

peptide, together with those of PE, PC, GPE, GPC and PS, compared with the results of the avian brains<sup>2</sup>.

The results show that the values of the phospholipid phosphoric esters (except GPC) are lower in placenta than in brain tissue. On the contrary the NH<sub>2</sub>-N-containing compounds (not reported in Table 1) are lower (except aspartic acid) in brain than in placenta extracts.

The presence of the phospholipid phosphoric esters in human placenta lead us to suppose that the metabolism of the phospholipid molecules is operative in this tissue.

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### Extraction and Purification of 'Queen Substance' from Queen Bees

QUEEN honeybees (*Apis mellifera*) secrete material which, distributed through the colony, affects the bees in two ways, inhibiting the development of ovaries in workers and influencing their behaviour by inhibiting queen rearing (that is, queen-cell construction)<sup>1</sup>. Carlisle and Butler<sup>2</sup> observed similarities between the 'queen substance' of honeybees in the form of an alcoholic extract and the ovary-inhibiting hormone of prawns (*Leander serratus*), and it is a justifiable extrapolation to suppose that this material has important physiological effects on animals of other phyla.

A method of quantitative assay based on the inhibition of the construction of emergency queen cells was devised by Butler and Gibbons<sup>3</sup>, and used by Butler and Simpson<sup>4</sup>, to demonstrate that 'queen substance' is contained in the mandibular glands of the queen honeybee. Using this test, the extraction and concentration of 'queen substance' have been

investigated and a crystalline material of high activity has now been isolated.

The head of the queen bee is a convenient raw material for extraction, and, after solvent extractions and partitions, an acidic fraction is obtained which is finally purified by chromatography. Quantities are given for 100 heads of mated queen bees. These were extracted in a Soxhlet apparatus with ethanol for 4 hr., the residue from the extract, about 150 mgm., was taken up in ether and water, the solutions were separated and the aqueous layer extracted with ether until a clear ether extract was obtained; the dried ethereal solution yielded approximately 90 mgm. The extract was partitioned between 6 ml. of 50 per cent aqueous ethanol and 6 ml. of light petroleum (boiling point 80–100°) and the petroleum layer was extracted again with 6 ml. of aqueous ethanol. The residue from the aqueous ethanolic solution, approximately 60 mgm., was taken up in 6 ml. of ether and extracted three times with 6 ml. of *N* sodium hydroxide. The alkaline extract was saturated with carbon dioxide, extracted with ether, acidified and again extracted with ether. The more strongly acidic fraction was separated into two fractions by chromatography on paper (stationary phase, water/methanol (1:3), mobile phase toluene). The fraction with *R<sub>F</sub>* approximately 0.5 was cut out and eluted with ethanol. The ether-soluble fraction of the extract was extracted into phosphate buffer pH 8.3 which was acidified and extracted with ether.

An extract obtained in this way (18 mgm.) yielded waxy crystals, melting point 45–50°, which could be recrystallized from aqueous methanol. The characteristic infra-red absorption spectrum (Fig. 1), paper-chromatographic behaviour, potentiometric titration and colour reactions indicate that the crystalline material is an  $\alpha\beta$ -unsaturated carboxylic acid, containing an unconjugated carbonyl group and with a molecular weight of the order of 190. It would appear to be closely related to the 10-hydroxy- $\Delta^2$ -decenoic acid present in royal jelly<sup>5</sup> and secreted in the mandibular glands of worker bees<sup>6</sup>. Tested on groups of bees, about 0.13  $\mu$ gm. per bee inhibited queen-cell production. The investigation is being continued and results will be published in detail elsewhere.

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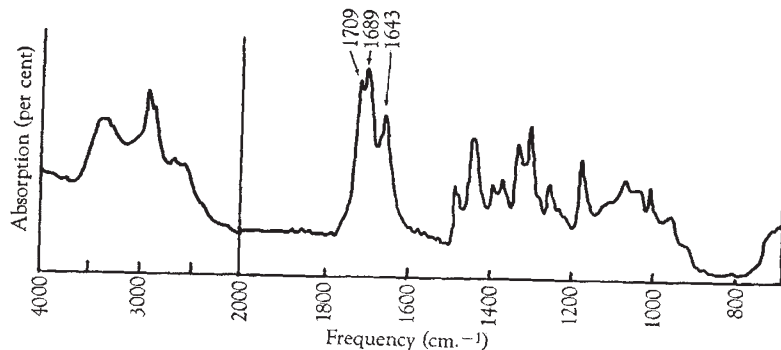


Fig. 1. Infra-red absorption spectrum of 'queen substance'. 160  $\mu$ m. in 40 mgm. potassium chloride. Perkin-Elmer Model 21; sodium chloride prism

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