Introduction

This book entitled *Hunger* deals with experimental studies of food intake and of the latter's role in nutritional homeostasis. Historically, from Aristotle until the first decades of this century, these studies have been limited and obscured by the fact that hunger was viewed only as a subjectively experienced feeling. Despite trivial evidence for relationships between this feeling and food deprivation, and for the relief of hunger by food intake, speculations and investigations have long focussed on the perceptual components of the hunger sensation. It was thought that such components, together with where they originate, were causes of both the hunger sensation and an induced state of the central nervous system associated with the acceptance and intake of foods. The notions of need, drive or motivation that create this state were unable to provide a mechanistic approach to solving the problem.

Only recently, rather than merely evaluating the intensity of hunger in man, investigators have begun to measure food intake. As in other fields of the behavioural neurosciences, this transition from subjective to objective studies was primarily the result of the development of experimental procedures and of techniques of measurements which had been applied to animal models. Such animal studies address the questions of how a combination of internal and external signals governs the selection and intake of foods, and how feeding behaviour is incorporated into the overall process of nutritional homeostasis. In the last few decades, experimental data have provided answers to these questions which go beyond the speculative theories of the past. This basic knowledge obtained from animal studies has permitted research to return, with a solid background, to humans. The results of their main conclusions will be reviewed in this short book.

It is self-evident that nutrition is the main requirement of all living systems. The first step and prerequisite of nutrition, and therefore of growth, self-maintenance and reproduction, is the selection from the environment and subsequent intake of substances designated 'nutrients'. Active feeding, that is to say feeding preceded by food-seeking behaviour, is a necessity of animal life. It is the main characteristic highlighting the difference between the animal and vegetal kingdoms. In the former, high selective pressure results from the essential survival value of feeding activity. It was and still is the most powerful agent in the evolution of

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species. Through natural selection, it was the origin not only of differentiation of organs devoted to eating but also of a somesthetic nervous system.

That natural selection is dependent on behaviour is often over-looked. Before mating, in order to be able to transmit their genetic heritage, animals have to survive until maturity thanks to a series of regulatory behaviours – among these the efficient feeding behaviour of themselves and their young. The best fit sensory and motor systems, and brain processing of neural input and output, have been selected and fixed through evolution. The least defect of these systems and the resultant failure of proper feeding eliminated individuals from being able to reproduce and could have led to the disappearance of whole species.

Man has emerged from this animal history presumably because he has developed the best capacities required to solve his problems of food sources and self-defence. The most important step in human evolution has been an extension of food seeking by the invention of the production of foods: cultivation and breeding. Presumably, there was the origin of social organization. Today there are still some societies in the world subject to the cruel laws of natural selection which operated in the first ages of humanity.

Out of this specific problem of 'hunger in the world', feeding behaviour – because it is the main daily condition of the maintenance of life – is highly socialized and a source of socialization. Many things could be and have been said by sociologists and anthropologists about the social role of the meal. The sociocultural aspect of food selection and food likes and dislikes superimposed on purely physiological determinants are of the greatest importance in human feeding behaviour. This makes it difficult for us to extrapolate results from animal studies. However, the role of sociocultural factors as external reinforcers of human food habits will often be stressed in this book.

The attribute of the human dimension is to develop a science, ethics and aesthetics. Food is an object of science; but also possesses a symbolic meaning. It is the basis for many rites in all religions. The offering of food belongs to ethical customs in many cultures. In addition to food science and ethics, man has made the preparation of foods an art, and feeding behaviour an aesthetic activity. Beyond, and often in opposition to, the physiological role of food and feeding (which will be treated in this book), preparing and tasting the *Grande Cuisine* is one of the most refined human behaviours.

1   Experimental techniques and procedures

A quantitative assessment and experimental study of feeding behaviour
has been made possible thanks to the development of appropriate
techniques and procedures. The latter have generally been associated
with the simultaneous control or manipulation of variables which affect
the intake of food or are consequences of this intake.

Assessment of food intake and parallel measurements in unaltered conditions

Animal models

The animal model which is used most often is the rat. Specific studies
have been carried out with other rodents (guinea pig, hamster, gerbil,
rabbit) and mammals (dog, pig, sheep and cattle). A limited number
of investigations have been made with monkeys and particular mention
must be made of experiments using hibernators such as deer-
mouse and ground squirrel. Comparisons have been made between
various strains of rat, often in association with genetic investigations.
Comparison of lean and obese littermates in heterozygous strains of rats
and mice which are genetically obese (Zucker *fa/fa* rats and *ob/ob* mice)
has been a fruitful tool in the investigation of the relation between food
intake and body energy balance.

Food intake measurements in animal models

Whatever other variables may be controlled or manipulated at the
same time, the most important measurement is that of the intake of
food itself. Surprisingly, until recently the short- or long-term
measurement of the amount eaten was made using techniques that
were far from perfect
  Feeding in animals (as will be more extensively discussed in Chapter
2) is essentially periodic in nature. Rats, like other animal species and
like human beings, are not nibblers but are meal eaters. Their feeding
habit, when allowed free access to a permanently available food source,
is a succession of bouts of eating (or 'meals') with meal-to-meal
intervals of satiety. Another condition of feeding, and of its measurement,
is the feeding schedule: experimental animals are presented with food
once or several times daily for periods of one or more hours. Finally,

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the feeding response studied and to be measured can be limited to one meal, as is the case in many studies on determinants of meal size.

The simplest technique for measurement, still commonly used, is the manual weighing of what remains in the food-cup at the end of a given period of observation. Spillage can be avoided or controlled by the use of food-cups with special openings. This simple technique is adequate when the feeding response to be measured is limited to a particular meal, but it is not adequate in medium- or long-term feeding *ad libitum* (free feeding).

Two methods have been developed which permit the automatic and quantitative recording of moment-to-moment feeding behaviour of rats and other rodents. The more long-standing is the recording of lever-pressing for food pellets (Skinner, 1930; Anliker & Mayer, 1956). Rats are trained to press a lever to obtain a specific amount of pelleted solid food (50 mg). Though better than the simple weighing of the food remains, this has nevertheless some defects and limitations. The rat has to be trained initially. The requirement to press a lever to obtain an arbitrarily chosen amount of food is an artificial condition which modifies the spontaneous microstructure of the eating behaviour. A comparison of feeding patterns recorded in the same rats by this and another technique (described below) shows differences in sizes, durations and rate of meal consumption recorded before and after elimination of lever-pressing (Kissileff, 1970). In addition to the crumbling of the meal brought about by delivery in pellet form, the technique excludes the sensory (visual and olfactory) action of the offered food in initiation and maintenance of the eating response. Finally, the lever-pressing technique excludes the use of various diets or regimens such as greasy food, powdered sucrose, and so on, which cannot be produced as pellets.

Eliminating the lever has been an improvement. In an apparatus called the 'eatometer', a rat or mouse makes the pellet fall by putting its head in a hole and, in so doing, interrupts an infrared beam (Kissileff, 1970; Wiepkema, 1971); these interruptions are recorded.

The best and most recent means of measuring food intake is continuous automatic graphic recording of the weight of food-cups. The original automatic electro-mechanical balance of Pokrovsky & Le Magnen (1963) has now been replaced by more compact devices. The food-cup, loaded daily with 50 g of food, is continuously weighed by a strain-gauge. The signal, from 0 to 10 mV, is proportional to the weight of the food-cup, and therefore to the amount of food eaten; it is graphically recorded to an accuracy of 0.1 g and a time resolution of some seconds. A set of 10 to 12 food-cup-weighing devices, each of which has been placed in an individual cage, is connected to a poly-

channel graphic recorder. In addition, signals may be recorded and pro-
cessed by computer (Geiselman *et al.*, 1979; Strohmayer *et al.*, 1980).

Such recordings of feeding pattern which allow meal consumption
and meal-to-meal intervals of satiety to be analysed raised the question
of how to establish criteria for identifying bouts of eating as 'meals'.
Rats eat in well-separated feeding episodes, but during these episodes
they exhibit short pauses in food-taking. A criterion was required to
distinguish such intra-meal pauses, followed by the continuation of the
same meal, from longer pauses indicating the termination of a meal and
followed by the start of a new meal. A statistical analysis of the
distribution of all pauses and interval durations in 200 daily recordings
showed a regular gap between groups of short pauses of 30–40 min. So,
a period of 30–40 min of no eating was taken as the best criterion for
separating the basic prandial events (Le Magnen & Devos, 1980*a*;
Devos, 1981). Thus, a meal is defined as a period of eating preceded,
and followed by, at least 30 or 40 min of no eating.

This technique employs solid food and only a single type of food is
offered at any one time. In other procedures, rats are presented with
a choice between two or more diets, each in a separate food-cup, as in
the classic investigations of self-selection of diets by C. P. Richter. More
recently, in studies of rats offered a multiple choice of highly palatable
foods termed 'cafeteria regimen', the use of food-cup-weighing recorders
in each cage has permitted detailed study of the feeding patterns (Rogers
& Blundell, 1980; J. Le Magnen & M. Devos, unpublished).

In many experiments, liquid diets are used instead of, or in addition
to, solid food. The short-term measurement of drinking is achieved by
recording the licking pattern through devices termed 'drinkometers'
(Stellar & Hill, 1952). Such recording of licks allows the investigator
to study the microstructure of the oral intake of a rat on a liquid diet
(Allison & Castellan, 1970). It had never been possible previously to
examine this microstructure of food intake in rats by recording chewing
and swallowing pattern.

Self-intragastric and self-intravenous feeding

These procedures described above concern the oral intake of foods.
Other techniques have been developed which allow us to study the
self-intragastric or self-intravenous intake of nutriments in rats, monkeys
and even in human subjects. In these sophisticated but now routinely
used techniques, the self-injection pattern is assessed by recording the
lever-pressing which instigates the delivery of a specified amount of the
experimental solution (Epstein & Teitelbaum, 1962; Snowdon, 1969;
Rowland *et al.*, 1975; Jouhaneau & Le Magnen, 1980).

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Parallel measurements in unaltered conditions

The feeding response of animal models may be measured with simulta-
neous control of other variables. In addition to the strain, age, and sex
of rats, the body weight is a major parameter. Body weights are taken
daily, generally when the cages are cleaned (preferably at the start of
the day). Rats are always housed in individual cages. Ambient tempera-
ture is kept either in a constant neutral zone (29 °C) or generally just
below (21 °C). A factor to be controlled is the dark–light cycle which
profoundly affects the daily feeding and underlying neurometabolic
pattern. Unless otherwise prescribed by the procedure, water is available
and its intake recorded by the reading of graduated drinking tubes or,
as mentioned above, by automated drinkometer recording.

A decisive advance in studying the involvement of feeding mechanisms
in body energy homeostasis has been obtained through a technique in
which the two components of energy balance (food intake and energy
expenditure) are measured simultaneously in rats over time. This
technique, initially developed in the late 1950s, has been described in
detail elsewhere (Le Magnen & Devos, 1970; Le Magnen *et al.*, 1973).
Briefly, the rat is placed in a closed chamber in which dry air is pumped
at a flow rate of 1.7 litre/min. Oxygen and CO_2 concentrations are
measured in the outflow by gas analysers, printed out, and eventually
recorded and processed in parallel by a computer. Within the chamber,
the rat has free access to its food-cup, which is inserted in the strain-gauge
weighing device. Hence its free-feeding pattern or short-term feeding
responses are recorded in parallel with fluctuations in respiratory
parameters. Through this indirect calorimetry and taking into account
the intake of metabolizable energy, the balance between energy intake
and energy expenditure may be evaluated. In addition alterations in the
$CO_2:O_2$ ratio (respiratory quotient) with food intake yields insights into
food metabolism.

Since the historical beginning of works on hunger, gastric secretions
and contractions have been studied and assessed in relation to hunger
sensations and/or food intake. The use of an air-inflated balloon to
record gastric contractions led to a famous amusing methodological
error. It was believed that the balloon recorded contractions of an empty
and 'hungry' stomach. It was later recognized that the contractions were
in fact elicited by the balloon in an attempt by the stomach to digest
it! More recently, rates of gastric emptying and intestinal transit and
absorption have been studied as possible variables controlling meal size
(Newman & Booth, 1981; Kalogeris *et al.*, 1983; Reidelberger *et al.*,
1983).

Another advance has been provided by taking parallel recordings of food intake and of the blood concentration of various metabolites and glucoregulatory hormones in free-moving rats. This technique uses chronically implanted cardiac catheters (Steffens, 1969*a*) and home cages equipped for such rats (Nicolaïdis *et al.*, 1974). Initially, rat blood samples were withdrawn every 10 min and free intake was recorded for 1 or 2 h (Steffens, 1969*b*). A more refined technique has been developed, in which a microflow of blood (0.9 ml/h) is withdrawn continuously throughout 12 h or more (Louis-Sylvestre & Le Magnen, 1980*a*). Replacement blood from a donor is given continuously to the experimental rats through a femoral catheter. The food intake *ad libitum* is automatically recorded by the food-cup-weighing device. The blood glucose level is continuously measured in the blood outflow by a glucose autoanalyser. In other experiments, using the same technique, insulin, glucagon or catecholamine levels can be determined on successive 2 min blocks of blood samples. Intrachronic intraportal and/or sus-hepatic venous catheters have also been used (Strubbe & Steffens, 1977*a*; Langhans *et al.*, 1982).

Food intake and parallel measurements in normal human subjects

All the above techniques are applied to animal models, mainly the rat. But what about human subjects?

The on-going control of daily voluntary food intake under laboratory or hospital conditions is a difficult problem. Various types of inquiries and interviews, particularly with obese people, have been proposed and discussed (Mayer *et al.*, 1965; Huenemann, 1972; Rolland-Cachera *et al.*, 1983). Restaurants and particularly self-service cafeterias are suitable for discrete observation of the choice of foods and amounts eaten (Coll *et al.*, 1979). In a canteen, it is even possible for an observer to record the microstructure (chewing and swallowing) of a consumer with a multichannel event recorder (Warner & Balagura, 1975).

Under laboratory conditions, the recording of food-taking from the plate, and in successive courses, has been carried out, as with rats, by a continuous monitoring of the weight of the plate by a computer (Kissileff *et al.*, 1980). Parameters such as the eating rate from the beginning to the end of the meal can be measured with this 'feeding machine'. A further step in the microanalysis of man's eating behaviour has been reached by the development of a technique for recording the chewing–swallowing pattern (Pierson & Le Magnen, 1970; Bellisle & Le Magnen, 1980). Subjects are offered a meal composed of bits of bread of constant volume and size. A layer of various items (butter, pâté,

jam, etc....) makes these pieces of bread palatable in different ways. A graphic recording of masticatory and swallowing movements, by sensitive equipment, allows the experimenter to analyse the human meal by using a series of parameters of the chewing–swallowing pattern.

Fortunately (or unfortunately), man can speak. Psychophysical methods have been applied to evaluate the intensity of hunger and satiety, or of pleasantness or unpleasantness of tasted foods. Category scaling, magnitude estimations or visual analogue ratings have been used as procedures in hunger and satiety ratings before and after a meal. Similar procedures have been extensively used to rate the level of pleasantness–unpleasantness as a function of the concentration of a particular tastant or odorant in a solution.

Such psychophysical studies must be used with caution. Generally, the evaluation of 'pleasantness' is performed by tasting a sample of the item but not swallowing it. Such a judgement of pleasantness is a misleading test of *palatability*, i.e. the ingestive response to the orosensory activity of a food. A 20% sucrose solution judged pleasant by tasting is judged strongly distasteful by the subject asked to drink 50 ml of this solution (F. Bellisle, unpublished).

Self-intragastric feeding has been investigated and measured in man (Jordan *et al.*, 1966). Subjects press a button and in so doing freely command the direct delivery of a liquid food into the stomach via an oesophageal tube. The flow rate is determined by the experimenter. The amount self-injected in a given state of the subject is measured, and compared with the amount drunk by controlled delivery through the mouth. Simultaneous oral and gastric deliveries at different and varying flow rates can also be studied using this technique.

Direct or indirect calorimetry has been used for people who have normal weight or are obese, particularly in the study of body energy homeostasis (Jequier, 1982/1983). The daily ratio of energy intake to energy output, and the heat increment of food, can be measured in subjects staying 24 h or more in a calorimetric chamber. In normal healthy subjects, blood parameters can also be measured in relation to hunger and satiety and to the intake of food. Twenty years ago, differences in arteriovenous blood glucose concentrations before and after a meal were measured by blood sampling (Mayer, 1953; Stunkard & Wolff, 1954). Now, under medical supervision, volunteers have changes in their blood glucose, insulin, and glucagon levels recorded for 1–2 h during food-taking (Bellisle *et al.*, 1983).

An original technique has been developed to test preferences and aversions in human neonates (Steiner, 1973), and has also been applied

to the rat (Grill & Norgren, 1978 *a*). It consists if a video-tape recording of the facial expressions of babies stimulated by olfactory or gustatory food-related stimuli. Rejection and acceptance are clearly distinguished.

Experimental manipulations

The above techniques and procedures developed to assess the intake of food may be used *per se* in normal subjects, whether or not other measurements are taken at the same time. The investigation of the mechanisms which govern food intake requires that there is an association between the same basic measurements of intake and various experimental manipulations of the normal state. These manipulations may affect the food, the gastrointestinal tract, the liver, the systemic compartment and blood contents, the adipose tissues (white and brown), and finally and essentially the brain and the somesthetic as well as the autonomic nervous system.

Diets

The short- or long-term free intake of many types of experimental diet may be compared with the intake of the complete stock-diet. Experimental diets differ from this control diet by changes in nutritive properties or in some sensory activities (smell, taste, texture) or both. The response to high fat diets (40–50% fats) has been studied. In addition to high fat content, this diet differs from the stock-diet in two ways: (*a*) its caloric density has 66% of calories provided by fats and (*b*) it has a low carbohydrate content. The respective effects of these simultaneous changes are not often clearly dissociated. In order to eliminate the effect of the high caloric density, a low caloric high fat diet has been prepared by addition of inert material (cellulose). High, low and free protein diets, and diets lacking only one essential amino-acid, have been prepared so that specific appetite for protein may be studied. Likewise vitamin-, Ca^{2+}- and Zn^{2+}-free diets, for example, have been used to test specific appetites for vitamins or minerals exhibited by animals deficient in these respects. Without changing the proportions of the three major macronutrients, the caloric density of the stock-diet is easily manipulated by the addition of non-metabolizable materials – cellulose or kaolin. Liquid diets of varying caloric densities have also been used to study the adjustment of caloric intake in man (Campbell *et al.*, 1971; Spiegel, 1973).

Various techniques and procedures have been used to dissociate the respective roles of sensory and nutritive properties of diets in the control of intake. The simplest procedure is the comparison of responses (1) to two diets identical in their sensory activities and differing in their

nutritive properties or (2) to two diets identical in their nutritive properties and differing in their sensory activities.

The comparison of intake of saccharin solution to that of sucrose or glucose solution is an example of the first type of response which has been highly fruitful in many investigations (Le Magnen, 1977*a*). A comparison of intake of solutions of NaCl and of LiCl has been also made (Nachman, 1963). Comparison of the course of intake of a quinine-adulterated toxic diet with that of a sucrose octoacetate-adulterated non-toxic diet is based upon the same rationale (Kratz *et al.*, 1978; Aravich & Sclafani, 1980).

Odour labelling of diets was first used in the 1930s (Harris *et al.*, 1933) to demonstrate learning of specific appetites on the basis of olfactory cues. The same procedure was used later by various researchers to demonstrate the learning of palatability induced by the post-ingestive nutritional activity of a food (Le Magnen, 1956; Booth, 1972*a*).

Another means of evaluating the role of sensory analysis of foods is to eliminate the sensory organ functions surgically. A complete ageusia is difficult to introduce by sectioning of the chorda tympani, and of the glosso-pharyngeal and pharyngeal branches of the vagus without impairing simultaneously the trigeminal and other non-gustatory afferents. Anosmia in rats is achieved either by a topical application of $ZnSO_4$ on olfactory mucosa or by surgical olfactory bulb ablations. The first method may give misleading results. It has been shown that regeneration occurs rapidly: olfactory responses are recovered after only 4–5 days (Larue, 1973). This reversibility of anosmia can be exploited.

Gastrointestinal negative feedback

Many techniques, surgical and otherwise, are used to test the role of the alimentary canal in determining the short-term food intake. The dissociation of (*a*) facilitatory and inhibitory actions of the food passing into the mouth from (*b*) the counteracting action of the stomach and intestine is obtained in various ways.

In rats, a condition of sham-feeding, i.e. of oral intake of foods which go out through an open fistula and then do not enter the stomach, is achieved by oesophagotomy (Mook, 1963) or by a gastric open cannula (Liebling *et al.*, 1975; Deutsch *et al.*, 1980). In the latter method, closing the cannula allows the experimenter to re-establish the normal oro-gastrointestinal transit for comparison (Antin *et al.*, 1977; Kraly *et al.*, 1978). Sham-feeding by mouth can be associated with infusions of a liquid food to the stomach and intestine or of various solutions which may or may not differ from what is being orally sham-fed. They are