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CARBOHYDRATES OF THE PROSTATE OF TWO AUSTRALIAN MARSUPIALS, *TRICHOSURUS VULPECULA* AND *MEGALEIA RUFA*

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Summary. Total carbohydrate, glucose, fructose and resorcinol-reactive material were estimated in homogenates of the prostates of brush-tailed possum (*Trichosurus vulpecula*) and red kangaroo (*Megaleia rufa*). Fructose was virtually absent from the gland in both species. Glucose occurred in quite considerable quantities in the posterior segment of the prostate of *M. rufa* (92·2 mg/100 g) and to a lesser extent in the small anterior segment of *T. vulpecula* (22·4 mg/100 g). The posterior prostate of *T. vulpecula* contained an unidentified material which reacted with the resorcinol reagent as fructose (51·2 mg/100 g) but did not appear to be fructose or a commonly occurring ketose.

INTRODUCTION

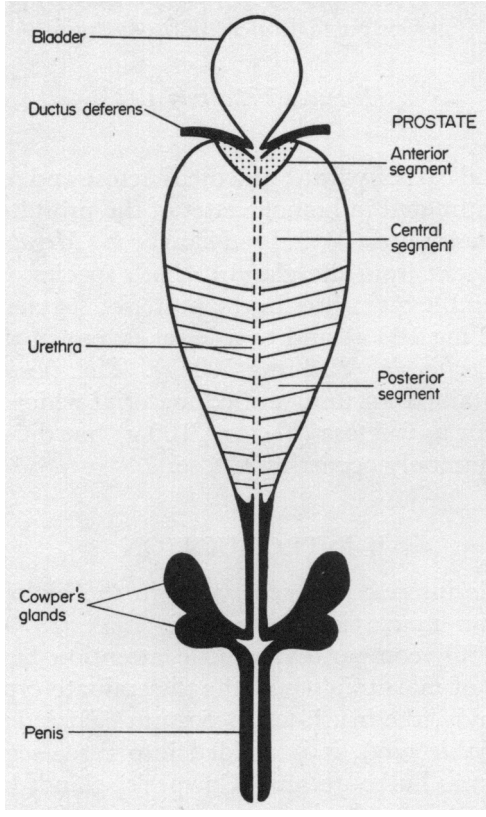
Despite considerable interest in recent years in the biology and biochemistry of female Australian marsupials (Sharman, 1959, 1970; Waring, Moir & Tyndale-Biscoe, 1966), comparatively little attention has been given to the male. The prostate of marsupials is of the disseminate type and in the species examined in this study, the brush-tailed possum (*Trichosurus vulpecula*) and the red kangaroo (*Megaleia rufa*), it is divided into three segments (Text-fig. 1). Marsupials also possess bulbourethral (Cowper's) glands but none of the other structures often found in eutherian species, e.g. seminal vesicles, coagulating glands or ampullary glands. In marsupials, the prostate is a relatively large organ which contributes the bulk of the seminal plasma and, since the carbohydrates of seminal plasma (notably fructose) have been found to be important in the metabolism of the spermatozoa of other species (see Mann, 1964), study of the reproduction of male marsupials has been initiated by examining the carbohydrates of the prostate gland.

MATERIALS AND METHODS

Prostate glands were dissected immediately after death from mature males which had been shot from wild populations. The specimens of red kangaroo were from Walgett, New South Wales, and were killed during August 1971.

The possum material was from animals shot in the Wentworth region of New South Wales during April 1972.

After dissection, the tracts were frozen in an insulated chest containing dry ice, returned to the laboratory and stored in a deep-freeze until homogenates could be made. While the glands were thawing, the bladder and any excess tissues were removed and the whole prostate was weighed. The gland was split along its length dorso-ventrally to reveal the three segments of the prostate, which were then separated and the central and posterior segments (including



TEXT-FIG. 1. Diagram of the prostate and associated structures of the red kangaroo (*Megaleia rufa*) and the brush-tailed possum (*Trichosurus vulpecula*), showing the three prostate segments.

the surrounding smooth muscle and the urethra) were weighed. Weighed portions of the actual glandular tissue of the three segments were homogenized in 10-ml vols of distilled water, using a plastic pestle driven by an electric motor and glass homogenizing tubes (TRI-R Instruments, Rockville Centre, New York, U.S.A.). Homogenates were deproteinized by adding 10 ml of 5% $ZnSO_4.7H_2O$ and 10 ml of 0·3 N-$Ba(OH)_2$. After centrifuging, deproteinized extracts were freeze-dried and the residue was dissolved in 3 ml of 80% ethanol. A known volume of this ethanol extract was evaporated to dryness under a stream of nitrogen and the residue was dissolved in 0·3 ml distilled water. This final

extract was frozen until assayed. Total carbohydrate was assayed by the anthrone reagent method of Brin (1966) with glucose standards. The resorcinol-HCl method was that of Roe (1934), using fructose standards. Glucose and fructose determinations were made using the enzymatic method of Klotzsch & Bergmeyer (1963).

RESULTS

The three segments of the prostate of *T. vulpecula* (Table 1) contained free carbohydrate, as revealed by the anthrone reaction, of which approximately half could be accounted for as glucose. The anterior segment had the highest

Table 1. The free carbohydrates of the prostate of the brush-tailed possum

Animal	*Segment of prostate*	*Weight of segment (g)*	*Total carbohydrate (anthrone-reactive material as glucose) (mg/100 g tissue)*	*Glucose (mg/100 g tissue)*	*Fructose (mg/100 g tissue)*	*Resorcinol-HCl-reactive material (as fructose) (mg/100 g tissue)*	*Weight of whole gland (g)*
TV 205	Anterior	—	45·0	15·5	1·0	4·8	21·1
	Central	15·0	17·8	8·6	0·4	2·1	
	Posterior	4·0	37·6	12·1	1·3	30·9	
TV 206	Anterior	—	71·9	31·4	4·6	2·9	25·6
	Central	19·2	15·1	7·1	0·7	0·7	
	Posterior	4·4	18·5	8·4	1·2	76·6	
TV 207	Anterior	—	47·9	26·7*	2·0*	0	32·1
	Central	25·4	9·3	5·4	0·4	0·9	
	Posterior	4·0	42·1	11·0	0·9	50·6	
TV 208	Anterior	—	56·3	18·2	2·7	1·4	26·8
	Central	19·4	19·2	4·1*	0·7*	3·8	
	Posterior	5·6	24·4	9·0	0·5	58·1	
TV 209	Anterior	—	50·7	20·0	1·8	10·1	23·5
	Central	18·2	13·6	5·0	0·7	2·6	
	Posterior	4·3	22·9	7·7	1·2	39·7	
Mean	Anterior	—	54·4	22·4	2·4	3·8	25·8
	Central	19·4	15·0	6·0	0·6	2·0	
	Posterior	4·5	29·1	9·6	1·0	51·2	

The results are the mean of two estimates on two separate homogenates.

* The mean of two estimates on one homogenate.

content of both free sugar and glucose. Fructose accounted for only 0·6 to 2·4 mg of the total sugar of the gland. Resorcinol-HCl-reactive material was present in very low concentrations in both the anterior and central segments. This was not the case, however, in the posterior segment where there was a considerable quantity of resorcinol-HCl-reactive material. This material was apparently not anthrone-reactive as the resorcinol-HCl assay significantly exceeded the anthrone-assayed material not accounted for as glucose or fructose.

Chromatography of extracts of the posterior segment of the prostate of the brush-tailed possum revealed the presence of glucose and an extremely fast-moving compound which reacted with aniline diphenylamine reagent but

failed to react with the other sugar reagents (Tables 2 and 3). The reaction with aniline diphenylamine reagent (ADP) was examined to find what part or parts of the mixture were actually involved in the reaction. If aniline was deleted from the reagent (DP), an identical reaction occurred (Table 3), this reaction requiring the presence of both diphenylamine and phosphoric

Table 2. Chromatographic characteristics of the resorcinol-reactive material in the posterior prostate of the brush-tailed possum: descending chromatography on Whatman No. 4 paper

Solvent	R_F*	R_G†
Isopropanol, water 4 : 1 (v/v)	0·89	1·78
l-butanol, ethanol, water 50 : 10 : 40 (v/v), top layer	0·88	3·95
l-butanol, ethanol, water, glacial acetic acid 50 : 10 : 39 : 1 (v/v), pH 3·6, top layer	0·90	4·60
l-butanol, ethanol, water, ammonia 50 : 10 : 39 : 1 (v/v), pH 11·2, top layer	0·52	2·71

$$* R_F = \frac{\text{Distance substance travels from origin}}{\text{Distance solvent front travels from origin}}.$$

$$\dagger R_G = \frac{\text{Distance substance travels from origin}}{\text{Distance glucose travels from origin}}.$$

Table 3. Colour reactions on paper of the resorcinol-reactive material in the posterior prostate of the brush-tailed possum

Reagent	*Reaction*	*Method*
ADP*	Yellow-green which changes to bright blue when the paper is washed in water	Smith (1960) except phosphoric acid to acetone solution = 1 to 20
DP	As ADP	As ADP except aniline deleted
Dische	Green-grey, turns yellow when washed in water	Deriaz *et al.* (1949)
20% Phosphoric acid	Red-grey	Edwards (1969)
1% H_2SO_4	Brown-grey, turns yellow when washed in water	Edwards (1969)

The unidentified material failed to react with: AD, A, D, and gave a very weak yellow colour with AP and P. It also failed to react with the following sugar reagents: silver nitrate (Trevelyan, Proctor & Harrison, 1950), anisidine (Hough, Jones & Wadman, 1950), naphthoresorcinol, resorcinol† (Forsyth, 1948) and aniline phthalate (Partridge, 1949).

* A, aniline; D, diphenylamine; P, phosphoric acid.

† Although the material failed to react with resorcinol reagent on paper, water eluants of the region contained resorcinol-reactive material of the same order of concentration as assayed in the unchromatographed extract.

acid (DP). This DP reagent is similar to the Dische reagent for 2-deoxyribose (diphenylamine in an acid solution) and on paper the unidentified material also formed a green colour product with Dische.

The posterior prostate of the red kangaroo (Table 4) was characterized by quite high concentrations of sugar, most of which was glucose. Fructose was

virtually absent from the gland and only small quantities of resorcinol-HCl reactive material other than fructose were present. The anterior segment of the prostate in the possum and the posterior segment in the kangaroo contained most of the free carbohydrate.

Table 4. The free carbohydrates of the prostate of the red kangaroo

Animal	*Segment of prostate*	*Weight of segment (g)*	*Total carbohydrate (anthrone-reactive material as glucose) (mg/100 g tissue)*	*Glucose (mg/100 g tissue)*	*Fructose (mg/100 g tissue)*	*Resorcinol-HCl-reactive material (as fructose) (mg/100 g tissue)*	*Weight of whole gland (g)*
MR 201	Anterior	—	28·5*	11·2*	3·9*	4·7*	46·0
	Central	19·1	45·1	8·3	1·3	4·8	
	Posterior	24·2	137·4	96·6	2·2	10·0	
MR 202	Anterior	—	32·1	10·5	1·7	4·9	74·3
	Central	33·1	50·6	4·7	0·5	6·4	
	Posterior	38·4	89·8	73·9	0·6	4·9	
MR 203	Anterior	—	15·0	7·7	2·3	4·5	41·6
	Central	17·0	22·7	5·8	2·3	4·4	
	Posterior	19·7	125·1	70·2	5·4	10·2	
MR 204	Anterior	—	12·6	8·8	1·5	3·3	55·8
	Central	24·1	52·9	10·6	0·6	7·0	
	Posterior	28·0	177·7	132·5	2·3	8·3	
MR 205	Anterior	—	26·2*	10·5*	0·0*	4·5*	22·7
	Central	10·7	56·0	6·4	0·9	10·5	
	Posterior	11·2	151·1	87·9	2·6	8·9	
Mean	Anterior	—	22·9	9·7	1·9	4·4	48·1
	Central	20·8	45·5	7·2	1·1	6·6	
	Posterior	19·9	136·2	92·2	2·6	8·5	

The results are the mean of two estimates on two separate homogenates.

* The mean of two estimates on one homogenate.

DISCUSSION

The failure of the unidentified material to react with carbohydrate reagents on paper is consistent with its failure to react with the anthrone reagent as described earlier. Comparison of R_F values obtained using the three butanol solvents suggests that the substance is acidic. A green Dische reaction is obtained with a number of aldehydes and ketoses (Deriaz, Stacey, Teece & Wiggins, 1949), but the Dische reaction with 2-deoxyribose is bright blue. Benzaldehyde and salicylaldehyde, which produce green colours with Dische, also form green products with DP. Both Dische colour reactions appear to be due to diphenylamine reacting with aldehydes either present before, or as the result of acid degradation (Deriaz *et al.*, 1949). Presumably, a similar mechanism is involved in the DP method.

The distinct red colour, formed with 20% phosphoric acid, does not help to characterize the unidentified substance, as a wide range of steroids (Edwards,

1969) and some sugars (e.g. fructose, sucrose and mannose) also react in this way. The u.v. spectra of the material in ethanol (high absorbance at wavelengths of approximately 200 nm and no absorbance peaks in the range 250 to 300 nm) rule out the substituted steroids, and leave as a possibility only the simple cholesterol-like forms. Cholesterol moves at the solvent front in the butanol : ethanol : water (5 : 1 : 4) solvent and is unreactive with diphenylamine phosphoric acid reagent. The material is, however, presumably not a monosaccharide since it fails to react with silver nitrate and other carbohydrate reagents. The reaction with resorcinol in the test-tube and Dische reagent on paper both suggest the presence of keto groups. Further chemical studies involving gas-liquid chromatography, infra-red spectroscopy, nuclear magnetic resonance spectroscopy and mass spectrometry are now in progress to characterize the material.

Although the posterior prostate of the possum contains quite considerable quantities of the unidentified resorcinol-reactive material, the segment is relatively small in comparison with the rest of the gland and it might be expected that the content of the resorcinol-reactive material in the semen would be lower than the assays of gland tissue might suggest. By contrast, the posterior segment of the red kangaroo prostate made up a major portion of the gland and thus it might equally be expected that its secretions, notably glucose, would be major constituents of the seminal plasma.

The absence of fructose from the major accessory gland of the two species examined is in marked contrast to findings in almost all eutherian species previously examined (Mann, 1946, 1964). Notable exceptions are the dog, 2 to <1 mg fructose/100 ml semen (Bartlett, 1962; Wales & White, 1965), the stallion, 2 mg fructose/100 ml semen, and the jackass, 3 mg fructose/100 ml semen (Mann, Minotakis & Polge, 1963). Glucose is not normally considered a major constituent of semen although on occasions high concentrations have been noted in rabbit, cock and man (Mann & Parsons, 1950; Mann, 1954; Eliasson, 1965). Glucose levels similar to those in the posterior prostate of the red kangaroo have been found in the seminal vesicle of the guinea-pig and coagulating glands of the rat and field vole (Fouquet, 1971).

Mann & Lutwak-Mann (1963) found levels of resorcinol-reactive material similar to those of *T. vulpecula* in the prostate of the American opossum, *Didelphis*, i.e. 3 mg/100 g in the anterior segment, 5 mg/100 g in the central segment and 44 mg/100 g in the posterior segment. They assumed this material was fructose but in view of the present observations, this may not be so. It would seem reasonable to suggest that the resorcinol-reactive material found in the posterior prostate of *Didelphis* is not fructose but possibly the same or a similar substance to that which occurs in the posterior prostate of the Australian possum.

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