THE CONTROL OF DIGESTION IN OCTOPUS II. THE ROLE OF INTERNAL STIMULI

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THE CONTROL OF DIGESTION IN *OCTOPUS*
II. THE ROLE OF INTERNAL STIMULI

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CÉPHALOPODE
DIGESTION
OCTOPUS
CONTRÔLE NERVEUX

RÉSUMÉ. — De la chair de Crabe partiellement digérée, placée directement dans le jabot d'*Octopus vulgaris*, stimule l'activité de la glande digestive, et la digestion suit son cours normal, comme c'est le cas pour un Crabe mangé par l'*Octopus*. La section de l'œsophage en avant du jabot n'affecte pas cette digestion normale. Par contre, de la cellulose placée dans le jabot ne déclenche aucune activité sécrétice dans la glande digestive. Si l'on introduit de petites pièces de chair de Crabe non prédigérées, la réponse de la glande digestive est variable; elle devient active si l'*Octopus* a une chance d'ajouter un peu de salive. Si de la cellulose ou des morceaux de Crabes sont placés dans le jabot, et si l'œsophage est ensuite sectionné entre le jabot et l'estomac, le jabot se remplit d'une grande quantité de salive. Les auteurs concluent que la glande digestive est activée par une substance de la digestion salivaire, alors que la sécrétion de la salive est provoquée par l'extension du jabot. Les possibilités d'un quelconque contrôle hormonal de l'activité de la glande digestive sont discutées.

CEPHALOPOD
DIGESTION
OCTOPUS
NERVOUS CONTROL

ABSTRACT. — Partially digested crab meals, placed directly into the crop of *Octopus* stimulate digestive gland activity and are processed by the gut in the same manner as meals ingested in the normal way. This result is not affected by severing the œsophagus upstream of the crop. Cellulose meals given in the same way do not stimulate a response by the digestive gland. The response to meals of chopped crab in seawater is variable; where the animal has a chance to add saliva, the digestive gland is activated. If cellulose or chopped crab meals are placed in the crop, and the œsophagus is cut downstream of this, copious salivary secretion fills the crop. It is concluded that the digestive gland secretes in response to some product of salivary digestion, while salivary secretion itself is evoked by expansion of the crop. The possibility of hormonal involvement in the control of digestive gland activity is discussed.

*** Correspondance and reprint requests to M.J.W. at Cambridge.**

INTRODUCTION

In an earlier account (Best & Wells, 1984) the control of digestion was considered, beginning with the effect of external stimuli (the sight of crabs, or contact with crabs) on the condition of the digestive tract. The effect of sectioning the sympathetic, abdominal and atrio-rectal nerves severally and individually was investigated. It was shown that while the abdominal and atrio-rectal nerves seem to play no part in the control of digestion the sympathetic nerves are important. If they are cut at the anterior end of the œsophagus the anticipatory response of the digestive gland no longer occurs. If the cut is made further back, severing the œsophagus below the level of the crop, and the animal is given a crab, the crop fills with saliva (and maybe a few fragments of flesh) but again there is no response by the digestive gland. The stimulus is abnormal, however, due to the very large volumes of saliva taken in with very little flesh, and it is possible that the effect of distension with a more normally constituted meal would be quite different.

One purpose of the experiments now to be reported was to examine the effect of food in the crop on further stages in the digestive process. A second was to examine the effect of food in the crop on salivary secretion. A third was to examine the nervous and/or hormonal pathways concerned.

MATERIALS AND METHODS

Octopus vulgaris Cuvier was used throughout, kept in individual tanks. The animals were fed daily on crabs. To ensure that the animals' digestive tracts were in a standard condition before any experiment, the individuals concerned were normally fed for at least 4 days at one 20-25 g crab per day, and then starved for 4 days before any tests were made; exceptions are specified in the Tables and Text. Details of operations in which the sympathetic, abdominal and/or atrio-rectal nerves were cut, have been described in Best (1981) and in Best & Wells (1984).

Various criteria can be used to assess whether digestion is proceeding normally. Those adopted here were :

1)The presence of "boules" of digestive enzymes in the main cells of the digestive gland. These are absent in starved animals, but appear in large numbers during the digestion of a normal crab meal. Boule synthesis can be stimulated simply by the sight of crabs (Best, 1981, Best & Wells, 1984). As will be shown below, it can also be caused by the presence of food in the gut.

2) The presence of brown fluid in the caecum. This originates in the digestive gland, contains digestive enzymes and is coloured because the gland also discharges "brown bodies" of waste matter in the early stages of digestion (believed to contain metabolites left over from previous meals) and later as the new meal is absorbed and processed.

3) The presence of red or brown strings of faeces mixed with mucous in the intestine.

4) The presence of considerable quantities of saliva in the anterior part of the digestive tract. In some of the experiments to be cited, the anterior œsophagus was cut or ligated, and this indication is missing.

5) Normal digestion and absorption is accompanied by a rise and fall in oxygen consumption, peaking about 2 hours after a crab is taken. (Wells, O'Dor, Mangold & Wells, 1983).

Cellulose, artificial and predigested meals

Internal stimuli presumably begin with the passage of food through the buccal apparatus. We could think of no way of imitating this without letting the animal feed itself. Food then accumulates in the crop. This is easier to imitate since food can be placed directly in the crop. The operational technique was similar to that used to cut the sympathetic nerves at the level of the anterior œsophagus. The octopus was anaesthetised in 2 % ETOH, and the sinus around the posterior salivary glands opened to reveal the gut. This was hooked up and a mush of food injected into the crop through a plastic tube inserted through a small slit in the œsophagus. The œsophagus was then ligated on either side of the slit, and the slit was sewn or the gut cut through. The salivary gland sinus was then closed.

In this manner meals of up to 10 or 15 ml could be placed in the crop. An alternative route, used when it was wished to avoid any possible damage to the anterior œsophagus, was to inject meals into the crop vita the posterior œsophagus, downstream of the crop and upstream of the stomach. The gut was again ligated on either side of the split in these experiments. The operation involves entry into a further blood sinus around the gut, and is essentially similar to that made to transect the gut at the level of the posterior œsophagus, as described in Best & Wells (1984).

"Meals" given in this manner were of three sorts

1. Cellulose meals : inert alpha cellulose, made up with filtered seawater into a mush, sufficiently fluid to inject down a 3-4 mm (internal diameter) tube.

2. "Predigested meals". A meal that got as far as the crop would, in the normal course of events, include saliva, which is used to loosen muscle attachments and lubricate the passage through the buccal mass and anterior œsophagus. *Octopus* saliva contains large quantities of proteolytic enzymes (Morishita, 1978) and digestion of these meals has already begun by the time this material enters the crop. To obtain such material, large octopuses were each fed several crabs. 40 mins later the animals were sacrificed, and the material in their crops taken. Since the donors were regularly larger than the future recipients, the fragments of food in their crop tended to be relatively large. To overcome this and produce a more readily injectable material, the predigested meal was homogenised. Predigested meals were freshly prepared and injected within 10 min of the donor's death.

3. **"Artificial meals"**, being chips of raw crab flesh, dissected out and suspended in filtered seawater as for the Cellulose meals above. The fragments were similar in size to those found in the crops of octopuses that had cleaned the flesh from the crabs themselves.

RESULTS

1. *Oxygen uptake following the placing of meals in the crop and the effect of anaesthesia*

The operation to place food in the gut takes about five minutes. Anaesthesia before this, in 2 % Ethanol, requires 6-7 minutes. Recovery is rapid when aerated seawater is passed over the gills, but the animal is left with an oxygen debt, so that oxygen consumption tends to remain elevated for the following hour or so. The processing of a crab meal is normally associated with a rise and fall in oxygen consumption, with a more or less abrupt peak at about 2 hours after injection. Maximum oxygen uptake can rise to 2-3 × resting levels.

Repayment of the oxygen debt incurred during anaesthesia overlaps and blurrs the digestive peak. To assess whether the digestive increase in oxygen uptake has occurred, it is necessary to compare the oxygen uptake of anaesthetised animals given "meals" with that of animals that were anaesthetised but not fed. Mann-Whitney "U" tests carried out on the oxygen data show that while predigested meals appear to cause increases in oxygen consumption ($p = 0.056$; the sample size was very small) no such changes occur after meals of cellulose, or crab flesh without saliva. The increase in oxygen consumption attributable to predigested meals is comparable with that of control octopuses feeding on crabs in a natural manner.

2. *The processing of cellulose meals placed in the crop*

Cellulose meals were placed in the crops of 6 animals through the anterior œsophagus which was then ligated. In 2 instances the meals were very small (2 g); in the rest they were of the "standard" 9-11 g size (typical values for naturally taken crab meals; about 50 % of a 20 g crab's flesh).

The animals were killed 6 hours later; there was never any sign of activity in the digestive gland (Table 1a). In one case there was a small amount of brown fluid in the caecum; elsewhere the guts were clear, part-filled with undigested cellulose.

It is plain that an inert meal of this sort does nothing to stimulate the digestive system downstream of the crop.

3. *The processing of predigested meals placed in the crop*

Predigested meals were placed in the crops of 6 octopuses by the anterior route, through the œsophagus in front of the crop. In 3 instances the œsophagus was ligated and cut; in the other 3 the œsophagus was then ligated, but not cut. Evidence from experiments on the anticipatory response of the digestive gland to crabs taken but not swallowed and the effect of nerves' section has indicated that ligation without section leaves the sympathetic nerves intact while preventing the entry of food and perhaps also saliva into the crop (Best & Wells, 1984).

When killed 6 hours later, all 6 of the animals had active digestive glands, with abundant "boules" in the digestive gland cells (Table 1b). The caeca of these octopuses were full of brown fluid, and in two instances the ducts were actively pumping fluid from the caeca into the digestive glands. There were strings of red or brown faeces in the intestines. In 3 out of the 5 instances in which oxygen was measured there was an apparent "digestive" peak, following an earlier anaesthetic increase, and the total oxygen uptake was higher than that of animals subjected to anaesthetic alone. API tests, carried out on the caecal fluid (Best & Wells, 1984) revealed a spectrum of enzymes matching those found in control octopuses that had eaten crabs in the normal manner.

In 3 instances there was still some undigested material in the crop, which suggests that digestion may be a little slowed by the anaesthetic. Otherwise, all the observations made agree in indicating essentially normal digestion.

In addition to the six experiments already cited two were made in which very small predigested meals (2-3 g instead of about 10 g) were placed in the crop before ligation of the anterior œsophagus. The effect of these was less marked, in that neither of the digestive glands showed the full "active" condition. Both, however, contained a few boules, distinguishing them from the digestive glands of starved controls.

4. *The processing of artificial meals*

The predigested meal experiments were repeated with a further series of octopuses, using "artificial" meals consisting of chopped crab in seawater.

There were 7 such experiments made, 4 with the œsophagus cut and 3 with the œsophagus ligated. To these can be added a further 8 similar experiments with octopuses that had the abdominal and atrio-rectal nerves cut; cutting these nerves makes no detectable difference to digestion (Best & Wells 1984).

The results are summarised in Table Ic. They are more variable than those obtained using predigested meals. Of the animals with the anterior œsophagus ligated and cut (groups 1 & 2 in Table Ic) only 3 had substantial numbers of boules in their digestive glands when killed six hours after feeding. Only one had brown "digestive gland" fluid in the caecum, and all save one had undigested fragments in the crop. Digestion apparently proceeded better in the 7 animals with the œsophagus ligated but not cut, or cut through and resewn (3 and 4 in Table Ic). 6 out of 7 of these had active digestive glands, 4 had brown fluid in their caeca and there was little or no undigested material in the crops.

As in the experiments with predigested meals, there were additional tests with very small (2-3 g) meals. The two animals concerned had the anterior œsophagus ligated. One subsequently proved to have

Table I. — The effect of meals placed in the crop, with the anterior œsophagus ligated or cut : condition of the digestive tract after six hours.

Number of animals	Crop	Caecum	Digestive gland	Oxygen uptake
a) Cellulose meals (α-cellulose mush in seawater)				
1 Oesophagus ligated				
6	Some food remains	Clear fluid	Inactive	Steady* in 3 tested
b) Predigested meals (removed from the crop of a donor)				
1 Oesophagus cut				
3	Brown fluid in 2	Brown fluid	Active	«Digestive» rise in 2 tested
2 Oesophagus ligated				
3	Some food remains	Brown fluid	Active	«Digestive» rise in 1 of 3
2 (small meals)	Empty	Clear fluid	Few boules	(not tested)
c) Artificial meals (chopped crab flesh in seawater)				
1 Oesophagus cut				
4	Some food remains	Clear fluid or empty	Some boules in 1 only	Steady*
2 Oesophagus, abdominal and atrio-rectal nerves all cut				
4	As above	Clear fluid Brown fluid in 1 «active» animal	Active in 2	As above
3 Oesophagus ligated				
3	As above	Brown fluid	Active	As above in 2 tested
2 (small meals)	Empty	Clear fluid	Active in 1	(not tested)
4 Oesophagus cut and resewn, abdominal and atrio-rectal nerves cut				
4	Empty	Clear fluid Brown in 1	Active in 3	Steady* (3 tested)

*ie. Anaesthetic effect only - see «Results» section 1

Further details of the performance of individuals animals from this series are given in Best (1981) a fully "active" digestive gland, the second had no boules in it.

It appears that digestion is more likely to proceed normally if material with saliva rather than undigested crab fragments is placed in the crop. All 6 of the animals fed large-predigested meals (Table Ib) had active digestive glands and even the two with very small meals produced a few boules. Only 10 out of the 17 animals in Table Ic (artificial meals) were secreting digestive enzymes at all. 4 of these belonged to category 4 (œsophagus cut and resewn). 4 further animals had the anterior œsophagus ligated but not cut (category 3). Experiments outlined below show that the presence of food in the gut stimulates salivary secretion. It is thus possible (and in 4 instances very likely) that some saliva was able to penetrate to the artificial meal in 8 out of the 10 instances in which digestive gland secretion occurred.

5. *Salivary secretion in response to artificial meals*

7 animals had artificial meals placed in the crop, through an opening in the posterior œsophagus, which was then ligated and cut. 3 of these octopuses also had the abdominal and atrio-rectal nerves cut.

When killed the crop was in every case distended with a massive quantity of clear fluid (30-50 ml.); the particulate artificial meal had been reduced to a fluid mush. The fluid could not have originated in the crop; 3 additional octopuses had the anterior œsophagus cut as well as the posterior, and the artificial meal remained unchanged and undiluted. API tests were not carried out on the fluid in these particular experiments. But a similar fluid produced in 5 octopuses by ligating the posterior œsophagus and then giving them crabs (Best & Wells 1984) was quite certainly saliva from the enzymes it contained and there is no reason to suppose that the fluid in the present instance was any different, quite apart from the observation that it digested the flesh in the crop.

The possibility that there is normally a considerable throughput of saliva in the gut (that is, that the massive accumulation in the crop was simply a result of blocking the posterior œsophagus) was examined by ligating and cutting the posterior œsophagus of 4 animals, without putting food in the crop. After 6 hours some fluid (5-12 ml cf. 30-50 ml noted above) was found in these controls. To compare the effect of this fluid with saliva, it was placed with an artificial meal and kept stirred, cooled in the circulating seawater so that it remained at an appropriate physiological temperature; no digestion occurred in 6 hours. Since the normal large volume of saliva would have caused considerable breakdown in this time, it was concluded that the fluid in the gut must be mainly seawater. It is possible that the animal normally passes one or two millilitres of fluid an hour through the gut : this could be useful as a way of flushing out metabolites passed down the digestive gland ducts.

6. *Cellulose meals with the posterior œsophagus cut*

Crab flesh in the crop evidently excites the secretion of saliva, which is swallowed. The effective stimulus could be chemical or physical, due simply to the stretching of the crop. This possibility was examined by injecting 6.5-11.5 ml of α-cellulose mush (see "methods" above) into the crop through the posterior œsophagus which was then ligated and cut as before. 3 animals were so treated. The crops of all three were hugely distended at death. API tests showed the enzyme spectrum to be typical of posterior salivary gland secretion.

7. *The condition of the digestive gland when meals are given and the posterior œsophagus cut*

Cellulose meals caused salivary gland secretion, but no signs of activity in the digestive gland (Table IIa). Artificial (crab flesh) meals, in contrast, caused boules synthesis in 4 out of 7 experiments (Table IIb). The difference between the two results is very striking.

The stimulus that causes boules secretion is, presumably, chemical, either a direct effect of some component of crab flesh, or a derivative of this, the result of salivary digestion. If the latter is true, the failure to excite when an artificial meal is placed in the crop and *both* outlets are cut (three such experiments were made, in addition to those listed in Table II) is not surprising; it does not prove the absence of a hormonal link, the stimulus itself could be inadequate because the octopus cannot add saliva to the crab flesh.

CONCLUSIONS AND DISCUSSION

The experiments reviewed above show that stimuli arising from the presence of food in the crop are capable of invoking activity in the digestive and posterior salivary glands. In the normal course of events these stimuli must add to the effects of external stimuli (the sight, and later the taste of crabs) in determining the response of the digestive apparatus.

The posterior salivary glands are large and can produce large volumes of a secretion that includes a number of strong proteases (Morishita, 1978). The secretion also includes Cephalotoxin, a glycoprotein neurotoxin that evidently penetrates the gills or the thin cuticle between the joints of crabs (Ghiretti,

Table II.— The effect of meals in the crop, with the posterior œsophagus cut.

Number of animals	Crop	Caecum	Digestive gland	Oxygen consumption
a) Cellulose meals				
Posterior oesophagus cut				
3	Distended with saliva	A little clear fluid	Inactive	No digestive rise (n = 2)
b) Artificial meals (crab flesh fragments in seawater)				
Posterior oesophagus cut				
4	Distended with saliva	Clear fluid	Some boules in one; others inactive	No digestive rise
Posterior oesophagus, abdominal and atrio-rectal nerves cut				
3	Distended with saliva	Clear fluid (1) or empty (2)	Boules in all 3, see text	No digestive rise
Further details of the performance of individual animals from the series are given in Best (1981)				

1959, 1960). The limp paralysed crab is held in the interbrachial web. If it is removed from the octopus at this stage and kept for an hour or so, the crab will literally fall apart, the carapace lifting away from the epiphragmal skeleton, and the limb joints pulling apart rather easily (Nixon, 1983). Evidently the salivary secretion dissolves muscle attachments and the octopus is able to rasp or suck out the contents of the crab, so that the cuticular remains are completely clean when discarded. If the posterior oesophagus is ligated and cut, and the octopus subsequently allowed to take a crab, the crop will fill with saliva, even though very little flesh is ingested (Best & Wells, 1984).

External stimuli are thus sufficient by themselves to invoke salivary secretion. So are internal stimuli. The presence of food in the crop, even of an inert 'food' like α-cellulose, will cause the animal to secrete and swallow saliva. The effective internal stimulus is thus mechanical; presumably what matters is expansion of the crop, since the cellulose mass must be almost weightless in seawater.

Internal stimulation of the digestive gland is, in contrast, chemical. Simple expansion of the crop by a cellulose meal injected via the anterior œsophagus (Table Ia) does nothing to stimulate enzyme production in the disgestive gland, in circumstances where a predigested meal is always effective (Table Ib). Artificial meals (Table Ic) were most effective in experiments where there was some possibility of contamination with saliva, so that the likely effective stimulus is some product of salivary digestion. In most of these experiments the anterior œsophagus was cut, so the effect is either mediated hormonally, or via the sympathetic nerves to the gastric ganglion. In this situation, the results of the 7 artificial meal experiments made with the posterior œsophagus cut are particularly interesting, because the operation breaks the nervous link to the gastric ganglion. In 4 out of the 7, boules were present in the digestive gland. In one of these cases only a few boules were present. But in two of the remaining 3, boules were found in substantial numberss and the third gland was fully active. Three of the 4 animals (including the fully active one) had the abdominal and atrio-rectal nerves cut as well as the sympathetics. There is thus at least a hint of chemical control here. The necessary stimulus to the digestive gland could be hormonal, or possibly one or more of the products of salivary digestion carried in the bloodstream. The failure to excite the digestive gland in experiments where the crop was isolated by cutting the œsophagus both above and below it (results, section 7 above) does not rule out chemical control, since the meals injected were saliva-free. The experiments need to be repeated with the possibly more adequate stimulus of a predigested meal.

The function of the abdominal and atrio-rectal nerves remains a mystery. They are not concerned in the pre emptive response of the digestive system to the sight or taste of food and they are not involved in transmission of stimuli from crop to digestive gland. Their removal does not even detectably alter food uptake or growth, at least in the first fortnight after nerves' section (Best & Wells, 1984).

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