hydrolysis products from groundnut phytosterolin[4] has revealed the presence of small quantities of stigmasterol and a saturated sterol.

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Isolation of the Antifungal Substance, 6-Methoxybenzoxazolinone, from Field Corn (*Zea mays* L.) in Canada

The presence of an antifungal substance which is inhibitory to the growth of *Fusarium moniliforme* (Sheld.) and *Gibberella zeae* (Schw.) Petch., two pathogenic fungi associated with root and stalk rot of corn in Ontario, has been reported[1]. The present communication reports the isolation and identification of the antifungal substance.

An ether extract was prepared from 9,000 gm. of corn plant tissue by the procedure reported previously. The ether was evaporated and the residue dissolved by boiling in 60 ml. triple-distilled water. On cooling, buff-coloured, needle-shaped crystals formed at 25°C. This crystalline material, at a concentration of 0·12 mgm. per ml. Czapek's agar, prevented growth of *G. zeae*, *Pyrenochaeta terrestris* (Hansen) Gorenz, Walker and Larson, *F. moniliforme* and *Diplodia zeae* (Schw.) Lév.

The crystallization procedure and the type of crystals obtained characterized this material as 6-methoxybenzoxazolinone as reported by Loomis *et al.*[2] and Smissman *et al.*[3] This identification was confirmed by Dr. E. Y. Spencer, Head of the Chemistry Section, Pesticide Research Institute, Research Branch, Canada Department of Agriculture, London, Canada.

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PHYSIOLOGY

Urokinase-Induced Fibrinolysis of Human Standard Clots

Studies with human fibrinolysis, either induced by pyrogens or appearing during thoracic surgery, together with other observations, indicate that very likely the first phase of an endogenous fibrinolytic reaction in man is the release of a plasminogen activator from the tissues into the blood stream. The question whether this activator can induce lysis of an intravascular clot directly was studied *in vitro* with human urokinase, a plasminogen activator excreted with the urine, as the enzyme source and human standard clots as substrate.

Urokinase was prepared as follows: 2,500–3,000 ml. of clear pooled urine was collected during the day, filtered (from inside to outside) overnight by gravity through a Coors porcelain filtering cylinder, porosity No. 1, size 1 (pore size diam. 13·5–15 μ). The next morning, the cylinder was emptied and then eluted by forcing fluids with suction from the outside to the inside in the following succession: 20 ml. of distilled water, which was discarded; 140 ml. of distilled water, which elutes thromboplastic material[1]; 40 ml. of 1 *M* potassium thiocyanate, which was discarded; 120 ml. of 1 *M* potassium thiocyanate, which elutes urokinase. This eluate was cleared by centrifugation and concentrated ten times by pervapouration in a 8 × 32-in. cellulose dialysing tubing. One end of the tubing was dialysed during the pervapouration (6 hr.). Several concentrated eluates, after being cleared by centrifugation and dialysed for 2 hr. against cool running tap water, were pooled and again concentrated by pervapouration (3 hr.). This preparation, 'Uro-100×', was again cleared by centrifugation, dialysed for 90 min. against distilled water and was then ready for use. The activity of the material was retained several days when it was frozen. Fibrinolysis occurred within 20–40 min. when 5 per cent 'Uro-100×' was added to human plasma and the mixture clotted with thrombin.

Standard clots were prepared as follows: Human *ACD*-bank blood plasma was mixed with 10 per cent 0·25 *M* calcium chloride, poured into the stem of a 'Klimax' protein sedimentation tube No. 46815 to exactly the 0·4 ml. mark, and allowed to clot at 37°. At least 1 ml. of the test solution was poured into the wide part of the tube on top of the clot filling the stem. A small glass bead was added, and the tube closed by insertion of a rubber stopper, allowing space for a small air bubble above the test solution. The tubes were chemically clean, siliconized, and the procedure, except for the urokinase, carried out under sterile conditions. The tubes were attached horizontally to a rocking device with a cycle of 30 sec. and a deviation for the horizontal of ten degrees in either direction, and placed in an incubator. The air bubble and the glass bead mixed the enzyme solution gently without touching the clot. With this arrangement, the relation of enzyme solution to the clot and its lysed product was kept constant within a few per cent during the experiment and prevented local accumulation of inhibitor deriving from the lysed clot. There was no marked progressive dilution of the test solution as in the fibrin plate method.

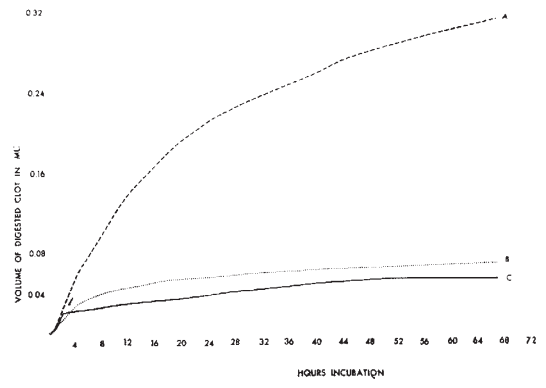

Fig. 1. Continuous fibrinolysis induced in human standard clots by human urokinase. Enzyme solutions: *A*, 10 per cent 'Uro-100×' in buffered saline; *B*, 10 per cent 'Uro-100×' in plasma; *C*, 10 'Uro-100×' in buffered saline, replaced after 2 hr. by saline (arrow). Abscissa; incubation time (hr.); ordinate, volume of clot digested