

A detailed account of these cultures will be published elsewhere.

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TAYLOR A. STEEVES
Society of Fellows of Harvard University.

I. M. SUSSEX
Department of Cryptogamic Botany,
University, Manchester.
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A Difference in *in vitro* Pancreatic Digestion between Animal and Plant Protein Feeds

MELNICK *et al.*¹ and Evans² found that the rate of enzymatic liberation of amino-acids from soy-bean proteins preheated at different temperatures provides an explanation for their different growth-promoting properties for the growing chick. We have used this principle to investigate the differences between the biological value of various types of protein feeding-stuffs for animals. In the course of this work we observed a property in which plant and animal proteins differ: pancreatic digests of plant protein feeds, but not of animal protein feeds, yielded considerable precipitates on addition of trichloroacetic acid.

The experiments were carried out with feeding-stuffs defatted with ether and dried at room temperature. 6-gm. samples of each feed were placed in 250-ml. Erlenmeyer flasks, 300 mgm. of 'Pancreatin' (Byla, Paris), 150 ml. of phosphate buffer (pH = 8.2; Sørensen) and a few drops of toluene were added. The flasks and contents were incubated at 37–38° C. for 48 hr. with frequent shaking. In one set of experiments the non-digested materials were filtered, washed several times with phosphate buffer (pH = 8.2) and the nitrogen content of the precipitates was determined by Kjeldahl digestion. In a second set the whole digests (together with the substrates) were acidified with hydrochloric acid (methyl red) and 25 ml. of 5 per cent trichloroacetic acid added. The precipitates were filtered and washed with hot water containing 0.5 per cent trichloroacetic acid.

The precipitates obtained by addition of trichloroacetic acid to plant protein digests contained much larger amounts of nitrogen than the non-digested residues by themselves, amounting to

20–25 per cent of the nitrogen contained in the original feeding-stuff. In the case of the pancreatic digests of animal-protein feeds, addition of trichloroacetic acid did not cause precipitation of any additional quantity of nitrogen.

It may be noted that in similar digestion experiments carried out with pepsin (pH 1.5), no additional nitrogen was precipitated by trichloroacetic acid, either in digests of plant proteins or in those of animal proteins.

Experiments to elucidate the chemical nature of these soluble nitrogen compounds precipitated by trichloroacetic acid are under way.

A. BONDI
YEHUDITH BIRK

Agricultural Research Station,
Rehovot.
March 21.

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Production of Gliotoxin in Unsterilized Soil

It has long been recognized that microbial antagonisms exist in the soil, and the possibility that this might be due to production of antibiotics has been suggested although never actually proved.

It has been shown that soil can be used as a medium for growth of many micro-organisms and also for antibiotic production, but in general sterilizing and supplementing with organic matter have been found to be necessary. Grossbard¹ has demonstrated the production of an antibiotic in autoclaved and also in partially sterilized soil, and Hessayon² has shown that activity can be produced in sterile unsupplemented soil. Gregory, Allen, Riker and Peterson^{3,4}, moreover, have demonstrated activity in unsterile soil which was, however, supplemented with highly nutrient organic matter. In these cases it was assumed, but not conclusively demonstrated, that the antibiotic formed in the soil was identical with that produced by the fungus in synthetic media.

The results presented here show the production of gliotoxin by *Trichoderma viride* in Wareham Heath soil, a highly acid podsol, in which all the microflora was present.

Unsterile soil was supplemented with dried clover, inoculated with a spore suspension of a gliotoxin-producing strain of *T. viride* and incubated at 25° C. for four days. The soil was extracted with ether and the extract used to demonstrate and identify the antibiotic.

Identification was demonstrated by a bio-assay based on paper chromatography. Chromatograms were run on the soil extracts, and solutions of pure gliotoxin on filter paper strips, and these were placed on agar seeded with *B. subtilis* as the test organism. After incubation of plates the distance moved by the active substance was demonstrated by the position of a clear zone of inhibition and the *R_F* values were calculated. The amount of gliotoxin present could be estimated roughly from the size of the inhibition zone.

	<i>R_F</i> values
Pure gliotoxin	0.60
Inoculated soil + 5 per cent clover	0.59
Inoculated soil + 2 per cent clover	0.59

Activity was produced only in inoculated unsterile soil to which dried clover had been added, and as

Feed	Total N-content of the fat-free feed (per cent)	Insoluble N after 48 hr. digestion with 'Pancreatin'	Insoluble and precipitated N after addition of CCl ₃ COOH to pancreatic digests
		In per cent of the N-content of the fat-free feed	
1. Fish meal (Norwegian)	11.6	40.1	40.1
2. Meat-meal (Argentinian)	11.4	25.0	25.3
3. Soy bean oil meal	7.7	10.3	30.0
4. Sesame oil meal	7.9	16.6	42.5