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Polysaccharide Digestion in Cheek Pouches of the Bonnet Macaque

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ABSTRACT. Bonnet macaques (*Macaca radiata*) possess cheek pouches which equip them to raid field crops. The pouches are regarded as the replacement of the first part of sacculated stomach of langur. The present study demonstrates that these pouches cause starch digestion also and that the secretions contain two different amylases or isozymes.

INTRODUCTION

Among members of Cercopethicidae, langurs have enlarged sacculated stomachs unlike the bonnet macaques which possess cheek pouches (NAPIER & NAPIER, 1967). Although the presence of sacculated stomach is attributed to herbivorous diet, the monkeys possessing cheek pouches despite their herbivorous nature, lack sacculated stomachs. It is suggested that the pouches replace the first compartment of the sacculated stomach (HOOTON, 1965). The cheek pouches act as food reservoirs and the food is ruminated at leisure. Food stored temporarily therein is expelled into the buccal cavity by voluntary contraction of the pouch walls; but when the food has been tightly crammed and the pouch is overdistended, the animal uses its fingers to dislodge it into the buccal cavity (RAHAMAN & PARTHASARATHY, 1969). The pouch is a useful adaptation for crop raiding monkeys (NAPIER, 1970) suggesting that the possession equips them to avoid harrassers and predators.

The present study is intended to ascertain whether these pouches have a physiological role as well in digestion.

MATERIAL AND METHODS

Ten bonnet monkeys, *M. radiata,* of both sexes were housed in cages sufficiently large to allow free movement. The animals were starved overnight and offered peeled but entire potatoes of known weight. When the pouches were sufficiently gorged and prevented voluntary expulsion of food, the animals were restrained from using fingers for 5 minutes and consequently prevented swallowing of semimasticated food. The pouch content was aspirated out and analyzed for the amount of starch digestion in comparison with control. The polysaccharides in the pouch content was estimated in the following way as described by HASSID and ABRAHAM (1957). The contents were centrifuged at 3,000 rpm for 10 minutes; to 1 ml clear supernatant 1 ml 60% KOH was added. 0.5 ml saturated Na_2SO_4 was added to the mixture followed by the addition of 1.1 to 1.2 volumes of 95% ethanol. The contents were stirred and recentrifuged at 3,000 rpm for 20 minutes. The precipitate was collected and dissolved in

2 ml distilled water, and reprecipitated with 2.5 ml 95% ethanol. The alcoholic supernatant was decanted and mixed with the mother liquor after centrifugation.

The purified polysaccharide was hydrolized in 6 ml 0.6N HCl by heating for 2–2.5 hours in a boiling water bath. The solution was cooled and neutralized with 0.5N NaOH with phenol red as the indicator. The neutral solution was diluted to appropriate volume and the sugar content was estimated according to CARROL, LANGLEY, and ROE (1956).

Sterilized cotton swab was introduced into the pouch of starved monkey to collect saliva. When the swab was soaked with saliva, it was taken out and squeezed into 5 ml 0.9% NaCl solution. The amount of saliva collected was indicated by the increase in NaCl volume. The amylase activity of the extract was estimated according to BERNFELD (1955). One ml extract was incubated for 3 minutes at 37°C with 1 ml 1% soluble starch (Merck). The enzyme reaction was interrupted by the addition of dinitrosalicylic acid reagent. The tube containing the mixture was heated for 5 minutes in boiling water and then cooled to room temperature. After addition of water to make 10 ml volume, the optical density of the solution containing the reduction product was determined photometrically using a green filter. The photometer used was Model CL 20A ELICO colorimeter (ELICO Pvt. Ltd. Hyderabad, India). A control was run with boiled saliva. A calibration curve was established with glucose to convert the colorimeter readings into milligrams of reducing sugar. The protein in the extract was measured by biuret method of LAYNE (1957).

The amylase activity of the saliva at different pH levels was studied using different buffers to detect the presence of different isozymes. 0.2M glycine-NaOH, acetate, phosphate and tris-HCl buffers ranging from 2.0 to 10.5 pH were prepared according to GOMORI (1955) for the purpose.

The end products of hydrolysis of starch were analyzed by paper chromatography. The enzyme substrate mixture was incubated for 3 hours at 37°C and was concentrated by evaporation over a boiling water bath. 10 µl of the mixture was subjected to paper chromatography using No. 1 Whatman filter paper and n-butanol-acetic acid-water (4:1:1 v/v) as solvent. The solvent was run for 48 hours at room temperature by the descending technique. The chromatograms were air dried and developed according to BACON and EDELMAN (1951). The position of end product monomers was identified by comparison with that of reference sugars.

The pouch was fixed in BOUIN's fluid and alcohol, sectioned and stained with hematoxylin and eosin for histological details. The sections were photographed and other data tabulated.

RESULTS

The buccal pouch was an extension of mucous membrane of the cheek wall and was covered with a muscular coat. In fixed state it extended to about 10 mm below the mandible and was U-shaped, opening anterodorsally and was loosely held. The cheek was smooth and could be distended to a great extent. The opening of the pouch was large enough to allow whole peanuts and the like to be tucked in. The histological section showed plenty of mixed glands with serous demilunes in the submucosa

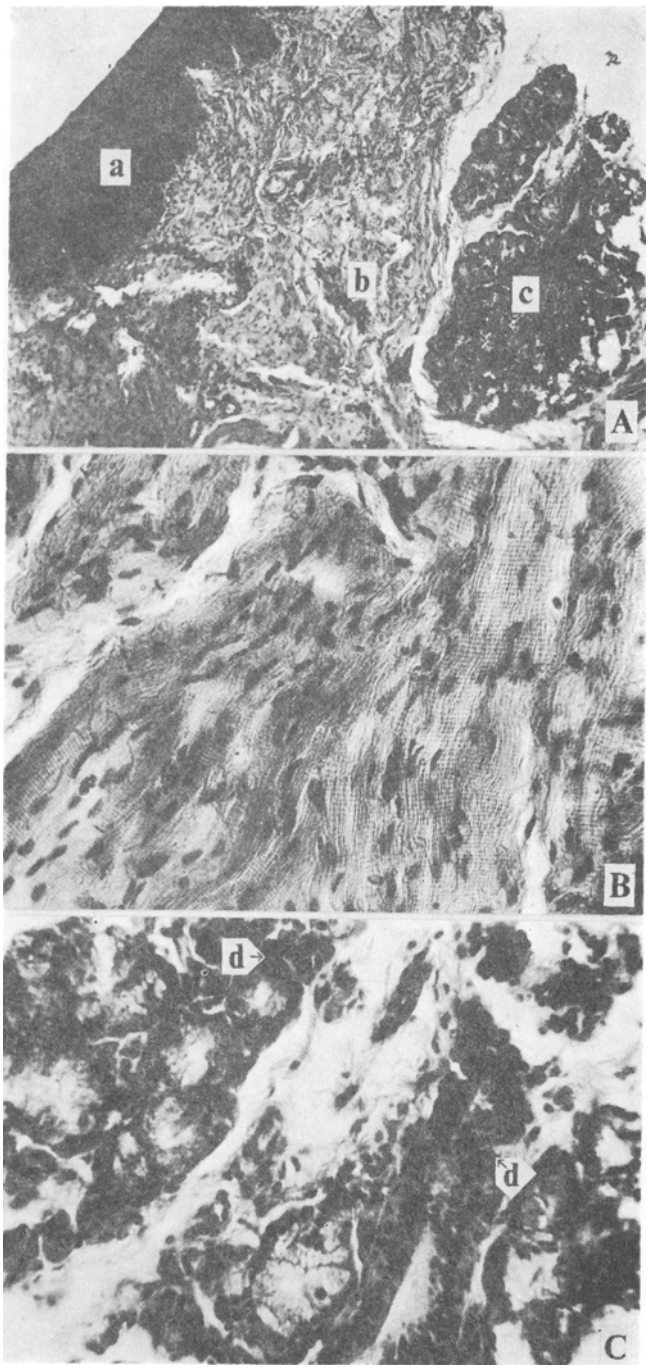

Fig. 1. Photomicrograph of cheek pouch wall of bonnet monkey. A. Cross section through pouch wall ×70, showing stratified squamous epithelium (a), striated muscle layer (b) and mixed secretory glands (c). B. b magnified ×280. C. c magnified ×280, showing serous acini (d).

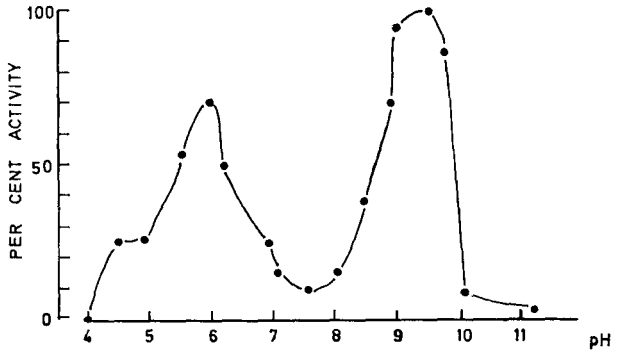

Fig. 2. Effect of pH on the salivary amylase activity of bonnet monkey.

(Fig. 1). The epithelium was stratified squamous and prevented possible damage to the pouch when it was overdistended. The muscles were striated which probably caused voluntary contraction of the pouch to expel food.

Under starved conditions the pH of saliva collected from cheek pouches ranged between 7.35 and 7.45 and 1 ml saliva contained 100–200 microgram protein (Table 1). The rate of amylase activity of saliva at neutral pH was 2.56±0.86 mg starch per 100 microgram protein per minute (Table 1).

Influence of pH on the amylase activity of saliva is illustrated (Fig. 2). The enzyme showed two peaks in pH activity; one on the acid and the other on the alkaline side. The acid pH was 5.9 while the alkaline pH was 9.5. The rate of hydrolysis was greater at alkaline than at acid pH.

On a chromatogram an *in vitro* saliva digest of starch showed glucose to be the end product of digestion.

Table 2 presents data on the rate of feeding and collection of pouch content. About 12.5±7.2 g potato was crammed in each pouch by one animal. It was estimated that the rate of potato consumption was 4.25±1.26 g/min/individual.

Starch/sugar ratio in the pouch was initially 10.3±6.5 which got reduced to 1.80± 0.93 after 5 minutes, indicating an increase in sugar level (Table 3).

DISCUSSION

Histologically, the cheek pouch of bonnet monkey did not differ much from that of rhesus monkey (GEIST, 1933), but for the rhesus macaque it has not been recorded whether the mixed type of glands and the demilunes observed in the bonnet monkey, were present. The presence of demilunes in the bonnet macaque indicates the possible secretion of zymogens and diastases as reported for serous cells of pancreas in other

Table 1. Composition and amylase activity of saliva of bonnet macaque.

Composition	Range	Mean±S.D.
pH	7.35 to 7.45	7.41± 0.04
Protein (μg/ml)	110.00 to 210.00	176.00±24.00
Amylase activity (mg glucose/100 μg/min)	2.01 to 3.08	2.56± 0.58

Table 2. Rate of potato deposition in the cheek pouch by bonnet macaque.

Particulars	Range	Mean±S.D.
g potato deposited in a cheek pouch by one monkey.	5.80–19.70	12.50±7.20
Rate of potato deposition (g potato/min/individual)	2.50– 5.50	4.25±1.26

Table 3. Changes in starch and sugar concentrations of semimasticated food in the buccal pouch.

Assay time	Ratio of starch/sugar
Initial	10.30±6.50
After 5 minutes	1.80±0 93

mammals (BLOOM & FAWCETT, 1968) and may thus cause starch digestion in the pouches.

The human saliva is slightly acidic (SCHMIDT-NIELSEN, 1946) but in the bonnet monkey it is slightly alkaline. Aklalinity is a medium favourable for starch digestion (PROSSER & BROWN, 1961). In ruminants and herbivores, the salivary pH is highly alkaline so that there may be a high and active amylase activity even in the buccal cavity (PROSSER & BROWN, 1961). Thus the monkey saliva appears to be more active in starch digestion than that of man (Table 1). The pH profile indicates (Fig. 2) two peaks in the amylase activity but the amylases have been reported to have only one pH peak activity (FISHER & STEIN, 1960; FRENCH, 1960). Vertebrate amylases are active in alkaline range (PROSSER & BROWN, 1961). The existence of two pH specific amylase activities in monkey may be due to presence of two different amylases or isozymes. It is also possible that the enzymes secreted by the demilunes and serous cells (Fig. 1) are responsible for the acid activity of saliva which is further supported by the fact that the demilunes secrete a carbohydrase too, probably a diastase (BLOOM & FAWCETT, 1968).

Amylases yielding glucose on hydrolysis are called alpha amylases and the ones yielding maltoses are beta amylases (FISHER & STEIN, 1960; FRENCH, 1960). Hydrolysis of starch by alpha amylase is a monomolecular reaction involving depolymerization of the substrate (BERNFELD, 1962) and the reaction occurs in two stages, first to maltose and maltotriose and then maltotriose to maltose and glucose (WALKER & WHELAN, 1960). The fact that bonnet saliva digests starch into glucose may show the presence of alpha amylases.

Data on starch/sugar ratios in the cheek pouch (Table 3) and on the rate of feeding and material collection (Table 2) illustrate the biological significance of cheek pouches in primates. Lowering of starch/sugar ratios shows that the digestion of starch is so rapid that about 50% of stored starch is digested within 5 minutes of its ingestion right at the site of cheek pouches.

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