar substances with strong ultra-violet absorption near 254 m μ ., two of which appear in adrenal venous blood.

The author would like to thank Mr J. Perkins and Mr D. N. Henty for the design and electrical fitting of the thermostatically controlled box used in these experiments, and the assistance of Mr K. Mourin at the operations.

He is also greatly indebted to Dr W. Feldberg and Dr R. K. Callow for much helpful advice and criticism; and to Prof. T. Reichstein for samples of pure steroids.

REFERENCES

- Brante, G. (1949). *Nature, Lond.*, **163**, 651.
 Brownell, K. A. (1950). *Fed. Proc.* **9**, 19.
 Burton, R. B., Zaffaroni, A. & Keutmann, E. H. (1950). *Science*, **111**, 6.
 Bush, I. E. (1950a). *Nature, Lond.*, **166**, 445.
 Bush, I. E. (1950b). *J. Physiol.* **112**, 10P.
 Butt, W. R., Morris, P. & Morris, C. J. O. R. (1949). *1st Int. Congr. Bioch. Abstr.* p. 405.
 Camber, B. (1949). *Nature, Lond.*, **163**, 285.
 Cornish, R. E., Archibald, R. C., Murphy, E. A. & Evans, H. M. (1934). *Industr. Engng Chem.* **26**, 397.
 Datta, S. P., Overell, B. G. & Stack-Dunne, M. (1949). *Nature, Lond.*, **164**, 673.
 Grollman, A. (1939). *J. Pharmacol.* **67**, 257.
 Hanes, C. S. & Isherwood, F. A. (1949). *Nature, Lond.*, **164**, 1107.
 Hartmann, F. A., Brownell, K. A. & Thatcher, J. S. (1947). *Endocrinology*, **40**, 450.
 Hartmann, F. A. & Liu, T. Y. (1950). *Fed. Proc.* **9**, 57.
 Hechter, O. (1950a). *Fed. Proc.* **8**, 70.
 Hechter, O. (1950b). *Fed. Proc.* **9**, 58.
 Kritchinsky, D. & Calvin, M. (1950). *J. Amer. chem. Soc.* **72**, 4330.
 Martin, A. J. P. (1950). *Ann. Rev. Biochem.* **19**, 517.
 Nelson, D. H., Reich, H. & Samuels, L. T. (1950). *Science*, **111**, 578.
 Nelson, D. H., Reich, H. & Zaffaroni, A. (1950). *J. biol. Chem.* **187**, 411.
 Pfiffner, J. J. & Vars, H. M. (1934). *J. biol. Chem.* **106**, 645.
 Pfiffner, J. J., Vars, H. M. & Taylor, A. R. (1934). *J. biol. Chem.* **106**, 625.
 Pfiffner, J. J., Wintersteiner, O. & Vars, H. M. (1935). *J. biol. Chem.* **111**, 585.
 Reichstein, T. & Shoppee, C. W. (1943). *Vitamins & Hormones*, **1**, 345.
 Reichstein, T. & Shoppee, C. W. (1949). *Discuss. Faraday Soc.* no. 7, 305.
 Selye, H. & Schenker, V. (1938). *Proc. Soc. exp. Biol., N.Y.*, **39**, 518.
 Spencer, A. G. (1950). *Nature, Lond.*, **166**, 32.
 Vogt, M. (1943). *J. Physiol.* **102**, 341.
 Wintersteiner, O. & Pfiffner, J. J. (1936). *J. biol. Chem.* **116**, 291.
 Zaffaroni, A., Burton, R. B. & Keutmann, E. H. (1949). *J. biol. Chem.* **177**, 109.
 Zaffaroni, A., Burton, R. B. & Keutmann, E. H. (1950). *Fed. Proc.* **9**, 250.

Kinetics of 'Acid' Phosphatase Action

By F. SCHÖNHEYDER

Department of Biochemistry, Aarhus University, Denmark

(Received 31 November 1950)

Prostate 'acid' phosphatase is present in normal human seminal fluid in very high concentration (Kutscher & Wolbergs 1935). The optimum pH for acid phosphatase in sperm was found to be 4-6.2 depending upon the experimental conditions (Kutscher & Wörner, 1936; Lundquist, 1947). Lundquist computed the dissociation constant of the enzyme-substrate complex (Michaelis constant)

from Kutscher & Wörner's experiments with β -glycerophosphate and on the basis of his own experiments with choline phosphate, phenyl phosphate and β -glycerophosphate.

Little attention has so far been paid to the reaction kinetics of the hydrolysis of phosphoric acid esters by acid phosphatase. In the present study reaction mechanisms are described which lead to simple

equations corresponding closely to the complete course of the hydrolysis of phenyl phosphate under varying experimental conditions.

EXPERIMENTAL

Enzyme. Solutions of unpurified acid prostate phosphatase from human sperm plasma in 2% NaCl were used. 1 ml. sperm plasma solution (about 3%) was added to 400 ml. substrate solution.

Substrate. Phenylphosphoric acid (m.p. 100°) dissolved in 2% NaCl was used. 0.01 g.mol. phenylphosphoric acid + 13 ml. *N*-NaOH were made up to 1 l. with 2% NaCl. Varying amounts of this stock solution were diluted with 2% NaCl to 400 ml.

Previous experience with a continuous titration technique in kinetic studies on the activity of pancreatic lipase (Schönheyder & Volqvartz, 1950) suggested the use of the same technique to follow the hydrolysis of phenyl phosphate at 22° and pH 5.62 ± 0.05. pH is registered by means of a glass electrode and a Radiometer potentiometer. On addition of enzyme to the substrate solution at pH about 5.60 a progressive increase in pH is observed, which is counteracted by addition of acid at the rate required to keep the pH constant. The acid (HCl) was added through a capillary tube connected with a Geissler burette (divisions 0.05 ml.). The pH was measured immediately after the addition of enzyme and followed during the whole experiment, an adequate amount of acid being added after each rise in pH above 5.67. For each addition of acid is obtained a series of corresponding values of pH and time. pH is plotted as ordinate with time in minutes as abscissa, and the time corresponding to pH 5.62 is determined; $t=0$ corresponds to the addition of enzyme. If the pH is then below 5.62 a certain time will elapse before this pH is reached. During this period some of the substrate is hydrolysed, and in order to obtain figures proportional to the acid bound it is necessary to add a correction to all readings on the burette. Careful determination of this correction is of greatest importance to the mathematical treatment of the experimental results. The pH is displaced in an alkaline direction because phenylphosphoric acid is stronger than phosphoric acid. Walker & King (1950) found pK_2 5.73 for phenylphosphoric acid. The writer's investigations confirm this value and also show that pK_1 is about 1. It was found that after complete cleavage of 1 mmol. phenyl phosphate 0.395 m-equiv. of HCl was needed to bring the pH back to 5.62. This value agrees fairly well with the amount calculated from the pK values of phenylphosphoric acid and phosphoric acid. At pH 5.62 the amounts of substrate present and of phosphate produced are large enough to keep the pH within the limits of 5.62 ± 0.05 by addition of HCl. On the other hand, the buffer capacity allows easy registration of changes in pH. Further addition of buffer substance to the enzyme-substrate mixture has therefore been omitted.

RESULTS

Optimum pH

The continuous titration technique is not suitable for the determination of the optimum pH, which was therefore determined by colorimetric measurement of the phenol liberated from phenyl phosphate.

It appears from the pH-activity curve (Fig. 1) that the pH used for the continuous titration experiments (5.62) was close to the optimum. The author is indebted to Dr P. Strøier Rasmussen for this unpublished experiment.

Fig. 1. Effect of pH on the activity of sperm acid phosphatase. Reaction mixture 5.9 ml. 0.01 M-phenyl phosphate in various buffers. The incubation was carried out at 37° for 15 min. The phenol released was determined by means of an adaptation of the King & Armstrong (1934) method for alkaline phosphatase.

Fig. 2. Effect of varying phenyl phosphate concentration upon the rate of hydrolysis by acid phosphatase. α = highest degree of hydrolysis measured. V, 0.499 mM; VI, 0.998 mM; VII, 1.496 mM; VIII, 1.995 mM.

Effects of varying enzyme and substrate concentrations

Fig. 2 shows experiments performed by the continuous titration technique, using the same amount of enzyme with four different concentrations of phenyl phosphate. The hydrolysis was followed nearly to completion, which is important if the

kinetic constants are to be determined with accuracy.

The progress of the enzymic hydrolysis does not follow either zero-order or first-order kinetics. However, for given concentrations of enzyme and substrate, the experimental results are fitted with great accuracy by the rate equation:

$$-\frac{ds}{dt} = \frac{as}{b+cs}, \quad (1)$$

whose integrated form is

$$at = b \ln \frac{s_0}{s} + c(s_0 - s) \quad (2)$$

(where s is the concentration of substrate at time t , s_0 the concentration at zero time, and a , b , c are empirical constants). The value of a depends only on the enzyme concentration, b is independent of enzyme concentration but is a linear function of the initial substrate concentration, s_0 ; while c is a true constant, whose value is independent of both enzyme and substrate concentration.

Writing $A = b/a$ and $B = c/a$, eqn. 2 becomes

$$t = A \ln \frac{s_0}{s} + B(s_0 - s). \quad (3)$$

For any given enzyme concentration, the values of A and B remain constant throughout the course of hydrolysis. Their values were determined by the usual method for the graphical solution of simultaneous equations. First $A = t/\ln \frac{s_0}{s}$ for $B = 0$, and $B = t/(s_0 - s)$ for $A = 0$ were computed for all observations. On a graph with abscissa A and ordinate B , straight lines were drawn through the points $(B, 0)$ and $(0, A)$ for each value of t . If the observations

fit eqn. 3, all the lines will meet in a point whose abscissa and ordinate are the required values of A and B . This method is far simpler than the 'method of least squares'.

In Table 1, the values of A and B determined in this way have been used to calculate by eqn. 3 the time, $t_{\text{calc.}}$, corresponding to each observed value of s .

Table 1. *Hydrolysis of 2.494 mM-phenyl phosphate, corresponding to 0.9838 mM-HCl. Exp. XII*

$t_{\text{obs.}}$ (min.)	HCl added (mmol.)	$t_{\text{calc.}}$ $\left(13.5 \ln \frac{s_0}{s} + 5.6(s_0 - s)\right)$
0.47	0.0252	0.49
1.20	0.0631	1.25
2.35	0.1135	2.29
3.33	0.1639	3.38
4.57	0.2144	4.52
5.70	0.2648	5.72
6.90	0.3153	6.98
8.26	0.3657	8.32
9.70	0.4161	9.76
10.80	0.4540	10.90
12.03	0.4918	12.11
13.33	0.5296	13.40
14.88	0.5675	14.79
16.20	0.6053	16.29
17.85	0.6431	17.92
19.79	0.6809	19.73
21.59	0.7188	21.74
24.05	0.7566	24.03
26.78	0.7944	26.71
29.50	0.8323	29.93
32.33	0.8575	32.53
35.35	0.8827	35.69
39.40	0.9079	39.71
45.00	0.9331	45.32
54.90	0.9584	54.83
69.00	0.9710	64.24
$\alpha = 98.7\%$		

Table 2. *Effect of substrate concentration (s_0) and addition of phosphate (p_0) on the rate of hydrolysis of phenyl phosphate*

(Same amount of enzyme used for each experiment.)

Exp. no.	s_0 (mm)	p_0 (mm)	B	$A_{\text{obs.}}$	$A_{\text{calc.}}$	$A_{\text{calc.}} \ln \frac{s_0}{s} + B(s_0 - s)$ t	$v_0 = \frac{s_0}{A + Bs_0}$
V	0.499	—	5.7	7.6	7.6	0.999	0.0478
VI	0.998	—	6.0	9.45	9.5	1.003	0.0646
VII	1.496	—	5.8	11.5	11.5	0.998	0.0741
VIII	1.995	—	5.6	13.5	13.4	0.995	0.0809
IX	2.494	—	5.8	15.4	15.4	0.997	0.0835
X	2.494	1.247	5.6	20.0	20.2	1.011	0.0734
XI	2.494	2.494	6.2	25.2	25.1	0.993	0.0613
Average			5.8			0.999	

Table 3. *Effect of enzyme concentration on the rate of hydrolysis of phenyl phosphate*

Exp. no.	s_0 (mm)	Enzyme	B	A	v_0	E	EB	EA
XII	2.494	Undil.	5.6	13.5	0.0908	1.087	6.1	14.7
XIII	2.494	50 %	11.6	26.4	0.0468	0.560	6.5	14.8
XIV	2.494	25 %	20.9	53	0.0234	0.280	5.9	14.8
Average							6.1	14.8

The results show close agreement with the observed times, $t_{\text{obs.}}$.

Table 2 (Exps. V-IX) shows that with a constant amount of enzyme, the value of A increases linearly with the initial substrate concentration s_0 , while the value of B remains constant. Table 3 gives the results of three experiments with the same substrate concentration and different amounts of enzyme. In agreement with eqns. 2 and 3, the observed values of A and B are inversely proportional to the enzyme

where k is the velocity constant for the break-up of the enzyme-substrate complex and K_m is the Michaelis constant. The integrated form of (5) is

$$t = \frac{K_m}{kE} \ln \frac{s_0}{s} + \frac{1}{kE} (s_0 - s) \quad (6)$$

(cf. Henri, 1903), which is formally similar to eqn. 3. The similarity is only apparent, however, since the value of A in eqn. 3 varies with the substrate concentration, while K_m/kE does not.

Fig. 3. Effect of varying phenol concentration upon the rate of hydrolysis of phenyl phosphate by acid phosphatase.

I (○), 2.494 mM-phenyl phosphate; II (□), 2.494 mM-phenyl phosphate + 1.247 mM-phenol; III (△), 2.494 mM-phenyl phosphate + 2.494 mM-phenol; IV (+), 2.494 mM-phenyl phosphate + 4.988 mM-phenol.

concentration E ; hence the products EA and EB are constants in the three experiments. (Since the pure enzyme is not available, the true value of E is unknown; in these calculations the value $E=1$ has been arbitrarily assumed for the enzyme concentration used in Exp. IX.)

If eqn. 1 is written as $-\frac{ds}{dt} = \frac{s}{A + Bs}$, then the initial velocity v_0 is given by

$$v_0 = \left(-\frac{ds}{dt} \right)_{t=0} = \frac{s_0}{A + Bs_0}. \quad (4)$$

The values of v_0 calculated in this way are given in Tables 2 and 3. With constant enzyme concentration, v_0 increases with s_0 , approaching a limiting value when $Bs_0 \gg A$ (Table 2). With constant substrate concentration v_0 is directly proportional to the enzyme concentration (Table 3).

Effects of phenol and orthophosphate

The above results show that the kinetics of acid phosphatase are more complicated than would be expected from the theory of Michaelis & Menten (1913). This theory predicts the rate equation

$$-\frac{ds}{dt} = \frac{kEs}{K_m + s}, \quad (5)$$

Fig. 4. Effect of varying phosphate concentration upon the rate of hydrolysis of phenyl phosphate by acid phosphatase equal to the amount used in experiments portrayed in Fig. 2. α = highest degree of hydrolysis measured. IX, 2.494 mM-phenyl phosphate; X, 2.494 mM-phenyl phosphate + 1.247 mM-phosphate; XI, 2.494 mM-phenyl phosphate + 2.494 mM-phosphate.

An explanation of the difference could be that the activity of acid phosphatase is inhibited by the products of the hydrolysis of phenyl phosphate, i.e. free phenol and orthophosphate. Fig. 3 shows that phenol, in concentrations up to 5 mM, has no effect on the kinetics. Fig. 4 shows that orthophosphate, on the other hand, is definitely inhibitory.

Table 2 (Exps. IX–XI) shows that with constant enzyme and substrate concentration, the addition of orthophosphate decreases the initial velocity v_0 . Also, the value of the constant A increases linearly with the initial concentration of orthophosphate, p_0 , while the value of constant B is unaffected. The data from all the experiments of Table 2, both with and without added phosphate, are found to fit the linear regression equation

$$A_{\text{calc.}} = 5.63 + 3.90 (s_0 + p_0), \quad (7)$$

as is shown by the good agreement between the values of $A_{\text{obs.}}$ and $A_{\text{calc.}}$ in Table 2.

It follows from eqn. 3 that

$$\frac{1}{t} \left[A \ln \frac{s_0}{s} + B(s_0 - s) \right] = 1.$$

The values of this expression, calculated from $A_{\text{calc.}}$ and $\bar{B}$ (the mean value of B in all experiments), are given in Table 2, column 7, and all lie between 0.993 and 1.011.

THEORETICAL

The following reaction scheme leads to equations analogous to eqns. 1–3, and is in good agreement with the experimental findings:

(where s = substrate, p = phosphate, f = phenol, e = free enzyme, (es) = enzyme-substrate complex, (ep) = enzyme-phosphate complex, and the k 's are velocity constants).

The equations for the formation of the enzyme-substrate complex (8) and its break-up into free enzyme and products (9) are identical with those of Briggs & Haldane (1925); but in addition the free enzyme is supposed to combine reversibly with phosphate to form an inactive complex (10).

From eqns. 8–10 we can at once write down the differential equations:

$$-\frac{ds}{dt} = k_1 es - k_2(es), \quad (11)$$

$$\frac{dp}{dt} = k_3(es) + k_5(ep) - k_4 ep, \quad (12)$$

$$\frac{df}{dt} = k_3(es). \quad (13)$$

In the steady state, $-ds/dt = dp/dt = df/dt$; hence the right-hand sides of these three equations may be equated.

Hence from eqns. 11 and 13,

$$k_1 es - k_2(es) = k_3(es),$$

or

$$\frac{e \times s}{(es)} = \frac{k_2 + k_3}{k_1} = K_s, \quad (14)$$

where K_s is the Michaelis constant or apparent dissociation constant of the enzyme-substrate complex (cf. Briggs & Haldane, 1925). Similarly, from eqns. 12 and 13 we obtain

$$\frac{e \times p}{(ep)} = \frac{k_5}{k_4} = K_p, \quad (15)$$

where K_p is the dissociation constant of the enzyme-phosphate complex.

Let E be the total enzyme concentration (free and bound). Then

$$E = e + (es) + (ep) = e \left[1 + \frac{s}{K_s} + \frac{p}{K_p} \right] \quad (16)$$

(from eqns. 14 and 15).

If p_0 and s_0 are the concentrations of p and s at zero time, then $p = p_0 + s_0 - s$; inserting this value of p into eqn. 16 and rearranging

$$e = E \left/ \left[1 + \frac{(p_0 + s_0)}{K_p} + \left(\frac{1}{K_s} - \frac{1}{K_p} \right) s \right] \right. \quad (17)$$

In the steady state $-\frac{ds}{dt} = k_3(es) = \frac{k_3}{K_s} es$.

Hence from (17)

$$-\frac{ds}{dt} = \frac{k_3 Es}{\left[K_s + (p_0 + s_0) \frac{K_s}{K_p} \right] + \left[1 - \frac{K_s}{K_p} \right] s}, \quad (18)$$

which on integration gives

$$k_3 Et = \left[K_s + (p_0 + s_0) \frac{K_s}{K_p} \right] \ln \frac{s_0}{s} + \left[1 - \frac{K_s}{K_p} \right] (s_0 - s). \quad (19)$$

This is analogous to eqns. 2 and 3 if we put

$$A = \frac{b}{a} = \frac{K_s}{k_3 E} + \frac{K_s(p_0 + s_0)}{k_3 E}, \quad (20)$$

$$B = \frac{c}{a} = \frac{1}{k_3 E} \left[1 - \frac{K_s}{K_p} \right]. \quad (21)$$

Hence if E is constant, B should be constant, while A should increase linearly with $(p_0 + s_0)$, which agrees with the experimental findings.

From eqn. 18 and the above expressions for A and B , it will be seen that when $p_o = 0$, the initial velocity is given by

$$v_o = \frac{k_3 E s_o}{K_s + s_o} = \frac{s_o}{A + B s_o}$$

identical with the Michaelis-Menten equation (5). Rearranging, this becomes

$$\frac{s_o}{v_o} = \frac{K_s}{k_3 E} + \frac{s_o}{k_3 E}. \quad (22)$$

Hence a plot of s_o/v_o against s_o should give a straight line with slope $1/k_3 E$, ordinate intercept $K_s/k_3 E$, and abscissa intercept K_s . Fig. 5 shows the data of

Fig. 5. Initial velocity-substrate concentration relationship (Exps. V-IX) plotted according to eqn. 22.

Exps. V-IX plotted in this way; the points lie very close to the straight line fitted by the 'method of least squares'. The value of the Michaelis constant, K_s , calculated from these data is 0.590 mm, and the value of $k_3 E$ is 0.104 mm/min. The true value of k_3 could only be determined if the enzyme concentration E were known in absolute units, which would only be possible if the pure enzyme were available.

From these values of K_s and $k_3 E$ and the experimentally determined constant B , the value of K_p can be determined by eqn. 21. Using the average

value $\bar{B}$ obtained from all the experiments in Table 2, the average value of K_p comes to 1.49 mm. Thus the affinity of orthophosphate for enzyme is about 0.4 times that of phenylphosphate.

DISCUSSION

All the experimental results agree with the equations derived from the reaction scheme suggested, which postulates a reversible inhibition of the enzyme by orthophosphate. This does not necessarily prove that the scheme is correct. The somewhat different reaction scheme:

can be shown to give rise to equations identical with (2) and (3), the integrated form of the rate equation being

$$h_3 E t = \frac{1}{h_1} [h_3 + h_2(s_o + p_o)] \ln \frac{s_o}{s} + \left[1 - \frac{h_2}{h_1}\right] (s_o - s). \quad (23)$$

This means that the empirical constants, from which h_1 , h_2 and h_3 may easily be calculated, will have a somewhat different significance.

Kinetic studies alone cannot decide which reaction mechanism is correct; the one given in the text is more in line with the mechanism usually postulated for hydrolytic enzymes. The problem may be solved when the pure enzyme is available; it should then be possible to decide whether the reversible combination with phosphate predicted by eqn. 10 actually occurs.

SUMMARY

1. By means of a continuous titration method adding dilute hydrochloric acid the kinetics of the enzymic decomposition of phenyl phosphate by unpurified acid prostate phosphatase at 22° has been studied. The effect of substrate concentration and excess of phenol and orthophosphate is reported. In all the experiments the hydrolysis was followed almost to completion. The pH optimum was determined by measuring the phenol liberated.

2. Two reaction mechanisms are put forward, both of which lead to equations that fit the experimental data with great accuracy over the whole course of the reaction.

REFERENCES

- Briggs, G. E. & Haldane, J. B. S. (1925). *Biochem. J.* **19**, 338.
 Henri, V. (1903). *Lois générales de l'action des diastases*. Paris: Thèse.
 King, E. J. & Armstrong, A. R. (1934). *Canad. med. Ass. J.* **31**, 376.
 Kutscher, W. & Wolbergs, H. (1935). *Hoppe-Seyl. Z.* **236**, 237.
 Kutscher, W. & Wörner, A. (1936). *Hoppe-Seyl. Z.* **239**, 109.
 Lundquist, F. (1947). *Acta physiol. scand.* **14**, 263.
 Michaelis, L. & Menten, M. L. (1913). *Biochem. Z.* **49**, 333.
 Schönheyder, F. & Volqvartz, K. (1950). *Biochim. Biophys. Acta*, **6**, 147.
 Walker, P. G. & King, E. J. (1950). *Biochem. J.* **47**, 93.

The Enzymically Catalysed Transfer of the Deoxyribosyl Group from One Purine or Pyrimidine to Another

By W. S. MACNUTT* (Fellow of the Jane Coffin Childs Memorial Fund for Medical Research)
Institute of Cytophysiology and Biochemistry, University of Copenhagen, Denmark

(Received 30 January 1951)

Since several of the natural deoxyribosides serve equally well as growth-promoting substances for certain bacteria (Kitay, MacNutt & Snell, 1949, 1950; Hoff-Jørgensen, 1949; Kocher & Schindler, 1949) it seems probable that in such organisms the administered deoxyriboside is converted into the other deoxyribosides of which the bacterial deoxyribonucleic acid is constituted. The only mechanism which has been established for the biological synthesis of nucleosides (Kalckar, 1947; Friedkin & Kalckar, 1950) would depict the interconversion as follows:

where R, R¹, etc. represent the purines and pyrimidines (or their precursors) which are characteristic of the deoxyribonucleic acid of the bacterium.

The experiments reported below were designed to test such a mechanism in representatives of the group of bacteria which require deoxyribosides for growth. Dialysed enzyme preparations from *Lactobacillus helveticus* S have been shown, by a series of indirect methods, to be capable of bringing about a wide variety of interconversions when incubated with a deoxyriboside and a free purine or pyrimidine. Contrary to expectation, deoxyribose-1-phosphate did not react, in this system, with purines or pyrimidines to yield deoxyribosides. These findings have been reported in brief (MacNutt,

1950). Enzyme solutions have been prepared from two additional bacteria, both of which require deoxyribosides for growth, and these, too, have been found to catalyse an exchange. The exchange of the deoxyribosyl group from one acceptor to another can hardly proceed through hydrolysis followed by resynthesis, since hydrolysed deoxyribose-1-phosphate was also inactive in this system. The enzyme preparation is therefore considered to be a trans-*N*-glycosidase, analogous to the trans-*O*-glycosidase from *Pseudomonas saccharophila* (Doudoroff, Barker & Hassid, 1947).

EXPERIMENTAL

Materials

All melting points reported are uncorrected. Optical rotations were measured in a 2 dm. tube.

Purines and pyrimidines. Commercial preparations (Schwartz Laboratories; Hoffmann La Roche and Co.) were used. 5-Methyleytosine was generously supplied by Dr H. K. Mitchell. It melted at 276–278° with gas evolution after softening at 268°. (Found in material dried at 100° over P₂O₅ for 4 hr. (1 mm.): C, 48.1; H, 5.8; N, 33.7. Calc. for C₈H₇ON₃: C, 48.0; H, 5.6; N, 33.6%). The picrate melted at 297–299° (decomp.).

4-Aminoglyoxaline-5-carbonamide hydrochloride was prepared from formamidomalonanamide hydrochloride (a gift from Dr D. W. Woolley) by ring closure (Shaw & Woolley, 1949). Buff-coloured material was obtained which decomposed at 253° without melting completely. The picrate decomposed at 237°.

* Present address: University Chemical Laboratory, Cambridge.