The release of prostaglandin from the oesophagus and the stomach of the frog (*Rana temporaria*)

Vogt & Distellkötter (1966) first reported the spontaneous release of a prostaglandin into fluid bathing the frog intestine. They also showed an increase of release of prostaglandin when the intestine was placed in distilled water. Bartles, Vogt & Willie (1968) showed that acetylcholine (10^{-5} g/ml) increased the concentration of free prostaglandin in frog intestine. The release of prostaglandin from the oesophagus and the stomach of *Rana temporaria* has now been investigated under conditions of rest and of electrical stimulation.

The tissue strips were stimulated electrically between two parallel electrodes (Birmingham & Wilson, 1963), using a frequency of 5 Hz at 0·2 m/s pulse width for oesophagus and 0·1 m/s width for stomach at maximal voltage (150v), in a bath containing 5 ml "frog" Krebs solution* at room temperature bubbled with 5% carbon dioxide in oxygen. Parallel experiments were made in which the tissues from one frog were set up for measurement of the spontaneous release of prostaglandin and the tissues from another frog were stimulated electrically. At hourly intervals for the whole day (5 h per day) the bath contents were removed for assay and replaced by fresh "frog" Krebs solution.

The five samples were pooled and acidified with *N* hydrochloric acid to a pH 2·0–2·5, then shaken up with an equal volume of diethyl ether for 2 min. The ether was then removed in a separator funnel. This process was repeated three times. The volume of ether collected in this way was evaporated to dryness in a stream of nitrogen and the residue was dissolved in Krebs solution (1 ml) and assayed on the rat fundus strip (Vane, 1957). Standard solutions of prostaglandin E_1 (1 mg/25 ml) were prepared and taken through the procedure just described.

The results (Table 1) are the outcome of five experiments on both stomach and oesophagus. The spontaneous release of prostaglandin was the same from stomach and oesophagus. Both tissues showed increased release on electric stimulation. On electric stimulation the release was greater from the oesophagus than from the stomach. The recovery of a known amount of prostaglandin E_1 by the method was 70–80%.

The released prostaglandin was identified according to Green & Samuelson (1964) and to Fleshler & Bennett (1969). These methods can only distinguish between E and F types of prostaglandins, but they do not differentiate between E_1 and E_2 or $F_{1\alpha}$ and $F_{2\alpha}$. The prostaglandin released from the stomach and the oesophagus in these experiments was predominantly E type.

The results presented here show that there was a small spontaneous release in the resting state from the frog oesophagus and stomach which could be increased by stimulation of the intramural nerves. This release was not as high as that reported by Vogt & Distellkötter (1966). The prostaglandin released by stimulation

Table 1. *Release of prostaglandin from the oesophagus and the stomach of* R. temporaria *in resting and electrically stimulated states. Means ± s.e. of means.*

State	Oesophagus	Stomach
Resting (Spontaneous)	1·33 μg/g (n = 5) s.e. ± 0·07	1·17 μg/g (n = 5) s.e. ± 0·26
Electrically stimulated	27·0 μg/g (n = 5) s.e. ± 1·78	6·16 μg/g (n = 5) s.e. ± 0·14

* 1 litre of "frog" Krebs solution = 700 ml of Krebs solution + 300 ml of distilled water; pH after gassing, 7·3.

may have come from the stores in the muscle itself or from the nerve fibres but the experiments do not allow a firm conclusion to be drawn about the origin of the prostaglandin E.

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Cannabis-induced vocalization in the rat

A disadvantage of previous methods of assaying the potency of cannabis preparations (Dixon, 1899; Gayer, 1928; Valle, 1967a,b) is that they cannot be used for determining effect of cannabis in rats. One possible approach is to use the degree of ataxia or catalepsy induced by the drug, another is to use vocalization as reported by Carlini & Kramer (1965). They found that, under the influence of cannabis, rats vocalize when they are touched.

We have set out to find if vocalization is a relevant indicator of cannabis effect. As reported by Boyd, Hutchington & others (1963) we also found that even low doses of cannabis produce a decrease in fixed ratio responding for food in rats. We have therefore compared the minimal doses necessary to affect vocalization with bar-pressing behaviour for food in a fixed ratio (FR) program.

Adult male albino rats of the Sprague-Dawley strain, weighing 300–350 g, 10 animals to each dose, were tested with cannabis extract (75% tetrahydrocannabinol, 7% cannabinol, 11% cannabidiol) or synthetic Δ^9- and Δ^8-tetrahydrocannabinol (THC) intraperitonally or orally, to assess the drugs' ability to dispose the animals to vocalization. The drugs were dissolved either in propylene glycol or olive oil. In an inhalation experiment, raw material (3·2% tetrahydrocannabinol, 1·2% cannabinol, 5·1% cannabidiol), as smoke, was tested, the rats being confined in a closed acrylic cage (22 × 15 × 15 cm) for 10–15 min after it had been filled with pure cannabis- or tobacco-smoke. The amount used varied between 0·60–0·80 g/rat.

Animals were tested in their individual cages. After the administration of the drugs, the rats were gently pressed with thumb and forefinger by the experimenter 2–4 times bilaterally behind their forelimbs on the ventral aspect of the frontal costal region every 5 min, to find the onset and duration of the vocalization behaviour and to see if habituation occurred.

In the fixed ratio experiment, six animals, trained at a FR30 schedule of reinforcement performing in daily sessions of 15 min, were used. The apparatus was standard operant conditioning equipment.

Vocalization could be produced in animals given: extract, in propylene glycol, in doses of 5 mg/kg, i.p. or more (one of ten animals did not vocalize at 10 mg/kg); extract, in propylene glycol or olive oil, orally in doses of 50 mg/kg or more; raw