The behavior of water here described seems to be a different phenomenon from "anomalous osmosis" through solid membranes which appears to require the presence of pores and of electrolytes.[2]

The gain in water in C is obviously only temporary. If left to itself the system would undoubtedly reach equilibrium with A and C identical in composition and in volume.

Since in certain respects guaiacol acts like some protoplasmic surfaces[1] it seems possible that similar phenomena may occur in living cells. If so these results have an obvious bearing on the movement of water in the organism and on methods of studying permeability. It becomes necessary to know to what extent a substance entering or leaving the cell carries water with it in the manner here indicated.

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"Acid" Phosphatase Activity of the Serum of Normal Human Subjects.

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Though the optimum for phosphatase activity of normal serum is in a distinctly alkaline range, pH-activity curves indicate slight but definite activity on the acid side of neutrality.[1] It has not been made clear whether these curves reflect the operation of a single enzyme over an unusually wide range in pH, or whether they are really composite curves representing the activities of 2 or more enzymes. Data to be presented suggest that the phosphatase activity of normal serum in the acid range is due largely to the presence of minute amounts of one or more phosphatases falling within the group classified by Folley and Kay[2, 3] as phosphomonoesterase A_2.

The method of King and Armstrong[4] proved adaptable to the estimation of "acid" serum phosphatase activity under conditions of hydrolysis most likely to approximate initial reaction velocities. The buffer-substrate employed was M/200 disodium monophenylphos-

2 *Cf.* Söllner, K., *Kolloid-Z.*, 1933, **62**, 31.

1 Roche, J., *Biochem. J.*, 1931, **25**, 1724.

2 Folley, S. J., and Kay, H. D., *Ergebn. d. Enzymforsch.*, 1936, **5**, 159.

3 Folley, S. J., and Kay, H. D., *Tabulæ biol. period.*, 1937, **6**, 268.

4 King, E. J., and Armstrong, A. R., *Canad. M. A. J.*, 1934, **31**, 376.

phate in Sørensen's M/10 citrate-HCl buffer adjusted to pH 4.8. After addition of serum, the pH of the reaction mixture at room temperature was 4.85 (glass electrode measurements) and therefore approximately 4.9 at the temperature of hydrolysis, 37°C. The optimum time of hydrolysis for satisfactory colorimetric readings was found to be 3-5 hours. This period may be shortened by using higher concentrations of substrate or by making photometric readings. However, time-activity curves indicated that after hydrolysis for even 5 hours, the deviation from linearity, while significant, was not sufficient to offset advantages of the method when applied to sera in certain diseases.[5]

The usual precautions against hemolysis were taken. Since the "acid" phosphatase activity of many sera falls after standing several days in the refrigerator, fresh sera were used throughout. The results are expressed in units per 100 cc serum, a unit being defined as that degree of phosphatase activity which at pH 4.9 and 37°C will liberate from the specified buffer-substrate solution 1 mg of phenol in 1 hour.

The phosphatase activity of 23 normal sera determined at pH 4.9 by this method ranged from 0.6 to 2.0 units per 100 cc serum (*vide* Table I for 12 representative values). The results obtained are due to enzymatic, not acid hydrolysis, as shown by the absence of scission in control experiments in which substrate-buffer mixtures were maintained at 37°C for 5 hours without the addition of serum. That the enzyme operating at pH 4.9 is not "alkaline" serum phosphatase, however, is suggested by the lack of correlation between the "alkaline" and "acid" activities of normal sera, notably in children (Table I). Sera in diseases presenting marked elevations in "alkaline" phosphatase activity show no proportionate rise in "acid" phosphatase activity (Table I).* Further, unlike "alkaline" serum phosphatase, the "acid" phosphatase activity of serum is markedly inhibited by M/100 NaF and is not accelerated by Mg ion (Table

[5] Gutman, A. B., and Gutman, E. B., *J. Clin. Invest.*, in press.

* If a single enzyme (*i.e.*, "alkaline" serum phosphatase) were operative and inactivated only in part at pH 4.9, values for "acid" serum phosphatase would be correspondingly lower than those at pH 9.0 but nevertheless proportional to them,[6] as occurs, for example, when hydrolysis is conducted at pH 7.6 (Kay's method). If a single enzyme were operative and wholly inactivated at pH 4.9, the phosphatase activity at pH 4.9 would, of course, be zero. It was found that after incubating for one hour at pH 4.9 (using M/10 citrate buffer), the phosphatase activity of serum brought back to pH 9.0 by addition of NaOH was not more than 25% and usually less than 10% of the value of untreated serum.

[6] Martland, M., and Robison, R., *Biochem. J.*, 1927, **21**, 665.

TABLE I.

"Acid" and "Alkaline" Phosphatase Activity of Serum.

(Substrate = M/200 disodium monophenylphosphate; buffer = M/10 Sørensen's citrate-HCl (pH 4.9); temperature = 37°C)
= M/20 sodium veronal (pH 9.0)

No.	Subject	Age	Sex	Phosphatase activity (mg phenol liberated in 1 hr, per 100 cc serum) pH 4.9	pH 9.0	No.	Subject	Age	Sex	Phosphatase activity (mg phenol liberated in 1 hr, per 100 cc serum) pH 4.9	pH 9.0
1	Normal adult	24	♂	2.0	8.3	9	Normal adult	25	♂	0.8	10.3
2	" "	31	♂	1.4	9.0	10	" "	25	♂	0.6	8.6
3	" "	49	♂	1.3	9.0	11	" child	4.5	♂	2.0	33.4
4	" "	28	♂	1.3	17.5	12	" "	4	♂	1.8	42.4
5	" "	29	♂	1.1	13.7	13	Advanced Paget's disease	63	♀	5.0	386.
6	" "	25	♂	1.1	14.4	14	" " "	55	♂	3.4	583.
7	" "	28	♀	1.0	6.8	15	Hyperparathyroidism	50	♀	2.3	134.
8	" "	23	♂	0.9	14.1	16	Obstructive jaundice	41	♀	1.7	143.

TABLE II.

Representative Experiments Illustrating "Acid" Phosphatase Activity of Normal Serum Under Varying Conditions of Hydrolysis.

Exp. No.	Substrate	Buffer Citrate-HCl	pH	Agent added	Time of hydrolysis hr	Phenol liberated, mg per 100 cc serum	Comment
1	M/200 phenylphosphate	M/10	4.9	0	3	4.2	Control
	M/200 "	M/10	4.9	M/100 NaF	3	1.4	Marked inhibition of "acid" phosphatase by fluoride
2	M/200 "	M/10	4.9	0	3	4.0	Control
	M/200 "	M/10	4.9	M/50 $MgCl_2$	3	3.5	No activation of "acid" phosphatase by Mg ion
3	M/40 α glycerophosphate	M/25	4.9	0	5	2.2	α glycerophosphate is not more rapidly hydrolyzed than β glycerophosphate
	M/60 β "	M/25	4.9	0	5	2.6	
4	M/200 phenylphosphate	M/10	4.9	0	3	3.9	Control
	M/200 "	M/10	4.9	M/1 propyl alcohol	3	3.9	No inhibition by alcohols

II). The "acid" phosphatase activity of serum, moreover, is less stable on standing than is "alkaline" serum phosphatase and is more slowly inactivated by heating to 60°C.

The "acid" phosphatase of normal serum was found to have a broad range of optimum activity from about pH 4.0 to pH 6.5. This, together with the characteristics summarized in Table II suggest that the enzyme concerned is best classified, for the present, as a phosphomonoesterase A_2. The available data indicate that "acid" serum phosphatase is not identical with erythrocyte phosphatase[1] or with the "acid" phosphatase found in normal prostate tissue[7] and in the skeletal metastases[8] and serum[5] of patients with disseminated prostate carcinoma. As regards phosphatases occurring in the liver and spleen,[9, 10] however, no serious inconsistencies are apparent at this time. These organs may be a source of the "acid" phosphatase content of the serum.

Conclusion. In addition to "alkaline" phosphatase, blood serum contains minute amounts of one or more "acid" phosphatases of the type classified by Folley and Kay as phosphomonoesterase A_2.

9900

A Micro Precipitin-Technic for Classifying Hemolytic Streptococci, and Improved Methods for Producing Antisera.

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The increasing practice of grouping hemolytic streptococci serologically by the use of the precipitin-reaction has stimulated us to devise a microtechnic whereby the accuracy of the original method is retained with a great saving of material. Potent sera are essential; and for the maintenance of a steady supply, it has been found advantageous to keep on hand rabbits which are repeatedly immunized and bled, with rest periods between the courses of treatment.

It is important to select suitable strains for immunization. The two methods of immunization employed lead to the production of precipitins for both the group-specific carbohydrate (C) and the

[7] Kutscher, W., and Wörner, A., *Z. f. physiol. Chem.*, 1936, **239**, 109.

[8] Gutman, E. B., Sproul, E. E., and Gutman, A. B., *Am. J. Cancer*, 1936, **28**, 485.

[9] Davies, D. R., *Biochem. J.*, 1934, **28**, 529.

[10] Bamann, E., and Riedel, E., *Z. f. physiol. Chem.*, 1934, **229**, 125.