Comparative Biochemistry and Physiology of Enzymatic Digestion

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Preface

It is with great regret that I report Professor Vonk's death in May 1982. We had been working together on the manuscript for some time and had agreed the final version of seven of the chapters. I have continued with the modification of his original draft and trust that the completed work would have met his criteria.

Following the response to his chapter on "Comparative Biochemistry of Digestive Mechanisms"[1] Professor Vonk was invited by Academic Press to write a book on this wide subject, but on reflection he decided to limit his account to digestive enzymes. Their distribution, properties and biological significance (Chapters 4–10) comprise the bulk of the text and some factors which influence enzyme action *in vivo* are further discussed in Chapter 11. The first three chapters consider the historical development of experimental methods in digestive physiology (Chapter 1), some properties of foods as substrates (Chapter 2) and structural aspects of metazoan digestive systems (Chapter 3). Aspects of food trituration, gut motility and the environmental, neural and hormonal factors which govern the production of enzymes and other digestive secretions are major subjects in themselves, as indeed are the absorptive processes of the gut wall. It has only been possible to consider these briefly here (Chapters 3 and 11), but all these factors have a considerable effect on the working environment of digestive enzymes *in vivo*.

It has been our intention to treat the material more from an experimental than from a theoretical standpoint. In the first chapter we have tried to convey how the early investigators designed their experiments and

[1] Vonk, H. J. (1963). *In* "Comparative Biochemistry" (M. Florkin and H. S. Mason, eds), Vol. 6, pp. 345–401. Academic Press, Orlando, New York and London.

what good results (for their time) they were able to obtain with primitive methods. In the later ones we have described experimental procedures and reassessed results when concepts and criteria of enzyme action and function have evolved considerably since the experiments were originally carried out. Only enzymes from metazoan animals are described here, and invertebrates and vertebrates are treated in separate chapters. The sequence of animal groups in the proteinase chapters is retained in those dealing with carbohydrases and lipases to facilitate comparison. The nature and range of available information on digestive enzymes varies widely from group to group of animals and from enzyme to enzyme, so that for some invertebrate groups, for example, no recent data on lipases can be given. Details of the pH of gut contents are given in the proteinase chapters. The incidence of pancreatic ribonucleases in vertebrates is discussed in Chapter 5 but the sparse and fragmentary nature of the data on invertebrate digestive ribonucleases warrants their omission at this time. Chapter 11 was designed to emphasize that digestion *in vivo* does not of necessity reflect the situation when a single purified enzyme and its substrate interact in the test-tube. It is hoped that this book will provide both an introduction to, and background information for, students and research workers interested in the digestive processes of animals and, via the bibliographies at the end of each chapter, facilitate their access to current literature in the field.

It is a pleasure to thank those who have been helpful. Authors, editors and publishers have generously permitted us to reproduce published material; acknowledgements to the individual authors concerned are given at the appropriate point in the text and a full reference to each source is given in the bibliography at the end of the relevant chapter. Many authors have facilitated our task by supplying reprints of their own work and access to their unpublished material. We are indebted to Ms. Emily Wilkinson and Mr Elwyn Davies of Academic Press for their advice, support and patience. Special thanks are due to our wives Aleida and Nesta for their forebearance and encouragement throughout the long gestation of this book, and without whom it could not have appeared.

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Contents

1 Historical and General Introduction

1. The Greek and Roman Eras

It has been widely accepted for a considerable length of time that animals must take in food and water in order to stay alive and that faeces and urine are regularly expelled from the body. The path followed by ingested food can be easily traced by simple anatomical investigation. Changes in the appearance and consistency of the food during passage through the alimentary canal are readily observed. Solid food is homogenized by chewing, and each swallowed aggregate entering the gut is termed a "bolus". These are subsequently converted to a semi-fluid viscous mass, the "chyme". These changes, readily visible in higher animals had, even in ancient times, drawn the attention of the "philosophers" who then speculated about their causes. Due to its relatively large dimensions, the stomach was considered to be the principal organ of digestion (the pancreas now holds this distinction). The efforts of the early investigators were concentrated on birds and mammals and the bulk of their speculation devoted to mammalian stomach function. Several hypotheses were advanced. Hippocrates (c. 460–377 BC) suggested that heat was the principal cause of digestion and compared it to cooking (*πεψισ*, hence the word "pepsin"), this view being endorsed some 500 years later by Galenus (c. AD 130–201). Pleistonicus (early in the third century BC) ascribed it to putrefaction.

Aristotle (384–322 BC) showed considerable interest in the comparative anatomy of the digestive system of vertebrates and some invertebrates (see Smith and Ross, 1910, 1912), but his overall concept of digestion is not clear. He considered the action of the stomach to be one of "concoction" (i.e. promoting the "ripening" of or "cooking" the food it contained). He pointed out that birds have beaks without teeth and are unable to grind their food, for which nature compensated by increasing the heat and efficiency of their stomachs. Food "difficult of concoction" required special multiple stomachs to reduce it to a smooth pulp. He described such stomachs in camels, sheep, cattle and goats. He also suggested that gut diverticula were sites where food could be stored and undergo putrefaction and concoction, naming the pyloric caeca of fishes and caeca of birds and mammals as examples. He thus not only favoured the heat and concoction theory but also considered that putrefaction might be involved in certain animals, and recognized that in some animals the teeth have an important mechanical role in the reduction of food, which they grind to a pulp.

Erasistratus (c. 330–250 BC) considered the conversion of food to chyme to be due to mechanical causes, namely the movements of the stomach and gut. He is reputed to have had the body of a living slave opened in order to observe these movements and seems to have been the first to examine digestion *in vivo*. He confirmed Aristotle's observation that movement of the epiglottis prevented the entry of food and fluid into the lungs via the trachea. (This disproved Hippocrates' theory that a small part of ingested fluid would go to the lungs.)

Asclepiades (c. 125–50 BC, a friend of Cicero) considered digestion to be a kind of solution, and was thus nearest to the modern concepts, if the term "solution" is extended to have chemical as well as physical significance.

Plato (427–347 BC), one of the greatest ancient philosophers, contributed surprisingly little to concepts of digestion. He suggested that the intestine is twisted to avoid a too rapid passage of the food, thereby preventing a too frequent urge for food which would have a harmful effect on mental performance.

2. Middle Ages and Later, Until 1750

Concepts of digestion were not significantly advanced during the 1500 years which followed Galenus's death. The digestive process was compared with fermentation by Paracelsus (1493–1541) and Van Helmont (1614–1699). Descartes (1596–1650) thought that fermentations in the stomach would release a strong acid like nitric acid which would further stimulate digestion. This is not dissimilar to the Pleistonicus concept of putrefaction, and led to the origin of the iatro-chemical school, founded by De La Boë Sylvyus (1614–1672), medical professor at Leiden. An alternative view held by the iatromathematical or iatromechanical school (Borelli, 1608–1679) supported the Erasistratus concept that movements of the gut were the principal mechanism of digestion. Boerhaave (1668–1738), a respected scientist of his time, held views intermediate between the two schools. Many authors on medical subjects in this long period commented on the phenomenon of digestion but this review will be restricted to those who made original observations or conducted experiments.

The Dutch physician De Wale (Walaeus, 1604–1649) opened dogs at different times after feeding and always found that the stomach tightly enclosed its contents. He also noted that different kinds of food were digested at different rates, and that food that had been dissolved left the stomach first.

Wepfer, municipal physician at Basle (c. 1679), conducted elaborate experiments to investigate stomach movements in dogs and cats. He killed his animals by poisoning them with extracts of *Aconitum napellus* (monkshood) and *Conium maculatum* (hemlock). He saw contractions, beginning at the middle of the stomach and running towards the pylorus, followed by gastric emptying through the pyloric sphincter, which then closed. In a wolf he noted repeated division of the stomach contents into two parts, just anterior to the pylorus. All the stomach movements ceased within seven to eight minutes of the animal's death. Peyer (quoted by Schiff, 1867) subsequently confirmed these observations. Benjamin Schwartz (also quoted by Schiff) stressed that stomach movements originate in the pyloric region, and the cardia and fundus are almost devoid of movement.

A new theory was advanced in 1766 by A. von Haller (1708–1777) who suggested that food in the stomach, especially if vegetable in nature, is macerated by the expansion of the water and air it originally contained. (This might facilitate subsequent penetration of the gastric juice although the effect for water is negligible, but for air there may be a 7% expansion for a 20°C difference between food and stomach temperatures.)

3. 1750–1850

Réaumur (1683–1757) conducted a series of elaborate experiments chiefly on birds (Réaumur, 1752). The stomach of birds has two chambers, the proventriculus which is anterior and contains numerous glands, and the posterior part, the gizzard, which is very muscular in grain eating birds, but less so in birds taking other types of food. Réaumur found the gizzard contained sand and small stones (in the turkey up to the size of a cherry) whose total weight exceeded that of the other gizzard contents. Sand and stones were retained in gizzards of birds kept in cages for a week without access to these materials. Since ingested glass tubes (outer diameter 9 mm, inner diameter 4·5 mm) and glass pearls were crushed and their fragments appeared polished, he concluded that this was caused by movements of the stomach combined with the grinding and scouring activity of the stones and grains of sand.

Subsequent experiments showed that plated iron tubes which could resist pressures of 40–140 kg without deformation were broken or bent in the gizzard of the turkey, but not in that of the goose. Gizzards were empty 24 h after feeding a turkey 24 walnuts and a male chick 18 hazelnuts, but if the animals were killed after 4 h all the nuts were found to be fragmented. Réaumur opened a number of animals but only observed gastric movements on one occasion, in a capon. Obviously these movements had stopped due to pain or killing, but he deduced the existence of strong contractions from the crushing of nuts and deformation of the tubes.

In order to find out whether the stomach juice had a dissolving function Réaumur fed birds with open-ended lead tubes filled with grain (raw or boiled) or meat. Attempts to demonstrate solution of the food in grain feeding birds were all negative, but in buzzards the meat was dissolved. After 24 h approximately 75% of the meat had been converted to a paste or had disappeared. The tubes (length 2·25 cm × 1·6 cm diameter) were not crushed although they could be easily bent by slight pressure of the fingers. In small chickens fed in this way, the meat was found to have disappeared from tubes which now contained some of their down feathers, suggesting there had been movement of the stomach and its contents. To check whether pressure in the tubes caused liquefaction of the meat, Réaumur closed the ends of the tube with wire. After this, liquefaction of the meat did not differ from that in tubes not closed in this way; in both after 24 h some 12% was still solid, the rest a thick paste. These observations were made by measuring the length of the solid column. Weighing confirmed these results. Pieces of femur from a young chicken enclosed in a tube were dissolved in 24 h in the stomach of the buzzard. When a piece of hard bone from a cow was administered, some 50% was dissolved after 24 h, and 90% after 48 h. Grain in the tube fed to the buzzard was hardly attacked.

To obtain samples of gastric juice Réaumur fed his buzzard with a piece of sponge. After the bird had vomited this the fluid was removed by pressure. Repeating this three times he obtained 150 "grains" (= 0·6 g) of juice. This had a salty rather than bitter taste and turned the colour of "papier bleu" (probably litmus) from blue to red and thus showed an acid reaction. Experiments on digestion of meat with this juice were unsuccessful. Probably the amount of fluid was too small; the meat became somewhat putrid. At this stage the buzzard died from unknown causes. Réaumur was unable to repeat his experiments due to lack of material but nevertheless had demonstrated a method which was to be successfully exploited later by Spallanzani. Réaumur had correctly identified the glands of the proventriculus as the source of this acid fluid. (These glands discharge in groups into papillae which are visible to the naked eye.) When pressed a small quantity of salty-tasting fluid was expressed. This fluid was not acid since it caused no change of colour (with litmus?) from blue to red (probably because acid secretion had not been stimulated by contact of the animal's mouth and stomach with food).

His experiments on mammals were somewhat limited. He found that a dog could partly digest bone but that thin tubes were not deformed in the stomach, indicating no appreciable gastric movement. Pieces of grass and straw in tubes fed to sheep were not digested. Réaumur concluded that in birds with a muscular stomach digestion is achieved only by trituration, and in birds with membranous stomachs (birds of prey such as the buzzard) only chemically. This applied to other animals with membranous stomachs including fish, reptiles, dog, horse, pig and man. In animals with intermediate stomachs digestion was by both mechanisms acting together.

Réaumur's experiments were repeated and largely extended by an Italian abbot and naturalist Lazzaro Spallanzani (1729–1799) whose book on digestion appeared in 1780. It was translated into French by J. Senebier (1783, reprinted 1784). An English translation appeared in 1784, a German one in 1785. His main achievement was to obtain large quantities of stomach juice (up to 400 g from a crow) and with this juice (and that of many other animals) to demonstrate digestion of meat *in vitro*.

In order to obtain the stomach juice a piece of sponge was pushed into the stomach of his animals and recovered after some hours. This was done by means of a thread fixed to the sponge, which was not needed in the case of birds of prey because they regularly vomit the feathers, hair and bones of ingested birds and mammals and so also the sponges. He proved that digestion is a purely chemical or physical process not dependent on a "vital force" and further extended these results by putting meat into the stomach of a freshly killed crow and demonstrating that this meat was digested just as well as in a living animal (the animal's body was exposed to the summer sun to maintain the body temperature). The same results were obtained with three cats, three dogs, a turtle and an owl, as well as with fishes, but these digested food more slowly. Moreover, Spallanzani observed the stimulating influence of an elevated temperature on digestion. He did not, however, find that boiling or temperatures higher than 60°C destroyed the activity of the juice on meat. In order to maintain a constant and natural temperature for his digestion experiments he often carried the sealed tubes which contained the mixtures in his armpit.

Spallanzani carried out many experiments to investigate digestion in the vertebrate series by feeding his animals perforated tubes filled with meat, bread and grain. He then allocated the animals into one of three groups according to the character of their stomach. The first group, with muscular stomachs, included the grain feeding birds, the second, with intermediate stomachs, birds like the crow and the heron and the third group, with membranous stomachs, contained fishes, frogs, salamanders, snakes, sheep, oxen, horses and man.

To investigate muscular stomachs Spallanzani studied turkeys, chicks, geese, ducks, pigeons and partridges, and confirmed the results reached by Réaumur on the contraction and forces exhibited by the gizzards of these animals. He agreed that the digestive juice does not originate from the gizzard, but he did not recognize as clearly as Réaumur that it comes from a part of the stomach situated anteriorly to the gizzard. (He considered the proventriculus to be the posterior part of the oesophagus.) Spallanzani clearly stated, from his own experimental evidence, that the stomach juice from these birds digested meat. Réaumur had previously denied this because the meat he had fed in tubes (which were not perforated) remained undissolved.

The crow and heron were selected to represent the "intermediate stomach" group. Spallanzani found that their gizzards exerted less force than those of the first group, but thin lead tubes were bent. By killing a crow 3 h after feeding he found that about 14 g of meat had been digested. However, if the meat was contained in the perforated tubes digestion was much slower. The tubes were vomited by the crow after 9 h, so that many experiments could be performed with the same animal. His herons hardly ever vomited the debris of their prey. Flesh of a frog and a fish was dissolved in 24 h with softening of the bones, which were completely dissolved on further digestion. He made the important discovery that digestion proceeds progressively from the surface of the food inwards. Indigestible fragments remained intact after the main part of the food had been completely digested.

Among the animals with membranous stomachs Spallanzani worked with owls, falcons and extensively with one eagle. He demonstrated that these animals do not digest grain. They absolutely refused to eat fruit even after a prolonged period of hunger. Meat was digested rapidly and also bones were dissolved (large pieces in 24–36 h). Birds of prey have a large crop, which does not digest, but only softens the meat. Whereas the stomach of the eagle could contain 90 ml of water, its crop could contain 1·14 litres.

Spallanzani carried out experiments with mammals including cats, dogs and man. The stomachs of some cats were removed and everted. When inflated with air (after having been wiped off) the mucosa became covered with fluid. This could be repeated several times with the same stomach. Glands, however, could not be observed. Dogs were fed with tubes (containing meat) hidden in a piece of meat, which was swallowed without chewing if the dogs were hungry. This was done to avoid forcible feeding and the danger of being bitten. The meat disappeared from the tubes, but bone and cartilage were only digested slowly and linen (cellulose) not at all. The tubes were not distorted, but hair was sometimes pushed into them, which pointed to some slight movements of the stomach contents.

Spallanzani rightly supposed that the stomach juice would be renewed continuously *in vivo* and that therefore digestion *in vivo* must proceed more rapidly than *in vitro*. He ingeniously confirmed his supposition by putting some meat in a vessel partly filled with stomach juice (obtained by the sponge method) of a crow. The juice trickled from the vessel through a narrow tube at the bottom and was slowly but continuously renewed by means of a funnel fixed above the vessel. Meat and bread were then digested much more rapidly than *in vitro* without renewing the juice (the "digestion" of bread must have been only a disintegration). In these *in vitro* digestion experiments he observed hardly any development of gas bubbles and noted the absence of a fetid odour even after a prolonged (24 h or more) stay in a sufficient quantity of stomach juice, and thus provided major evidence against the fermentation concept of digestion which was largely accepted before and during his time.

As the structure of the gastro-intestinal tract in man is homologous to that of other mammals it seemed to Spallanzani that digestion in man would resemble that in the mammals investigated so far. However, this was difficult to prove as only some of these experiments could be repeated in man and then not without some risk. He decided to carry out these experiments on himself and swallowed small linen bags (of one to three layers) and wooden tubes (11 mm × 6·75 mm) containing bread or meat or cartilage. Digestion (or at least disappearance) of the contents from the tubes could only be judged after their appearance in the faeces. In order to get an idea of the time necessary for the digestion of these foods the experiments had to be repeated many times, as the duration of their stay in the body could not be controlled.

When bags were used chewed pigeon meat (4 g) was digested in 19 h, turkey meat (34 g) in 36 h. If boiled and chewed beef was placed in the perforated tubes 1·5 g was digested in 17 h, 2·5 g in 22 h. These procedures were repeated with many kinds of meat. Bread (3·5 g) had disappeared from a bag (one layer) in 23 h, but the same weight in a three layered bag was not cleared in the same time. Chewed meat was digested more rapidly than unchewed and boiled more rapidly than unboiled, but figures were not given. Cartilage was digested in 85 h (weight not mentioned) and sinews after 97 h. Soft bones decreased in weight during passage through the digestive tract but the weight of a ball of hard bone, 6·75 mm in diameter, was unchanged after 33 h. The wooden tubes were not deformed