Table IV. Analysis of variance and least significant differences for the chlorogenic acid data

Variation source	df	SS	MS	VR	95% LSD
Time effect	3	0.0509	0.0170	0.2901	
Shock effect	4	1.0241	0.2560	4.3686[a]	0.24
Error	12	0.7031	0.0586		

[a] F = 97.5%.

observed. A possible explanation for this observation is, since tannins are associated with cytoplasmic organelles[5], that tannins are not formed and, therefore, not stored in broken cells. Broken cells, or lesions, are characteristic in material shocked at 30 and 40 ψ, sometimes occurring after 20 ψ, but have not yet been observed after 10 ψ. Unfortunately, the values obtained for the 20 ψ exposure for both tannic acid and chlorogenic acid are believed to be anomalous because of equipment failure. The reason for this belief is based on the chlorogenic acid result, since the same tuber provided material for both assays. That is, the synthesis of chlorogenic acid is known to be linear with increasing oxygen concentrations reaching a maximum at about 20% and levelling with further increases[10]. Since the shock tube is loaded with air in the constant volume loading chamber, the air gets compressed with higher shock levels. Since the load volume is kept constant, oxygen concentration becomes variable depending on the shock level.

There was a progressive increase in chlorogenic acid with increasing shock levels (Table III), thereby agreeing with ZUCKER and LEVY's[10] work on the effect of oxygen concentrations on chlorogenic acid synthesis in potato tubers. Premature lignification has been observed in shocked pea roots but only after a 40 ψ shock exposure. The roots of tubers for the 30 and 44 ψ shock exposures were slightly brownish, possibly indicating premature suberization of the roots. Chlorogenic acid may also act as a plant growth regulator[11]. Metabolically, it is a competitive inhibitor of IAA-oxidase, and is considered to be effective both in the catabolism and anabolism of auxin[12–14]. It is known that increased concentrations of auxin (or auxin-like substances) have an inhibitory effect on plant growth[15]. Observations that plant growth is reduced after shock treatment[7,16] may possibly be explained by increased concentrations of chlorogenic acid[17].

Résumé. Les tanins de l'acide chlorogénique s'accumulent immédiatement après que les racines d'ignames ont été soumises à des chocs de 10 à 44 ψ (0.6–2.66 kg/cm^2), consistant en une pulsation de la pression comparable à une rafale d'air dans un tube. L'accumulation des tanins est inversement proportionelle à la pression. Mais, l'acide chlorogénique s'accumule progressivement avec élèvation du niveau du choc.

SYLVIA A. MURRAY

1522 Willow Street, Alameda (California 94501, USA),
20 July 1970.

[10] M. ZUCKER and C. C. LEVY, Pl. Physiol. *34*, 108 (1959).
[11] R. L. WAIN and H. F. TAYLOR, Nature, Lond. *207*, 167 (1965).
[12] S. RABIN and R. M. KLEIN, Arch. Biochem. Biophys. *70*, 11 (1957).
[13] S. A. GORDON and L. G. PALEG, Pl. Physiol. *36*, 838 (1961).
[14] P. E. PILET, Phytochemistry *2*, 617 (1964).
[15] D. J. MORRÉ and J. BONNER, Physiol. plant. *18*, 635 (1965).
[16] S. A. MURRAY and C. L. NEWCOMBE, Radiat. Bot. *11*, in press.
[17] Acknowledgment. This work was supported in part by funds from Advanced Research Projects Agency, Department of Defense, through the U.S. Geological Survey, under ARPA Order No. 938. Special thanks are expressed to Dr. C. L. NEWCOMBE of San Francisco State College, and Dr. H. T. SHACKLETTE of the Geological Survey for making this study possible.

Potentiation of Haemolysis by the Combined Action of Phospholipase A and a Basic Peptide Containing S-S-Bonds (Viscotoxin B)

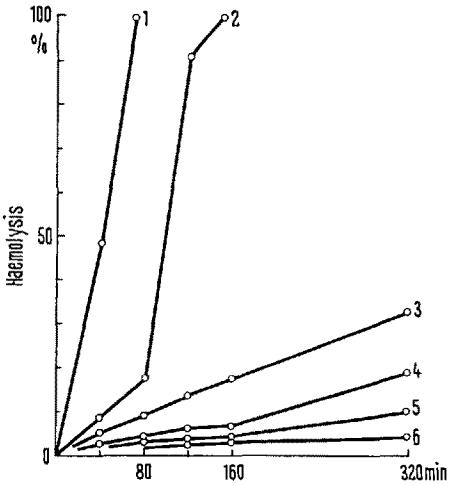
Although red cells contain lecithin in their membranes and are susceptible to lysis by lysolecithin, they are not lysed by phospholipase A. However, in the presence of a basic peptide fraction of cobra venom, the direct lytic factor (DLF) which is a weak haemolysin by itself, phospholipase A becomes strongly haemolytic and cleaves membrane phospholipids[1]. Recent results from this institute have indicated that the action of DLF depends on the presence of disulphide bridges[2,3]. It was further suggested that the potentiating effect is due to an alteration of the membrane structure caused by interaction of DLF with SH groups of membrane constituents, and that the combination of basic charge with disulphide bonds is a general structural feature of peptides which enable phospholipase A to cause haemolysis[3].

This hypothesis was put to the test with viscotoxin B, one of a group of related peptides which have been isolated from the European mistletoe, *Viscum album* L. These peptides have S–S-bonds and a net positive charge[4]. In an earlier publication, a crude viscotoxin preparation has been reported to cause haemolysis, besides having effects on the heart and circulation[5]. The preparation of viscotoxin B was kindly supplied by Dr. G. SAMUELSSON, Stockholm. Phospholipase A was separated from bee venom according to the procedure of HABERMANN and REIZ[6]. Heparinized guinea-pig blood was centrifuged and the packed cells were washed 3 times with 1% NaCl solution. They were finally suspended in 0.01 M phosphate buffer pH 7.3 containing 0.15 M NaCl and 0.45 mM $CaCl_2$ (20 times the original blood volume). Each 0.25 ml of phospholipase A solution (2×10^{-5} g/ml) and of various concentrations of viscotoxin B were added to 4.5 ml red cell suspension. The

[1] E. CONDREA, A. DE VRIES and J. MAGER, Biochim. biophys. Acta *84*, 60 (1964).
[2] P. PATZER and W. VOGT, Naunyn-Schmiedebergs Arch. Pharmak. exp. Path. *257*, 320 (1967).
[3] W. VOGT, P. PATZER, L. LEGE, H.-D. OLDIGS and G. WILLE, Naunyn-Schmiedebergs Arch. Pharmak. *265*, 442 (1970).
[4] G. SAMUELSSON, T. OLSON, T. MELLSTRAND and B. PETTERSON, Second International Symposium on Animal and Plant Toxins, Toxicon, *8*, 150 (1970).
[5] H. F. ZIPF, Arch. exp. Path. Pharmak. *209*, 165 (1950).
[6] E. HABERMANN and K. G. REIZ, Biochem. Z. *341*, 451 (1965).

mixtures were incubated at 37 °C and portions of 1 ml each were taken at various time intervals for photometric estimation of haemolysis.

As seen from the Figure, viscotoxin B has a weak direct haemolytic effect when acting in concentrations of 10^{-4} or 5×10^{-4} g/ml for prolonged times. Phospholipase A at the final concentration of 10^{-6} g/ml is practically non-haemolytic. When both substances are present simultaneously, complete haemolysis occurs in 80–160 min. Thus a pronounced potentiation is apparent, similar to the synergistic effect of phospholipase A and DLF.



Effect of phospholipase A (final concentration 10^{-6} g/ml) and varying concentrations of viscotoxin B on washed guinea-pig erythrocytes. Ordinate: % haemolysis. 1. Phospholipase A + 5×10^{-4} g/ml viscotoxin. 2. Phospholipase A + 10^{-4} g/ml viscotoxin. 3. 5×10^{-4} g/ml viscotoxin alone. 4. 10^{-4} g/ml viscotoxin alone. 5. Phospholipase A alone. 6. Control without haemolysins.

By demonstrating that viscotoxin B has the anticipated enhancing action, our results support the view[3] that the phospholipids in the red cell membrane, normally protected from attack by phospholipase A, can be exposed by structural changes induced through reaction of S–S-groups with membrane constituents. The cationic charge, which has been found earlier also to be essential[1,3], may serve to attract the peptides to the proper membrane sites where they can react.

Zusammenfassung. Viscotoxin B, ein basisches Peptid mit Disulfidgruppen, zeigt in der Hämolyse den gleichen synergistischen Effekt mit Phospholipase A wie der direkt lytische Faktor des Kobragiftes. Dieses Ergebnis stützt die Hypothese, dass Zellmembranen durch Reaktionen ihrer Protein-SH-Gruppen so verändert werden können, dass Phospholipase A sonst unzugängliche Membranphospholipide angreift.

P. G. Lankisch and W. Vogt[7]

Department of Biochemical Pharmacology, Max-Planck-Institut für experimentelle Medizin, D-34 Göttingen (Germany), 28 August 1970.

[7] We thank Miss Astrid Jörgensen for skilful technical assistance, and Dr. G. Samuelsson for viscotoxin B.

Oxidation of Vanillin by Cream Xanthine Oxidase and its Inhibition by Allopurinol

Recent research has directed attention of biochemist and pharmacologists to aldehydes, which rather than inactive transitory intermediates of oxidation of alcohol, and of amines, have come into prominence of their own, and are now considered important metabolites, endowed with significant biochemical activity[1–5]. Several enzymes (alcohol dehydrogenase, aldehyde oxydase and dehydrogenase, and xanthine oxidase) can metabolize aldehydes to the corresponding acids; other pathways are also available for metabolizing aldehydes[6].

In connection with an investigation dealing with the metabolism of 'seroton-aldehyde' and other metabolites of biogenic amines[7], the oxidation of aldehydes by purified cream xanthine oxidase (E.C. 1.2.3.2; Worthington) was studied. The selected substrate was vanillin (3-methoxy-4-hydroxy-benzaldehyde) which is of special interest due to its similarity to catecholamines (Figure 2). This compound has been shown to be a substrate for xanthine oxidase by several investigators[8–10], but its behavior in the tetrazolium reduction assay for xanthine dehydrogenase[11,12], nor the effect of the specific competitive inhibitor of xanthine oxidase, allopurinol[13], has not been investigated.

Vanillin presents a broad absorption peak at 345 nm, which is absent in vanillic acid (Figure 1); the disappearance of this peak can be utilized for spectrophotometric assay of xanthine oxidase activity with vanillin as substrate (Figure 2).

Allopurinol (4-hydroxypyrazolo-[3,4-d]pyrimidine) is a structural analog of hypoxanthine, which explains its inhibiting activity with that substrate. It is well known,

[1] S. G. A. Alivisatos, Neurosci. Res. Progr. (Nov. 1968).
[2] V. E. Davis and M. J. Walsh, Science *167*, 1005 (1970).
[3] G. Cohen and M. Collins, Science *167*, 1749 (1970).
[4] R. A. Lathi, E. Majchrowicz, Biochem. Pharmac. *18*, 535 (1969).
[5] J. W. Ridge, Biochem. J. *88*, 95 (1963).
[6] W. W. Westerfeld and R. J. Bloom, in *Alcohol and Alcoholism* (Ed. R. E. Popham; University of Toronto Press, Toronto 1970), p. 4.
[7] R. Fried and S. Alivisatos, in preparation.
[8] V. A. Booth, Biochem., J. *32*, 494 (1933).
[9] G. F. Edwards, J. Ass. Agric. Chem. *33*, 855 (1950).
[10] S. Kuramoto, R. Jenness, S. T. Coulter, J. Dairy Sci. *40*, 187 (1957).
[11] R. Fried, Analyt. Biochem. *16*, 427 (1966).
[12] R. Fried and L. W. Fried, in *Methods in Enzymatic Analysis* (Ed. H. U. Bergmeyer), 2nd edn (Gev.) 1970.
[13] P. Feigelson, J. D. Davidson and B. K. Robins, J. biol. Chem. *226*, 993 (1957).