IDENTIFICATION OF BEHAVIORALLY SIGNIFICANT VOLATILE COMPOUNDS IN THE ANAL GLAND OF THE RABBIT, *Oryctolagus cuniculus*

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Abstract—An investigation of the anal gland volatiles of the male wild rabbit *Oryctolagus cuniculus* has been made in which the heart rate of adult male rabbits, positioned to sniff the effluent from a gas chromatographic capillary column, has been employed as an indicator for the presence of constituents of likely behavioral significance. The examination by this means of the headspace volatiles of homogenized anal glands using gas chromatography and gas chromatography—mass spectrometry has implicated a number of saturated and monounsaturated normal and methyl-branched aldehydes in the C9–C12 range.

Key Words—rabbits, *Oryctolagus cuniculus*, cardiac responses, anal glands, aldehydes, headspace collection, gas chromatography, GC-MS.

INTRODUCTION

Earlier studies have established that the odor of anal gland secretions of the wild rabbit, *Oryctolagus cuniculus*, has a function in territorial marking (Mykytowycz, 1968). The general chemical composition of the secretions has been described (Goodrich and Mykytowycz, 1972), and more recently various fractions of secretions from this gland were tested for their behavioral significance using subadult and nestling rabbits (Hesterman et al., 1976). The acid and neutral fractions elicited the strongest behavioral response in the
form of avoidance. Measurements of heart rate in nestlings showed a statistically significant correlation between deceleration of heart rate and strength of the behavioral response. These results suggested that changes in heart rate can be used to detect differential responses of rabbits to various chemical components of the same secretion.

In this study the change in heart rate in adult male rabbits located to sniff the outlet of a capillary column in a gas chromatograph and then in a gas chromatograph-mass spectrometer (GC-MS) unit, has been used to indicate the presence of volatile compounds in the anal glands, which may be behaviorally active.

METHODS AND MATERIALS

General Approach

In view of the low concentration of volatiles in the secretion from the anal glands of rabbits and the difficulty involved in collecting adequate amounts of the secretions, it was necessary to use pooled whole glands. These were homogenized in saline solution and headspace collections of the volatiles were made by techniques developed for the examination of food flavor volatiles.

Preliminary gas chromatographic examination of the headspace volatiles were made on a glass SCOT column coated with a polar phase (Carbowax 20M) with two human observers monitoring the effluent by nose. The presence of compounds with strong “rabbit-like” odors was thereby established. The next step was to use the heart-rate response of rabbits located to sniff the same column effluent to indicate the positions in the complex chromatogram (Figure 2) of compounds of likely behavioral activity. Guided by this information fractions were collected from the more significant areas in repeated runs of the chromatograph. These fractions were then rechromatographed on a glass SCOT column of lower polarity (Silicone SF96) which was coupled to a mass spectrometer and with the rabbit again positioned to monitor the effluent. The high resolution achieved by transferring narrow fractions from the polar to nonpolar columns ensured that the rabbit was exposed mostly to well-resolved constituents. Mass spectra recorded concurrently formed the basis of the identification of significant components.

Sample Preparation

Homogenates of pooled anal glands from adult male rabbits (20 g equivalent to approximately 20 glands) were prepared in saturated saline solution (40 ml) at 0°C using an Ultra-Turax high-speed homogenizer (Janke