1. Introduction

It is surprising that disciplines in behavioural science such as psychophysiology and clinical psychology have not placed more value on the use of saliva in the assessment of emotional states and stress. Methodological research in these fields has focused mainly on the development of electrophysiological measurement methods, whereas body fluids as carriers of psychophysiological indicators have been marginalized. The three body fluids which are most easily obtainable for analysis are plasma, urine and saliva. To obtain a sufficient number of plasma samples demands either several venepunctures or insertion of an indwelling catheter. Through their traumatic nature, these procedures might cause by themselves a certain degree of stress and therefore confound results. Furthermore, the presence of trained medical personnel is required. In contrast, urine samples can be obtained non-invasively and are painless. Problems may arise due to incomplete voiding of urine from the bladder, leading to the mixing of urine formed during different time spans. The temporal resolution of changes in parameters gained by urine cannot be satisfactorily displayed, since the filling of the bladder requires some time. Also, a certain amount of privacy has to be assured for the subjects to collect samples. This intimacy coupled with urinary sampling often acts as a deterrent to subjects.

However, saliva samples can be collected in a completely non-traumatic manner at discrete time points from subjects in places and situations which do not require privacy. In the early seventies, Brown suggested changes of saliva parameters to be regarded as an "index of specific states of psychopathology, and as a sensitive measure in the evaluation of the effectiveness of psychotherapy and chemotherapy in psychiatric patients" (Brown, 1970, p. 66). Some effort has been undertaken to establish salivary parameters as useful measures in psychophysiology. However, apart from the analysis of hormones in saliva, such as cortisol and DHEA (Kirschbaum & Hellhammer, 1994; Vining & McGinley, 1987), not many other components have been taken into consideration as meaningful physiological markers in psychophysiological research.

A specific area of interest within psychophysiology is stress research. Stress is not only a ubiquitous phenomenon determining the course of modern life, but may occasionally become detrimental if encountered and/or experienced in too high a dose or too frequently. It is therefore the goal of stress research to better understand this burden, and to develop measures to assess it. However, stress has proven to be an elusive concept. There are many different concepts of stress that have been formulated in the last decades. Concomitantly, operationalization of stress-related changes in both mind and body is not easy to develop. Since there is a need for the examination of stress, academic interest and research attention are focused on this phenomenon. In stress research, subjects might be examined either in the field or in the laboratory. Wherever the setting is chosen, valid and reliable measures for changes that are associated with stress must be applied. But not only validity and reliability are an issue; also simple handling and easy obtainment of a stress measure is of utmost importance. Therefore, a wide array of possible parameters indicating stress-related changes in subjects have been proposed. Some of them have disappeared into oblivion, others have endured decades and are still used. Although there are a number of parameters used as indicators for changes of the sympathetic nervous system, for example, these parameters do not correlate well with each other (Grassi & Esler, 1999). Thus, there seem to be differential processes that are responsible for this observation. Since stress is a multi-faceted phenomenon, it requires a multidimensional measurement approach. As a consequence, the canon of psychobiological parameters should be enlarged.

In his editorial of the Spring issue of *Stress and Health*, Theorell describes four good reasons why the search for biological stress markers should be intensified (Theorell, 2003, p. 59):

1. Biological markers are less sensitive to exaggeration (which may result in spuriously strong indications of adverse stress) than questionnaires or interviews.

2. Conversely, biological markers provide an opportunity to circumvent the problem of denial (which may result in spuriously weak indications of adverse stress).
3. People are interested in their own stress biology.
4. For researchers, parallel studies of perceived conditions/symptoms and biological changes are of central importance to the deeper understanding of stress.

One parameter that has been suggested to reflect stress-related changes in our body is the salivary enzyme alpha-amylase. Alpha-amylase release is known to be elicited by activation of the autonomic nervous system which controls the salivary glands. Those function both as the site of production and storage of alpha-amylase. Physiological stressors as well as psychological stressors have been used to show stress-related changes in alpha-amylase. However, despite the existence of numerous studies examining this relationship, conflicting results have been reported. Some of these inconsistencies might be explained by the different stressors used, others by methodological factors such as sample size, measurement of alpha-amylase, etc.

The aim of this work is to undertake a careful assessment of whether alpha-amylase is suited as a parameter worth being incorporated in a canon of psychobiological parameters measuring stress. To that end, we conducted a series of experiments which are described in detail in this volume. In the following chapter, stress as a concept and the anatomy and physiology of stress systems are described. The third chapter concentrates on the anatomy and physiology of the salivary glands. Their innervation and release of salivary proteins are focused. The main interesting protein, alpha-amylase, is described in chapter four. Not only its characteristics but also its relation to stress are treated in this chapter. Chapter five is devoted to some methodological considerations that have to be taken into account when measuring alpha-amylase. In this chapter, some smaller studies of our own are described shortly that might illustrate some of the problems that should be considered. Chapter six to eight describe a total of three studies that have been conducted to assess alpha-amylase as a valid and reliable parameter in stress research. The first study (chapter six) applied a standardized psychosocial stressor to establish a pattern of stress-related alpha-amylase release. In the second study (chapter seven), the same paradigm was used to measure concomitantly other "classical" stress parameters, such as cortisol and catecholamines, to compare the effects stress might exert on these parameters and amylase. The third study (chapter eight) is dedicated to the methodological question of whether there is a diurnal pattern of alpha-amylase release that might confound measurement of the enzyme. Finally, in chapter 9, our results are summarized and discussed in a broader context.

2. Stress

In this chapter, the broad concept of stress is narrowed down to some of the most important descriptions of this phenomon. Furthermore, a short description is given on the physiological mechanisms that underlie the stress concept.

2.1 The stress concept

As one of the pioneers in stress research, Walter Cannon introduced the term homeostasis, which he defined as "coordinated physiological processes which maintain most of the steady states in the organism" (Cannon, 1929). Homeostasis means the sum of regulation mechanisms that maintain physiological equilibrium. According to Cannon, the essential homeostatic system in our body is the sympathetic nervous system. Its main function is to buffer against stress-induced disturbances of homeostasis and therefore to assure survival of the organism.

Some years later, Hans Selye gave a detailed description of the reaction of the body that is shown when homeostasis in the organism is threatened (Selye, 1955, 1993). He defined this reaction by three successive steps:

1. an initial *alarm reaction* which goes together with an activation of the sympathetic-adrenomedullary (SAM) system and the hypothalamic-pituitary-adrenal (HPA) axis
2. a *resistance phase* during which local adaptive reactions triggered by neuroendocrine signals encounter stress and
3. an *exhaustion phase* which is characterized by a gradual decrease of restistance to stress.

Selye coined the term "general adaptation syndrome" (GAS) to describe the succession of these steps, and the concurrent combined adaptive changes of structure and function on the level of the adrenals (hypertrophy), the gastrointestinal tract