movements reached a surprising amplitude. The fish started swimming blindly in a distressed condition and often dashed against the glass jar wall with eyes fixed in one position. This process continued for about 20 min. Ultimately, the stimulus for swimming gradually vanished and the fish settled at the bottom of the jar slightly on its side with fins extended. At this time the opercular movements were occurring at a rate of one hundred and five each minute, which indicated a normal respiratory condition. Soon afterwards the fish displayed characteristic movements, and its posterior half was seen to bend to one side, though occasionally the fish struggled to regain its normal condition. Gradually, the mouth closed, and with this closure the opercular movements ceased and the fish died. This survival of the two fish for 81 days and 102 days in a sealed jar completely cut off from outside atmosphere indicates that the fish are changing to anaerobic respiration.

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"Pyrethrum" in Peonies

In 1962, it was reported that the petals of *Paeonia albiflora* Pall. contain considerable quantities of "pyrethroids"[1]. The chemical evidence was based on ultra-violet absorption maxima and on the supposed isolation of chrysanthemum mono- and di-carboxylic acids. Evidence that the compounds isolated from the peony were not identical with the pyrethroids that occur in *Chrysanthemum cinerariaefolium* is the reported melting point (205–206°) for the diacid. Naturally occurring (+)-chrysanthemum dicarboxylic acid has a melting point of 164° (ref. 2).

We have carried out a careful examination of dried flowers of *Paeonia albiflora* from various sources using extraction with acetone and solvent partition techniques[3]. Also fresh, freeze dried flowers were extracted at 4° C with 60°–80° light petroleum and the solvent removed at ambient temperature on a rotary evaporator, followed by partition[3].

The yellow oils isolated by these techniques were examined by gas-liquid chromatography using an electron capture detector and columns of either '*S.E.* 30' on 'Ballotini' or neopentyl glycol succinate on 'Chromosorb *W*'. In no cases were compounds corresponding to the known pyrethroids detected in even millimicrogram amounts.

The extracts and concentrates were also examined by thin-layer chromatography on silica gel 'HF_{254}' and silyer nitrate impregnated plates. In addition to the normal ascending techniques[4], low temperature chromatography and the *BN* chamber[5] were also used for separation.

With these techniques none of the six known pyrethroids or their decomposition products was found. Direct readings of ultra-violet spectra from the plates were carried out using the Zeiss chromatographic spectrometer for thin-layer chromatography, according to Stahl[5].

A nitromethane concentrate was chromatographed on a silica gel plaster-of-Paris column using 8 per cent ethyl acetate in 80°–100° light petroleum[3]. Fractions were examined by gas-liquid and thin-layer chromatography and infra-red and ultra-violet spectroscopy. No compounds having a pyrethroid structure were detected.

Finally, extracts were examined by a highly sensitive bioassay with *Aedes aegyptici* larva[4]; no insecticidal activity was detected.

We do not consider that the experimental evidence in the previous paper[1] is rigorous enough to establish the presence of "pyrethroids" and our work suggests that the claim is unfounded.

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Auxin-induced Elimination of a Protein in Bean Hypocotyl

It has been reported that electrophoretic protein patterns of hypocotyledonary hooks of bean (*Phaseolus vulgaris*, L., var. 'Burpee's Stringless Greenpod') were altered when the extracted proteins were incubated with the auxin indolyl-3-acetic acid (IAA)[1]. The alteration consisted of the complete elimination of one protein band. It seemed useful to determine whether this elimination of a protein could occur *in vivo* in tissue segments treated with IAA.

Hypocotyledonary hooks (1 cm) were excised from 7 day old bean seedlings grown in vermiculite in the dark at 23° C and were floated in the dark in various solutions. All incubation solutions were buffered with 0·005 molar potassium phosphate buffer, *p*H 7·0. The length of incubation was varied from 2 h to 12 h. After incubation, the hooks were rinsed, blotted dry and used immediately for protein extraction or were stored at −20° C until required.

Protein was extracted by grinding the tissue in the cold in 0·1 molar sodium borate buffer, *p*H 8·1 (1 g tissue : 2 ml. buffer). The homogenate was squeezed through four layers of cheesecloth and centrifuged for 15 min at 7,000*g*. The supernatant was divided into three equal portions. One aliquot was applied directly to gel columns for electrophoresis. A second aliquot was dialysed 48 h against a 0·002 molar borate buffer, *p*H 8·1, and was then electrophoresed. The third aliquot was adjusted to 70 per cent saturation with ammonium sulphate, centrifuged for 15 min at 7,000*g* and the pellet was dissolved in a 0·1 molar borate buffer, *p*H 8·1. This solution was then used for electrophoresis, which was performed on columns of 7·5 per cent polyacrylamide gel using a 'Canalco' disc electrophoresis assembly. *Tris*-glycine buffer, *p*H 8·3, was the electrolyte. The proteins investigated were those migrating towards the anode at 4 m.amp/column.

Fig. 1 shows four electrophoresis columns, each representing proteins of differently treated tissues. The dark region at the top of the columns is actually composed of three dense protein bands as revealed by microdensitometry. The region of interest is immediately below these