slow evacuation no such transition occurs, the blue glow rising to a constant value throughout the explosion region. If evacuation is stopped at any stage after crossing the limit, the blue glow slowly fades. A further slight evacuation brightens it up momentarily, and this process can be repeated many times.

The phenomenon leads to experimental difficulties only when observing the second limits of very weak mixtures. To obtain any explosion at all in this region, the rate of evacuation must be faster than is desirable for accurate manometer reading, and also under these conditions the explosion lag, as indicated above, must become a significant source of error. This uncertainty tends to prevent the accurate determination of the constants c and F of the explosion limit equation[2], values from the second limit being much greater than those derived from the first. Furthermore, the unknown error due to explosion lag might well be the cause of the observed but unexpected temperature dependence of the third-body coefficient a^2, since the necessary rate of withdrawal increases both with ratio of oxygen to hydrogen and with temperature.

A. C. EGERTON
D. R. WARREN*

Department of Chemical Engineering
and Applied Chemistry,
Imperial College,
London, S.W.7.
June 30.

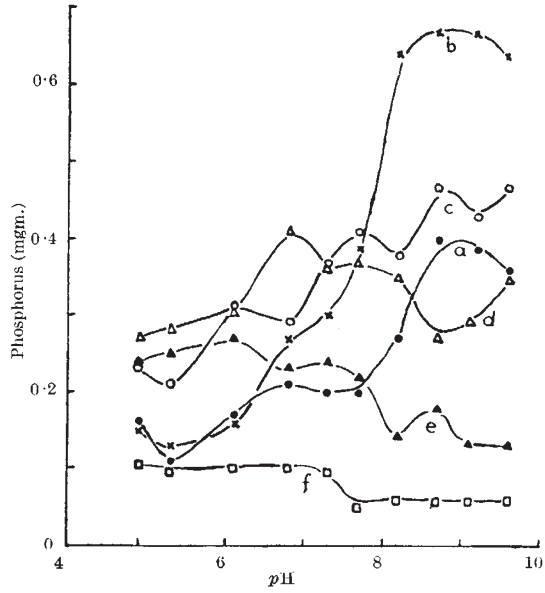
* Present address: Aeronautical Research Laboratories, Fisherman's Bend, Melbourne.

[1] Baldwin, R. R., and Precious, R. M., *Nature*, **169**, 290 (1952).
[2] Egerton, A. C., and Warren, D. R., *Proc. Roy. Soc.*, A, **204**, 465 (1951). Warren, D. R., *ibid.*, A, **211**, 86, 96 (1952).
[3] von Elbe, G., and Lewis, B., *J. Chem. Phys.*, **9**, 194 (1941).

Zinc, Ionic Equilibrium and Phosphatase Activity

CONTROVERSY has centred around the nature of the active groups influencing the activity of alkaline phosphatases of higher animals[1]. In a recent communication[2], I suggested on the basis of results obtained with the alkaline phosphatase of the fungus *Penicillium chrysogenum*, *Q*-176, that the activity of the enzyme is influenced by both zinc and magnesium ions and that the relative proportion in which these ions existed in an active state determined the *p*H of optimum activity.

I have further investigated this problem with alkaline phosphatases prepared from ox kidney and intestines by the method described by Thoai, Roche and Roger[3], and the results obtained by me go to support my earlier observations that both zinc and magnesium ions and the relative concentration in which they are present exert considerable influence on the activity of the enzyme. From the accompanying graph, it will be apparent that while cyanide at higher concentrations inhibits activity at all the *p*H's tested, the enzyme shows increased activity on the acid side at intermediate concentrations of cyanide and on the alkaline side at very low concentrations. I have also observed that by the addition of magnesium ions to the enzyme inhibited by 0·002–0·004*M* cyanide, activity was increased on the acid side to a considerably greater extent than at other *p*H's, whereas a similar addition of zinc resulted in the restoration of activity at all the *p*H's at low, and on the alkaline side at intermediate, concentrations of zinc. In



Effect of varying concentrations of cyanide on ox kidney alkaline phosphatase at different *p*H's. Each substrate contained neutralized sodium cyanide at the following concentrations: (*a*) nil, (*b*) 0·0002 *M*, (*c*) 0·0004 *M*, (*d*) 0·001 *M*, (*e*) 0·002 *M*, (*f*) 0·004 *M*

both cases there was an optimum concentration at which the activity was maximum, higher concentrations of zinc especially bringing about general inhibition of activity at all *p*H's.

The complete inhibition of activity of the enzyme at all the *p*H's on the addition of cyanide at the higher concentrations and the effect of addition of zinc in increasing concentrations to the cyanide-inhibited enzyme in restoring the activity first at all the *p*H's and thereafter at the more alkaline *p*H's show that zinc ions are necessary for activity at alkaline and acid *p*H's. Other heavy metals, such as manganese, iron or cobalt, had no appreciable effect in restoring the activity of the cyanide-inhibited enzyme. It is well known that magnesium ions are necessary for full activity of the enzyme on the alkaline side, and I have observed that this is so only when zinc ions are also present, for the cyanide-inhibited enzyme is only partially activated by magnesium on the alkaline side.

From these findings, I suggest that there is no entity like 'acid' or 'alkaline' phosphatases, but that by merely changing the equilibrium between the prosthetic ions or groups of the enzyme, activity can be established at any *p*H desired. Admittedly, the phenomenon as it applies to enzymes in higher animals is more complex than the one observed for the enzyme in *P. chrysogenum*, *Q*-176. The results of this investigation will be published in due course.

V. SADASIVAN

Haffkine Institute,
Parel,
and
Municipal Laboratory,
Bombay, 1, India.
March 11.

[1] Cloetens, R., *Biochem. Z.* **308**, 37 (1941); **310**, 42 (1941); *Arch. Int. Pharmacodym.*, **68**, 419 (1942). Hove, E., Elvehjem, C. A., and Hart, E. B., *J. Biol. Chem.*, **134**. 426 (1940). Thoai, N. v., Roche, J., and Roger, M., *Biochim. Biophys. Acta*, 1, 61 (1947). Abul-Fadl, M. A. M., and King, E. J., *Biochem. J.*, **44**, 435 (1949); *Arch. Biochem. and Biophys.*, **37**, 172 (1952).
[2] Sadasivan, V., *Nature*, **169**, 418 (1952).
[3] Thoai, N. v., Roche, J., and Roger, M., *Biochim. Biophys. Acta*, 1, 61 (1947).