ABSTRACT: The autonomic nervous system maintains internal homeostasis by regulating cardiovascular, thermoregulatory, gastrointestinal, genitourinary, exocrine, and pupillary function. Testing and quantifying autonomic nervous system function is an important but difficult area of clinical neurophysiology. Tests of parasympathetic cardiovagal regulation include heart rate analysis during standing (the 30:15 ratio), heart rate variation with deep breathing, and the Valsalva ratio. Tests of sympathetic adrenergic vascular regulation include blood pressure analysis while standing, the Valsalva maneuver, sustained handgrip, mental stress, and cold water immersion. Tests of sympathetic cholinergic sudomotor function include the sympathetic skin response, quantitative sudomotor axon reflex test, sweat box testing, and quantification of sweat imprints. Pupil function is tested pharmacologically and with pupillographic techniques. Tests of gastrointestinal and genitourinary function do not satisfactorily isolate autonomic regulation from their other functions. The available tests have various sensitivities and ease of administration. They are typically administered in a battery of multiple tests, which improves sensitivity and reliability, and allows probing of various autonomic functions. © 1997 American Association of Electrodiagnostic Medicine. Published by John Wiley & Sons, Inc. Muscle Nerve 20: 919–937, 1997

Key words: autonomic function; head-up tilt-table testing; 30:15 ratio; Valsalva ratio; sinus arrhythmia; sympathetic skin response; quantitative sudomotor axon reflex; thermoregulatory sweat test; sweat imprint test

AAEM MINIMONOGRAPH #48: AUTONOMIC NERVOUS SYSTEM TESTING

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The autonomic nervous system is a complex neural network maintaining internal physiologic homeostasis, especially cardiovascular, thermoregulatory, gastrointestinal (GI), genitourinary (GU), exocrine, and pupillary. Disordered function is manifested by such problems as orthostatic hypotension, heat intolerance, abnormal sweating, constipation, diarrhea, incontinence, sexual dysfunction, dry eyes, dry mouth, loss of visual accommodation, and pupillary irregularities. Autonomic failure is paramount in multiple system atrophy (which also causes cognitive, cerebellar, extrapyramidal, and pyramidal failure) and in pure or progressive autonomic failure (which does not cause other neurologic failure). Autonomic failure may accompany diseases of the central nervous system (CNS) such as Parkinson’s disease and other neurodegenerative diseases, trauma, vascular diseases, neoplastic diseases, metabolic diseases such as Wernicke’s and cobalamin deficiency, multiple sclerosis, and various medications. It occurs in many neuropathies such as diabetes, Guillain–Barré syndrome, Lyme disease, human immunodeficiency virus infection, leprosy, acute idiopathic dystonia, amyloidosis, porphyria, uremia, alcoholism, and familial neuropathies such as Riley–Day syndrome, Fabry’s disease, and familial amyloidosis. It occurs in diseases of the presynaptic neuromuscular junction such as botulism and myasthenic syndrome. The autonomic nervous system is divided into two opposing systems, the sympathetic and parasympathetic. The sympathetic nervous system exits from the thoracolumbar regions of the spinal cord and synapses at prevertebral and paravertebral ganglia. Preganglionic fibers are myelinated, relatively short, and cholinergic; postganglionic fibers are unmyelinated, long, and primarily adrenergic, except for the innervation of the sweat glands, which are cholinergic. The parasympathetic nervous system travels with the...
third, seventh, ninth, and tenth cranial nerves and the sacral spinal roots. Preganglionic axons are myelinated and have long peripheral projections to cholinergic synapses in ganglia located close to the end-organs; postganglionic axons are short and cholinergic. Afferent conduction originates in receptors in the viscera and conducts, along somatic and autonomic nerves to initiate local, segmental, or rostral reflexes. The central autonomic network is a complex network in the CNS integrating and regulating autonomic function.

Testing autonomic function is an important area in clinical neurophysiology. A variety of tests measure autonomic function (Table 1). These tests are reviewed in this report.

**TESTS OF CARDIAC AND VASCULAR AUTONOMIC REGULATION**

**Anatomy and Physiology.** Sympathetic and parasympathetic fibers innervate the atria, ventricles, coronary arteries, and resistance vessels of the peripheral circulation. Sympathetic activity increases heart rate, increases myocardial contractility, dilates coronary vessels, and constricts resistance vessels; parasympathetic activity does the converse except it has little influence on peripheral vasculature. Afferent activity originates in arterial baroreceptors located in the carotid sinus, aortic arch and various thoracic arteries, cardiac mechanoreceptors, and pulmonary stretch receptors. Regulation is by a negative-feedback system. Increasing activity of the afferent pathway results in decreasing activity of the sympathetic efferent pathway and/or increasing activity of the parasympathetic efferent pathway and vice versa. For example, increases of blood pressure and cardiac output increase the activity of the afferent pathway, which reflexly inhibits sympathetic activity or activates parasympathetic activity or both. Conversely, decreases in blood pressure and cardiac output decrease afferent activity, which reflexly allows excitatory compensatory responses.

**Recording Heart Rate and Blood Pressure.** Since heart rate reflexes occur within seconds of a perturbation, beat-to-beat heart rate analysis is necessary, and this comes from analysis of electrocardiograms (ECGs). While an ECG is usually recorded on commercial ECG machines, it is easily recorded on standard electromyographic (EMG) equipment. ECG signals have low-frequency content, so the filters of the amplifiers must be low; low-frequency (high pass) filters of 1–5 Hz and high-frequency (low pass) filters of 500 Hz are sufficient. Slow oscilloscope sweep rates or continuous printing is needed to record the 1–2 min epochs required for most tests. Many modern electromyographs do not have continuous printers, and signals must be collected before printing. Computerized programs for signal analysis of ECG are present in some systems. Standard limb or precordial placement of the recording electrodes are used. Some laboratories place the active electrode over the midline posteriorly between the points of the scapulae and the reference over the left midaxillary line. Heart rate is inversely related to R-R interval (interval between QRS complexes) and is easily calculated from it; heart rate (R-R/min) equals paper or sweep speed of the recording system (mm/s) multiplied by a correction factor 60 (s/min) divided by the R-R interval (mm/R-R interval). All tests presuppose the patient is in normal sinus rhythm. If premature beats occur, both that R-R interval and the subsequent one (which may have a compensatory pause) must be excluded from analysis.

Blood pressure is usually recorded by a standard inflatable sphygmomanometer. The level of measurement must be maintained at the level of the heart in all postures to avoid hydrostatic pressure effects of the column. Beat-to-beat blood pressure measurements formerly required invasive intraarterial recording, but modern photoplethysmographic (Finipres) devices generate waveforms similar to intraarterial recordings and allow noninvasive recording. There is some dispute over the concordance of Finipres determinations with direct intraarterial blood pressure determinations, but it is reliable if sufficient attention is paid to cuff application and temperature standardization.

**Cardiovascular Responses to Standing and 30:15 Ratio.** 

**Indications.** Studying blood pressure changes to standing is indicated in testing the integrity of the sympathetic adrenergic function. Studying heart rate changes to standing (30:15 ratio) is indicated in testing the integrity of parasympathetic cholinergic (cardiovagal) function. The tests are indicated in progressive autonomic failure syndromes, orthostatic intolerance including syncope, postural tachycardia syndrome, and other diseases where autonomic cardiovascular regulation may be altered.

**Normal Responses.** In addition to gravitational changes from upright posture, standing induces an exercise reflex and mechanical squeeze on both venous capacitance and arterial resistance vessels. The immediate effect is squeezing capacitance vessels by postural muscles, which displaces blood toward the heart, and increases, not decreases, venous return and cardiac output. Similarly, the postural muscles squeeze resistance vessels,
which increases blood pressure. These changes stimulate the baroreceptors, and there ensues a pronounced neurally mediated reflex, which decreases sympathetic outflow, releases vasoconstrictor tone, decreases total peripheral resistance by up to 40%, and drops blood pressure by up to 20 mmHg; these changes last 6–8 s. The heart rate increases immediately upon standing and continues to rise for the next several seconds, whereasupon it slows to a maximal extent by 20 s.23 The initial cardiac acceleration upon standing is an exercise reflex which withdraws parasympathetic tone, and subsequent changes are baroreflex-mediated changes which enhance sympathetic tone. Pharmacologic testing suggests most of the heart rate changes are parasympathetic withdrawal. The subsequent steady-state is controlled not only by autonomic reflexes but also by humoral mechanisms, capillary-fluid-shift mechanisms, and renal-body-fluid control mechanisms.

**Protocol.** Examination of postural blood pressure and heart rate is the most fundamental and simple test of autonomic regulation. Measurements should be obtained after the patient has been resting and inactive, preferably for 20 min, since responses are different after a few minutes than after 20 min of supine rest. The blood pressure and heart rate are recorded at baseline and then serially for 1–3 min after postural standing. The ECG allows determination of the 30:15 ratio, which is the ratio of the longest R-R (slowest heart rate) occurring about 30 beats after standing, divided by the shortest R-R (fastest heart rate), which occurs about 15 beats after standing.23,24

**Normal Values.** Orthostatic hypotension is a reduction of systolic blood pressure of at least 20 mmHg or diastolic blood pressure of at least 10 mmHg within 3 min of standing.18 But other norms allow greater changes: more than 30 mmHg systolic, 20 mmHg diastolic or 20 mmHg.60 Orthostatic tachycardia is a sustained increase of heart rate of more than 25 beats per minute (bpm) or a resting tachycardia of greater than 110 bpm with further increases of greater than 20 bpm or to greater than 140 bpm. The 30:15 ratio as originally defined is normally greater than 1.04 and abnormal if less than 1.0.23 More precise age-related norms are: 10–29 years, >1.17; 30–49 years, >1.09; 50–65 years, >1.03.100

**Clinical Comments.** The advantages of the tests are that they are simple, direct, easily accessible, quantitative, and clinically relevant. The disadvantage is that the underlying physiology is complex and interpretation must be cautious, results may lack reproducibility, and autonomic failure must be advanced before abnormality is detected (poor sensitivity). The hallmark of orthostatic hypotension from autonomic failure is the absence of compensatory tachycardia. This indicates failure of both the cardiac and vascular reflexes. A rare syndrome called orthostatic tachycardia is characterized by normal or minimally decreased blood pressure but dramatic increases in heart rate upon standing; this may represent focal or multifocal autonomic failure.38,59,80

The diagnosis of either orthostatic hypotension or tachycardia is only tenable after medical conditions are excluded. These conditions include hypovolemia such as dehydration, diuresis, hemorrhage, or vomiting; medications such as alpha and beta antagonists, venodilators, peripheral dopa agonists; metabolic dysfunction such as adrenal insufficiency, hypothyroidism, and thiamin deficiency; septic shock; and prolonged bed rest with physical deconditioning. (Baroreflex failure is an uncommon problem caused by disruption of the reflexes regulating blood pressure. It is manifested by volatile or labile hypertension, tachycardia, palpitations, headache, inappropriate diaphoresis, pallor and flushing, and resembles pheochromocytoma. The disrupted baroreflexes are caused by such problems as previous transection of the glosopharyngeal nerve, neck irradiation, and resected bilateral carotid body tumors.56,73)

**Head-Up Tilt-Table Testing.** **Indications.** Studying the responses to tilt-table testing is indicated in testing the integrity of the autonomic cardiovascular reflexes (early responses occurring in 30–60 s) and also the integrity of neurocardiogenic reflexes (late responses occurring in 30–60 min). The autonomic cardiovascular reflexes (early responses) are similar but not identical to standing; blood pressure is regulated by sympathetic adrenergic function, and heart rate is regulated by parasympathetic cholinergic (cardiovagal) function. The test is indicated in progressive autonomic failure syndromes, orthostatic intolerance including syncope, postural tachycardia syndrome, and other diseases where autonomic cardiovascular regulation may be altered. Most laboratories assess autonomic cardiovascular responses either to standing or tilting on a table but not both. The neurocardiogenic reflexes (late responses) are the focus in cardiology laboratories, and testing them is indicated in recurrent unexplained syncope in able to establish a diagnosis of neurocardiogenic syncope.1,40,74

**Normal Responses.** The responses to tilt-table testing differ from active standing because the exercise reflex and the mechanical squeeze on the capaci-
tance and resistance vessels are less. Upon changing from a recumbent to upright position on a tilt-table, there is a 25–30% shift of venous blood from the central to the peripheral compartment; 50% of the change occurs within seconds. This results in decreased cardiac filling pressures, and the stroke volume is decreased by up to 40%. This decreases afferent activity from the sensory baroreceptors, and the heart rate rises, first from withdrawing parasympathetic activity and then from increasing of sympathetic activity. The sympathetic activity increases vascular tone and total peripheral vascular resistance. Overall, the cardiac output only drops 20%, and blood pressure is largely maintained. Blood pressure and heart rate are subsequently maintained during prolonged periods of upright posture.

**Protocol.** ECG is monitored continuously and blood pressure either is measured serially with a standard sphygmomanometer at 1 min and every 3 min thereafter, or measured continuously if a continuous monitoring device is available. The test is performed after a standard period of supine rest, since the responses are different following 1 min than 20 min of preceding rest. In autonomic laboratories, after baseline ECG and blood pressures are established, the patient is positioned at an incline of 80° from the horizontal on a tilt-table with a foot board for weight bearing and monitored for a period of 3–5 min. In cardiology laboratories, the patient is positioned at an incline of 60° and monitored for about 45–60 min. Some laboratories use different degrees of tilt and different durations. Infusion of isoproterenol, a beta adrenergic agonist that increases ventricular contraction and may activate depressor reflexes, has been used in the past to increase the diagnostic yield of the test, but is no longer recommended.

**Normal Values.** Orthostatic hypotension is a reduction of systolic blood pressure of at least 20 mmHg or diastolic blood pressure of at least 10 mmHg within 3 min. In cardiology laboratories investigating unexplained syncope, a positive response is syncope or presyncope associated with a significant drop of blood pressure (usually greater than 30 mmHg systolic or 20 mmHg mean arterial pressure) or bradycardia.

**Clinical Comments.** In autonomic laboratories, the advantages of tilt-table testing are that it is simple, more easily standardized than standing since less physiologically complex, and quantitative. It is helpful in achieving upright posture in patients with motor difficulties such as Parkinson’s disease and multiple system atrophy. The disadvantages are that the underlying physiology is artificial compared to standing, and special equipment and space are needed. In cardiology laboratories, tilt-table testing evaluates neurocardiogenic syncope and its treatment. Neurocardiogenic (vasovagal, vasodepressor) syncope, often known as simple faint, is characterized by hypotension accompanied by paradoxical bradycardia. It is called neurocardiogenic to emphasize that autonomic reflexes are present (and overactive, in contradistinction to autonomic failure where they are failing and there is little or no compensatory tachycardia with hypotension) and to distinguish it from cardiogenic syncope. Neurocardiogenic syncope may occur spontaneously or reflexly from stress, fear, micturition, defecation, phlebotomy, coughing, or sneezing. The mechanism involves the Bezold–Jarisch reflex. This reflex originates in the cardiac mechanoreceptors which are sensitive to ventricular wall tension—increased wall tension such as from a vigorous contraction gives rise to increased afferent activity, which activates parasympathetic reflexes and withdraws sympathetic ones to cause bradycardia, vasodilation, and hypotension. Tilt-table testing has shown neurocardiogenic syncope is the cause of 50–75% of patients with recurrent unexplained syncope, 67% of patients with recurrent unexplained seizure-like episodes unresponsive to anticonvulsants, and 71% of patients with recurrent vertigo associated with syncope or near syncope.

**Heart Rate Variation with Respiration (Sinus Arrhythmia; R-R Interval Analysis.** **Indications.** Studying heart rate variation with respirations is indicated in testing the integrity of the parasympathetic cholinergic (cardiovagal) function. The test is indicated in progressive autonomic failure syndromes, orthostatic intolerance including syncope, postural tachycardia syndrome, and other diseases where autonomic cardiovascular regulation may be affected, including peripheral neuropathies.

**Normal Responses.** The variation of heart rate with respiration, often known as sinus arrhythmia, is generated by autonomic reflexes. In 1973, Wheeler and Watkins quantified it to measure autonomic innervation of the heart, and the test has been refined subsequently. Inspiration increases heart rate, and expiration decreases it (see Fig. 1). The variation is primarily mediated by the vagus innervation of the heart. Transection or freezing of the vagus nerve in animals and parasympathetic blockade (with agents such as atropine) abolish sinus arrhythmia, while sympathetic blockade (with beta blockers) has little effect. Pulmonary stretch receptors as well as cardiac mechanoreceptors and possi-
bly baroreceptors contribute to regulating the heart rate variation. Sinus arrhythmia is influenced by several important factors.\textsuperscript{10,11,22,53,68,101} It decreases with age; it increases with slower respiratory rates and reaches a maximal around 5 or 6 respirations per minute; it decreases with hyperventilation and hypocapnia; it decreases with increasing resting heart rate; it also decreases in cardiac failure, pulmonary disease, and CNS depression.

\textbf{Protocol.} A variety of protocols for quantifying sinus arrhythmia has been established.\textsuperscript{10,11,20,49,68} While some protocols quantify sinus arrhythmia with the patient breathing normally or after a single deep breath, most require deep breathing at 5 or 6 breaths per minute, a rate which maximizes variation and allows sensitive quantification. In quantifying heart rate variation, calculations can be performed on the R-R intervals or on the heart rate. Multiple numeric calculations have been performed\textsuperscript{20,22,35,65,87}: maximal-to-minimal differences, maximal-to-minimal ratios, maximal-to-minimal variation expressed as a percent of the mean, means with standard deviations, mean consecutive differences, mean circular resultants, and so on. The mean variation over several respiratory cycles [also called the inspiratory (I) to expiratory (E) difference] and the E to I ratio are the simplest and most commonly used calculations.\textsuperscript{49}

A simple protocol is to have the patient supine with the head elevated to 30°. The patient breathes deeply at six respirations per minute, allowing 5 s for inspiration and 5 s for expiration. Care should be taken lest the patient hyperventilate—hypocapnia reduces variation. The maximal and minimal heart rates within each respiratory cycle and the mean variation are determined. The E to I ratio or index can be calculated as the sum of the six longest R-R intervals of each of the six respirations divided by the sum of the six shortest R-R intervals. Alternatively, it may be calculated as the average of the six ratios of the longest R-R interval and shortest R-R interval within each of the six respirations. The E to I ratio can be performed as a single deep respiration (5 s inspiration and 5 s expiration).\textsuperscript{85}

\textbf{Normal Values.} Normal values by age for 6 per minute deep breathing expressed as average (or mean) maximal-to-minimal variation in bpm are: 10–40 years, >18 bpm; 41–50 years, >16 bpm; 51–60 years, >12 bpm; 61–70 years, >8 bpm.\textsuperscript{49,53} Normal values by age for single deep breaths expressed as E to I ratio (lower 95th percentile) are: 16–20 years, >1.23; 21–25 years, >1.20; 26–30 years, >1.18; 31–35 years, >1.16; 36–40 years, >1.14; 41–45 years, >1.12; 46–50 years, >1.11; 51–55 years, >1.09; 56–60 years, >1.08; 61–65 years, >1.07; 66–70 years, >1.06; 71–75 years, >1.06; 76–80 years, >1.05.\textsuperscript{85}

\textbf{Clinical Comments.} The main advantages to using heart rate variation with respiration are that it is sensitive, reproducible, quantitative, simple, fast, and readily obtained on most electrophysiologic equipment; it has become one of the most utilized autonomic function tests. The main disadvantages are that it is influenced by numerous variables, many different protocols exist, and several different methods of quantification exist. In patients with autonomic failure syndromes such as multiple system...
atrophy and progressive autonomic failure, abnormalities are detected in 80–85% of patients. In diabetics, abnormalities are detected in up to 67% of patients—the diagnostic yield increasing as patients progress from asymptomatic through symptomatic polyneuropathy to symptomatic autonomic neuropathy. Abnormalities have been reported in 39% of uremic patients, about 67% of patients with peripheral neuropathy and clinical dysautonomia, 28% of patients with distal small fiber neuropathy, in patients with amyotrophic lateral sclerosis, and in extrapyramidal and cerebellar disorders.

Valsalva Maneuver and Valsalva Ratio. Indications. Studying blood pressure changes during the Valsalva maneuver is indicated in testing the integrity of sympathetic adrenergic cardiovascular function. Testing heart rate changes during the Valsalva maneuver (Valsalva ratio) is indicated in testing the integrity of parasympathetic cholinergic (cardiovagal) function. These tests are indicated in progressive autonomic failure syndromes, orthostatic intolerance including syncope, postural tachycardia syndrome, and other diseases where autonomic cardiovascular regulation may be affected, including peripheral neuropathies.

Normal Responses. The Valsalva maneuver consists of respiratory strain which increases intrathoracic and intraabdominal pressures and alters hemodynamic and cardiac functions. The Valsalva maneuver is recorded by invasive monitoring of the intraarterial blood pressure. Levin monitored the heart rate alone without monitoring blood pressure during the Valsalva maneuver and calculated a ratio of the fastest heart rate to the slowest as a way of noninvasively quantifying the procedure. Newer photoplethysmographic monitoring devices are able to record beat-to-beat blood pressures noninvasively as well as heart rates, thus allowing easier evaluation of the maneuver.

The Valsalva maneuver has four phases (see Fig. 2A). Phase I occurs at the onset of strain—there is a transient, few-second-long increase of the blood pressure caused by increased intrathoracic pressure and mechanical squeeze of the great vessels. There are no neurally mediated changes in heart rate. Phase II occurs with continued straining. It has two subphases. In early phase II, venous return decreases, which results in decreasing stroke volume, cardiac output, and blood pressure. In about 4 s, late phase II, blood pressure recovers back toward baseline levels. This recovery stems from increased peripheral vascular resistance from sympathetically mediated vasoconstriction. This is blocked by alpha adrenergic blockade with phentolamine. Throughout phase II, heart rate increases steadily. The increased heart rate results initially from vagal withdrawal. This is evidenced by the fact it is abolished by muscarinic blockade with atropine, and subsequently by increased sympathetic activity, as evidenced by the fact it is abolished by beta blockade with propranolol. Since selective adrenergic blockade has its most specific impact on the blood pressure rise in late phase II, this is a sensitive index of sympathetic adrenergic function. Phase III is the opposite of phase I—it occurs with release of the strain, which results in a transient, few-second-long blood pressure decrease caused by mechanical displacement of blood to the pulmonary vascular bed, which had been previously under increased intrathoracic pressure. There are no neurally mediated reflex changes in heart rate. Phase IV occurs with further cessation of the strain. The blood pressure slowly increases and heart rate decreases. Since the blood pressure rises to above and the heart rate falls to below baseline levels, it is often called the “overshoot.” This generally occurs about 15–20 s after the
strain has been released and may last for 1 min or longer. The mechanism is increasing venous return, increasing stroke volume, and increasing cardiac output, which return the blood pressure and heart rate back toward baseline. The Valsalva ratio (see Fig. 3) is the ratio of the maximal heart rate in phase II to the minimal heart rate in phase IV. This may be calculated easily as the ratio of the longest R-R interval during phase IV to the shortest of phase II. The most important factors that affect the Valsalva ratio are age, position of the patient, expiratory pressure, duration of the strain, and medications.\textsuperscript{3,8,49} These must be standardized in test protocols and normative values.

Protocol. A number of protocols for determining the Valsalva ratio have been used.\textsuperscript{3,8,49} The patient is supine or with head slightly elevated to about 30°. Most labs have the patient strain against 40 mmHg applied for 15 s by blowing into a mouthpiece attached to a sphygmomanometer. The system should have a slow leak to ensure the patient strains continuously and not falsely maintain pressure by glottic closure of the airway or tongue closure of the mouthpiece. Following cessation of the Valsalva strain, the patient relaxes and breathes at a normal comfortable rate. The ECG is monitored during the strain and 30–45 s following its release. The maximal heart rate of phase II actually occurs about 1 s following cessation of the strain, and that is generally taken as the maximal heart rate. The minimal heart rate occurs about 15–20 s after releasing the strain. The ratio of the maximal-to-minimal heart rate is determined as a simple ratio. After a brief rest, the maneuver is repeated until three ratios are determined. The largest ratio of the three is the most meaningful, since this represents the best performance of the autonomic function. Beat-to-beat blood pressure monitoring with photoplethysmographic recordings adds sensitivity, since the heart rate ratios alone may be normal but not corresponding blood pressure changes, especially in early and mild sympathetic adrenergic failure.\textsuperscript{76}

Normal Values. Some laboratories regard a Valsalva ratio of less than 1.2 as abnormal, 1.2–1.45 as borderline, and greater than 1.45 as normal.\textsuperscript{3,46} Since the Valsalva ratio decreases with age, however, age specific norms are more precise: 10–40 years, >1.5; 41–50 years, >1.45; 51–60 years, >1.45; 61–70 years, >1.35.\textsuperscript{49,53} If photoplethysmographic recordings of the blood pressure are available, blood pressure drops of greater than 20 mmHg during early phase II in conjunction with either absent phase IV or absent late phase II are considered abnormal (see Fig. 2B).\textsuperscript{7,8,49}

Clinical Comments. The main advantages of the Valsalva ratio are that it is sensitive, reproducible, quantitative, simple, fast, and readily obtained on most electrophysiologic equipment. It has become one of the most utilized autonomic function tests. The main disadvantage is that without photoplethysmographic recordings of beat-to-beat blood pressure, the compensatory heart rate is being recorded instead of the true stimulus, blood pressure, and this can lead to occasional spurious results. For example, exaggerated drops of blood pressure during early phase II from sympathetic adrenergic failure produce an exaggerated tachycardia, which can normalize the ratio to the otherwise abnormal bradycardia in phase IV. Or, lack of overshoot in phase IV can lessen the bradycardia and decrease the Valsalva ratio.\textsuperscript{7} Another disadvantage is that the test may be difficult for debilitated patients. In patients with autonomic failure syndromes such as multiple system atrophy and progressive autonomic failure, abnormalities are detected in over 90% of patients, but the clinical subtypes are not distinguishable.\textsuperscript{17,72} In diabetics, abnormalities are detected in up to 67% of patients—the diagnostic yield increasing greatly as patients progress from asymptomatic through symptomatic polyneuropathy to symptomatic autonomic neuropathy.\textsuperscript{9,10,68}

Miscellaneous Tests. Blood Pressure Response to Sustained Handgrip. Sustained muscle contraction causes blood pressure and heart rate to increase. The mechanism involves the exercise reflex, which

\begin{figure}[H]
\centering
\includegraphics[width=\textwidth]{valsalva_ratio.png}
\caption{R-R intervals during the Valsalva maneuver. During phase II, the period of strain, R-R intervals decrease (heart rates increase) and during phase IV, after the strain is released, R-R intervals increase (heart rates decrease) beyond baseline to form the “overshoot.” The “Valsalva ratio” is the ratio of the maximal to the minimal R-R interval.}
\end{figure}
withdraws parasympathetic activity and increases sympathetic activity. The blood pressure changes are regulated by sympathetic adrenergic vascular function, and the heart rate changes are regulated by parasympathetic cholinergic (cardiovagal) function. The test requires the patient to apply and maintain grip at 30% maximal activity for up to 5 min if possible, although 5 min may be adequate. The diastolic blood pressure should rise more than 15 mmHg; 11–15 mmHg rise is borderline. The rise is relatively independent of age and absent in diabetic and uremic neuropathy. There is less experience with this test, and it has not been widely studied.

Blood Pressure Response to Mental Stress. Mental stresses such as arithmetic, sudden noise, and emotional pressure cause sympathetic outflow to increase with it blood pressure and heart rate. It has been used as a measure of sympathetic efferent function that has the advantage of not requiring direct afferent stimulation, but the test lacks sensitivity.

Blood Pressure Response to Cold Water Immersion (Cold Pressor Test). This is an older test of autonomic function. The patient submerges a hand in ice water, and subsequently there is a rise of blood pressure. The afferent limb of the reflex is somatic, and the efferent limb is sympathetic. The problems with the test are that it is difficult for many patients to maintain the hand in ice water for the requisite period of time, and the test lacks sensitivity, since many normal subjects do not have a significant rise of blood pressure.

Skin Vasomotor Testing. Skin blood flow can be measured by using laser Doppler velocimetry. Maneuvers which cause vasoconstriction include inspiratory gasp, standing, and Valsalva. The response largely, but not solely, depends on postganglionic sympathetic cutaneous adrenergic function. The problems with skin vasomotor testing are marked fluctuations and noisiness of the baseline and large coefficients of variation.

Plasma Catecholamine Levels and Infusion Tests. Since upright posture induces vasopressor responses which are sympathetic and adrenergic, plasma norepinephrine levels nearly double. In a preganglionic sympathetic disorder such as multisystem atrophy, resting supine norepinephrine levels are normal but fail to rise when standing because of the lack of preganglionic drive. In postganglionic sympathetic disorders such as progressive autonomic failure, resting supine norepinephrine levels are low and fail to rise when standing. Since metabolic clearance of norepinephrine varies, sensitivity of the test may improve using indices of norepinephrine biosynthesis. Various infusions such as norepinephrine, tyramine, isoproterenol, phenylephrine, edrophonium, vasopressin, and angiotensin have been studied to evaluate autonomic responses, including afferent baroreflex sensitivity.

Newer Techniques. Newer techniques attempt to assess the cardiac parameters directly controlled by autonomic regulation instead of indirect parameters such as blood pressure, which are assessed by the tests currently available. Blood pressure is the product of total peripheral resistance and cardiac output, which in turn is the product of heart rate and stroke volume. The standard method of measuring cardiac output is invasive, using thermodilution techniques. Newer methods are noninvasive and include Doppler echocardiographic techniques, pulse contour analysis, and impedance cardiography. Another line of investigation has been spectral analysis of heart periods, using sophisticated signal analysis methods such as Fourier transformations. High-frequency components may be indicative of parasympathetic activity, and low-frequency components may be mixed sympathetic and parasympathetic. Wide ranges of normal values probably limit the clinical value of spectral analysis of heart periods, and the usefulness of these techniques in a clinical setting is undemonstrated.

TESTS OF THERMOREGULATORY FUNCTION

Anatomy and Physiology. Thermoregulation is controlled by the sympathetic nervous system, with the parasympathetic system playing a minor role. Sympathetic sudomotor fibers, the only sympathetic postganglionic fibers that are cholinergic, innervate sweat glands to regulate evaporative heat loss. Sympathetic vasomotor fibers cause vasoconstriction of cutaneous vasculature comprised of abundant arteriovenous anastomoses in the dermis, which shunts blood flow away from the surface to reduce convective heat loss. The control of pilomotor function is rudimentary in humans; contraction reduces surface area, which limits convective heat loss. Afferent activity arises from thermosensitive neurons located within the hypothalamus skin, abdominal viscera, spinal cord, and brain stem. Thermoregulation is controlled in the hypothalamus, where a set-point is established by a balance between the activities of the thermosensitive neurons. When body temperature goes below set point, heat is generated by shivering, and heat loss is minimized by cutaneous vasoconstriction and piloerection. When body temperature exceeds set-point, heat is dissipated by heat loss through sweating, cutaneous vasoconstriction, and release of piloerection.
Sympathetic Skin Responses (Peripheral Autonomic Surface Potential).

Indications. The sympathetic skin response (SSR) test is indicated in assessing the integrity of peripheral sympathetic cholinergic (sudomotor) function. It is indicated in progressive autonomic failure syndromes, diseases where thermoregulation may be affected, peripheral neuropathies where autonomic or other small fiber involvement is suspected, distal small fiber neuropathies, and possibly in diseases with sympathetically maintained pain.

Normal Responses. Electrodermal activity reflects sympathetic cholinergic sudomotor function which induce changes in resistance of skin to electric conduction. The changes are regulated by activity in the sweat glands of the dermis and may be generated by presecretory rather than secretory activity. Electrodermal activity is brought out either directly or reflexly; a direct response is recorded by stimulating a peripheral nerve or sympathetic trunk, which evokes a time-locked potential. Because of the difficulty achieving the high threshold for activation of the unmyelinated C fibers and the unavoidable simultaneous activation of pain fibers, direct responses are not studied in a clinical setting. A reflex is brought out indirectly by a wide variety of stimuli which perturb the sympathetic nervous system and bring out the potential—they do not directly evoke it. Many modalities of stimulation suffice to elicit the potential reflexly: commonly, for example, electric depolarization of a sensory nerve in the digit which startles the subject. Other eliciting stimuli include startling auditory sound or deep inspiratory gasps. The morphology of the potentials are mono-, bi-, or triphasic and are variable from stimulus to stimulus (see Fig. 4). Potentials are symmetric in homologous body regions. The potentials in the hands have larger amplitudes and shorter latencies than those in the feet. The latency is about 1.5 in the hand and about 2 s in the foot following an eliciting stimulation. The major contributor to latency is the efferent conduction along the sudomotor pathways, which are small unmyelinated C fibers. Since conduction along these fibers is all or none, latencies are not generally meaningful. Amplitudes are difficult to define because of the variable morphology of the potential, but an increasing number of studies indicate usefulness of this parameter.

Protocol. The SSR can be easily recorded on most standard EMG equipment. The potential has low-frequency content and the amplifiers of the recording equipment, especially the low-frequency (high pass) filter, must be set as low as possible, such as 0.1 or 0.5 Hz. Increasing the low-frequency (high pass) filter from 0.1 Hz to 1.0 Hz may begin to filter and thereby attenuate the potential. Some equipment can record direct current, which is best except for drift of the baseline. A high-frequency (low pass) filter of 500 or 1000 Hz is sufficient. The gain should be set to record a potential which is 500 µV to 3 mV peak-to-peak amplitude, and the sweep is set to record 5 s after the stimulus. Recordings are obtained simultaneously from the hands and feet. The active electrodes are placed in the palm or sole and the reference over the dorsum of the respective body part. All skin surfaces are cleaned but not abraded. Temperatures are standardized to over 30°C, preferably over 32°C. Standard disk electrodes are employed, although silver/silver chloride electrodes are better. Electrolyte gels (not pastes) are used. As noted, many modalities of stimulation suffice, but commonly electric depolarization of a sensory nerve in the digit (either contralateral or ipsilateral to the recording site) is used. Two common pitfalls lead to an erroneous conclusion that the potentials are absent: inadequate stimulation and habituation. Both can be guarded against by having the stimuli intense (slightly noxious such as to cause startle or blinking), irregular, and infrequent. Patients can be asked to

![FIGURE 4. The sympathetic skin response from hands (channels 1 and 2) and feet (channels 3 and 4). The potentials have larger amplitudes and shorter latencies in hands than feet and are symmetrical in homologous regions.](image-url)
indicate when the stimuli are strong but tolerable. The stimuli are delivered randomly, looking for a blink or slight withdrawal to indicate a startle showing the stimulus is sufficiently strong. Several diligent attempts should be made to record the potential before deciding it is absent. Other eliciting stimuli include startling sound or deep inspiratory gasps. The best potentials are chosen for measurement, since they represent the "best" sudomotor response. Averaging should not be performed, since the latency and morphology vary from one recording to the next and this may cause phase cancellation.

**Normal Values.** The SSR is age dependent. It is normally present in both hands and both feet in subjects under the age of 60 years, but only 50% of feet and 73% of hands in subjects older than 60 years. As discussed above, the SSR is controlled by conduction along unmyelinated fibers, and latency measurement is not useful. Initially regarded as an all-or-none phenomenon, now as techniques are refined, amplitude criteria are defined. According to Hoeldtke, latency in hands is 1.6 ± 0.1 s, amplitude in hands is 1.3 ± 0.2 mV, latency in feet is 2.1 ± 0.1 s, and amplitude in feet is 0.8 ± 0.1 mV. According to Knezevic, latency in hands is 1.5 ± 0.1 s, amplitude in hands is 0.5 ± 0.1 mV, latency in feet is 2.1 ± 0.2 s, and amplitude in feet 0.1 ± 0.04 mV. According to Drory, mean latency in hands is 1.5 s, mean amplitude in hands is 0.450 mV, mean latency in feet is 1.9 s, and mean amplitude in feet is 0.15 mV.

**Clinical Comments.** The main advantages of the SSR are that it is sensitive, reproducible, semiquantitative, simple, fast, and readily obtained on most electrophysiologic equipment. The main disadvantages are that it is only semiquantitative, may be difficult to elicit or be habituated and thereby be mistaken as abnormal, and it involves neural pathways beyond the postganglionic sudomotor ones. In patients with autonomic failure syndromes such as multiple system atrophy and progressive autonomic failure, abnormalities are detected in 88% of patients, but there is no usefulness in distinguishing between the clinical subtypes. In diabetics, abnormalities are detected in up to 66–83% of patients—the diagnostic yield increasing as patients progress from asymptomatic through symptomatic polyneuropathy to symptomatic autonomic neuropathy. Abnormalities have been reported in 67% of uremic patients, 50% of patients with peripheral neuropathy, 85% of patients with neuropathy and clinical dysautonomia, 58% of alcoholic neuropathies, and 10% of patients with distal small fiber neuropathy. The SSR is comparable in its sensitivity to the quantitative sudomotor axon reflex test (QSART) (discussed below) for the detection of autonomic dysfunction, and there is concordance. In tests comparing the SSR with tests of cardiovascular reflexes, they were nearly comparable, with the cardiovascular reflexes being slightly more sensitive than SSR to dysfunction. Not all investigators, however, advocate the value of this test in assessing sudomotor function.

**Quantitative Sudomotor Axon Reflex Test.** Indications. The QSART is indicated in assessing the integrity of peripheral sympathetic cholinergic (sudomotor) function. It is indicated in progressive autonomic failure syndromes, other diseases where thermoregulation may be affected, peripheral neuropathies where autonomic or other small fiber involvement is suspected, distal small fiber neuropathies, and in sympathetically maintained pain states.

**Normal Responses.** Sweat gland function can be assessed by direct activation from intradermal injection of methacholine, which activates muscarinic receptors; sweat output is measured by weighing filter paper. Alternatively, sweat gland function can be assessed by indirect activation from axon reflexes. Axon reflexes are generated when acetylcholine activates nicotinic receptors in the sudomotor fiber terminal, sending impulses antidromically to branch points and then orthodromically back to remote neurosecretory synapses. In this way, the sudomotor axon is stimulated chemically, not electrically, by acetylcholine iontophoresis. The QSART quantifies postganglionic sympathetic sudomotor function by measuring dynamic sweat output in response to activation from axon reflexes. Several regions of the body may be sampled to obtain topography.

**Protocol.** QSART requires a multicompartamental sweat capsule and a sudorometer (see Fig. 5). The different compartments of the sweat capsule allow for both stimulation and pickup of the sweating response. Stimulation requires a constant current generator which applies a current over one of the compartments to iontohoresed acetylcholine onto the skin. The acetylcholine is iontophoresed at 2 mA for 5 min. Since this also stimulates sweat glands directly, the site of evaluation of the subsequent sweating responses is remote from the site of stimulation in a different compartment. Measurement requires a sudorometer, which measures humidity. Low-humidity nitrogen gas is piped through the sudorometer to measure baseline or input humidity. The gas passes into the sweat compartment of the sweat capsule through an intake port and then exits the compartment through a different port. The gas passing over
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<td>2</td>
<td>Needs special equipment; stimulates sweat glands directly</td>
</tr>
<tr>
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<td>2, 3</td>
<td>2</td>
<td>Needs special equipment; not well validated</td>
</tr>
</tbody>
</table>

BP, blood pressure; CN, cranial nerves; QSART, quantitative sudomotor axon reflex test, HR, heart rate.
*Modified from Ref. 49.
†Arbitrary scale of 1–4: 1 is lowest or worst and 4 is highest or best.
the skin is humidified by the sweat. It passes back to the sudorometer where the humidity is remeasured. The differences between the output and input humidities are recorded and quantified as a function of time. Recording continues for 5 min after cessation of the iontophoresis. The sweating promptly occurs about 1 to 2 min after the stimulation and generally returns to baseline about 5 min after the stimulation has ceased. The multicompartmental sweat cells are placed over four locations: the foot laterally in the distribution of the sural nerve, the distal leg proximal to the medial malleolus in the distribution of the saphenous nerve, the proximal leg just distal to the fibular head in the distribution of the peroneal nerve, and in the forearm medially in the distribution of the medial antebrachial nerve. The patient needs to be supine, comfortable, and warm.

**Normal Values.** Males have similar latencies to females but larger sweat outputs. Age in general reduces the sweat output but it is variable by sex and site. In general, the onset of the sweating response is between 1 and 2 min, depending on site. For males, the median sweat output is about 2–3 µL/cm² (approximate range 0.7–5.4 µL/cm²) depending on site of stimulation. For females, the median sweat output is 0.25–1.2 µL/cm² (approximate range 0.2–3.0 µL/cm²) depending on site. The morphology of the sweat output is also important: in addition to being reduced or absent, sweat output may be abnormally persistent or excessive, often with associated short latencies; these abnormalities may be seen in painful neuropathies such as diabetes, mild or early neuropathies, and florid reflex sympathetic dystrophy, and reflect the altered axonal excitability of these conditions.

**Clinical Comments.** The main advantages of the QSART are that it is sensitive, reproducible, accurate, quantitative, involves directly the postganglionic neurosecretory unit, allows delineation of proximal-to-distal topography, and provides a dynamic record of sudomotor function over time. The main disadvantages are that it is time-consuming, requires special equipment, and is not widely available. In patients with autonomic failure syndromes such as multiple system atrophy and progressive autonomic failure, abnormalities are detected in 67% of patients, but there is no utility in distinguishing between the clinical subtypes. In diabetics, abnormalities are detected in up to 58% of patients—the diagnostic is comparable to tests of vagal function, and both tests together are more sensitive to the detection of autonomic neuropathy than either one alone.

Abnormalities have been reported in 83% of patients with peripheral neuropathy, with the legs and distal limbs showing more abnormality than the arms and proximal limbs, 80% of patients with distal small fiber neuropathy, and in myasthenic syndrome. The test has been useful in defining a high incidence of autonomic abnormalities in extrapyramidal and cerebellar disorders. The concordance of QSART testing and SSR testing has been discussed above. The QSART and a related test measuring resting sweat output together may be useful in diagnosing reflex sympathetic dystrophy and predicting which patients will respond to sympathetic block, although the interpretation has been questioned.

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**FIGURE 5.** The sudorometer and attachments used in quantitative sudomotor axon reflex testing (QSART). Reproduced with permission from Ref. 49 (Low PA: Laboratory evaluation of autonomic failure, in Low PA (ed): Clinical Autonomic Disorders: Evaluation and Management, Boston, Little, Brown & Co, 1993, pp 169–197).
Thermoregulatory Sweat Test. Indications. Thermoregulatory sweat test (TST) is indicated in assessing the integrity of sympathetic cholinergic (sudomotor) function. It is indicated in progressive autonomic failure syndromes, other diseases where thermoregulation may be affected, peripheral neuropathies where autonomic or other small fiber involvement is suspected, distal small fiber neuropathies, and possibly in sympathetically maintained pain states.

Normal Responses. The TST evaluates the overall autonomic regulation of body temperature, specifically cooling, by providing a physiologic stimulus, heat, and recording the body’s ability to dissipate it by sweating. Thus, it assesses central preganglionic as well as peripheral postganglionic function. It requires a heat environment where the patient is warmed and an indicator to observe and record the sweating response. The overall topography of sudomotor function is recorded.

Protocol. The patient is placed in a controlled heat environment of about 45–50°C and relative humidity of 45–50%. In general, the core body temperature is warmed to 38°C, or 1.4°C above baseline. Since core body and surface temperatures are not always concordant, infrared heaters warm skin surface temperature to 39–40°C. The patient has an indicator powder such as alizarin red mixed with corn starch and sodium carbonate applied to the body. Other indicators that can be used include iodinated cornstarch or iodine solution, which is painted on the body. An older indicator called quinizarin is no longer available in the United States because of skin irritations. These indicators change color when wet to indicate areas of sweating. Alizarin red, for example, is light orange when dry and changes to purple when wet. As the body is warmed, the resulting sweating is recording. After 35–45 min the sweating response is maximal. The patient’s sweating pattern is recorded and the pattern and percent of surface body area sweating are noted.

Normal Results. Normally, sweating is generalized, symmetrical, and with variable involvement of the proximal limbs and legs. Abnormal patterns include distal anhidrosis, segmental or regional anhidrosis, focal anhidrosis (such as in a peripheral nerve or dermatomal pattern), or global anhidrosis.

Clinical Comments. The main advantages of the TST are that it is sensitive and physiological, assesses overall thermoregulatory function centrally and peripherally, and directly maps the topography of sudomotor function. The main disadvantages are that it is time-consuming, requires special equipment, is messy and only semiquantitative, may be hard on the patient, and can cause skin irritation. When used in conjunction with the QSART, it may be possible to separate preganglionic from postganglionic lesions. If both the QSART and TST are abnormal, the lesion is more likely than not postganglionic. If the TST is abnormal but the QSART is not, the lesion is likely to be preganglionic. In patients with autonomic failure syndromes such as multisystem atrophy and progressive autonomic failure, abnormalities are detected in most patients, but there is no utility in distinguishing clinical subtypes. Abnormalities have been reported in 72% of patients with distal small fiber neuropathy. The test has been useful in defining a high incidence of autonomic abnormalities in extrapyramidal and cerebellar disorders.

Sweat Imprint Tests. Indications. Sweat imprint tests are indicated in assessing the integrity of sympathetic cholinergic (sudomotor) function. They are indicated in progressive autonomic failure syndromes, other diseases where thermoregulation may be affected, and peripheral neuropathies where autonomic or other small fiber involvement are suspected.

Normal Responses. Sweat imprint methods measure the sweat output at a fixed time after a stimulus by visualizing and quantifying sweat droplets secreted by sweat glands. The sweat glands may be directly stimulated by a cholinergic agonist or else stimulated by way of the axon reflexes similar to the QSART. Since sudomotor innervation is necessary for sweat gland function (they do not follow Cannon’s law of denervation hypersensitivity), absent or subnormal responses imply denervation whether or not the stimulation is achieved by axon reflexes or directly.

Protocol. A variety of methods of sweat imprint tests are summarized in the review by Kennedy. The stimulation to sweat gland secretion may be physiologic, such as by heat or exercise, or direct, such as by cholinergic agents. The latter are the most controllable and can be applied by either intradermal injection or iontophoresis. The cholinergic agents include acetylcholine, pilocarpine, or methacholine. Kennedy’s protocol involves iontophoresis of pilocarpine onto 1 cm² of skin by applying 2 mA direct current for 5 min. Once stimulated, sweat output is visualized by a variety of techniques. Indicator dyes can be applied to the skin such as the starch powders or iodine solutions used in the thermoregulatory sweat tests. Hard copies are made by bond paper, paper towel absorption, or photographic techniques. An alternative to indicator dyes is plastic and silicone imprints. The plastic or silicone is ap-
plied as a thin film and removed when hardened; sweat droplets are recorded as holes or imprints in the material and then can be quantified by various counting methods. Each droplet corresponds to the duct of one sweat gland. Kennedy’s protocol calls for Silastic material to be spread over the region 5 and 20 min after pilocarpine iontophoresis and allowed to harden, usually within 3 min. The volume of sweat is estimated by measuring the number of droplets and their area. The Silastic mold is transilluminated under a dissecting microscope and the sweat droplets are measured. The mold may be photographed and the droplets digitized on a digitizer pad. Recently, the analysis has been automated using a system with a video camera, which projects the mold onto a screen where a software program performs the operations.42 Typically, two sites are evaluated, the dorsum of the hand and foot. In a recent study by Stewart comparing direct sweat gland stimulation with axon reflex stimulation,89 direct stimulation was slightly more sensitive to the detection of abnormality (55% abnormal with a 95% specificity) than axon reflex stimulation (50% abnormal).

Normal Values. In the hand, there are 311 ± 38 sweat glands per cm², the lower limit of normal being 255; the sweat droplet size is 25 ± 6 units (arbitrary), the lower limit of normal being 19. In the foot, there are 281 ± 38 sweat glands per cm², the lower limit of normal being 235; the sweat droplet size is 28 ± 7 units (arbitrary), the lower limit of normal being 19.41

Clinical Comments. The main advantages of the sweat imprinting techniques are that they are sensitive, reproducible, accurate, and quantitative. The main disadvantages are that they are time-consuming and require special equipment. Unlike the QSART, which evaluates the dynamic sweat output over time, the various imprint tests evaluate the sweat output at a fixed time after stimulation. Unlike the QSART, which requires direct stimulation of the sudomotor axon in the evaluation, the various imprint methods require direct sweat gland stimulation and presume abnormal function reflects denervation. In diabetics, 24–36% had abnormally low sweat function in the hand and 56–60% had abnormalities in the foot. The abnormalities correlated with other tests of small fiber function, such as thermal sensation, but not with tests of large fiber function, such as vibratory sensation and nerve conduction studies.41,89 These findings are similar to those obtained with SSR and QSART.

**MISCELLANEOUS TESTS OF AUTONOMIC REGULATION**

**Tests of Exocrine and Pupillary Regulation.** *Anatomy and Physiology.* Sympathetic and parasympathetic fibers innervate the pupils, the ciliary muscles, Mueller’s muscle in the eyelid, and the exocrine glands controlling lacrimation and salivation. Sympathetic activity causes pupil dilation and contraction of Mueller’s muscle in the upper lid. Parasympathetic activity causes pupil constriction, accommodation, lacrimation, and salivation. The afferent pathway for the pupil is the optic nerve, and central integration is in the dorsal midbrain and Edinger–Westphal nucleus. The afferent pathway for exocrine function is primarily along the trigeminal nerve and central integration is in the brain stem and central autonomic network.

Indications. Studying pupillary function is indicated in evaluating parasympathetic cholinergic and sympathetic adrenergic function. It may be useful in establishing and localizing the site of abnormality of Horner’s syndrome, Adie’s syndrome, anisocoria, and syndromes of light-near dissociation. Lacrimation and salivation testing may be useful in objectifying abnormality in patients with complaints of dry eyes and dry mouth. These problems may occur either in isolation or as part of more generalized autonomic failure.

Normal Responses and Protocols. Evaluating pupil function is often done by pharmacological testing. Pupil constriction and accommodation are tested with parasympathomimetic agents. Pilocarpine and methacholine are parasympathomimetic agents which act directly on cholinergic parasympathetic constrictor muscles to cause pupillary constriction. In dilute amounts (pilocarpine 0.125% or methacholine 2.5% solution), they cause minimal constriction, but when there is parasympathetic denervation, there is denervation hypersensitivity, and the pupil constricts. A diluted pupil that does not constrict to pilocarpine 1.0% is pharmacologically dilated. Pupil dilation is tested with either parasympatholytic agents (which also block accommodation) or sympathomimetics. For example, epinephrine acts directly on sympathetic adrenergic dilatory muscles to cause pupillary constriction. In dilute amounts (pilocarpine 0.125% or methacholine 2.5% solution), they cause minimal constriction, but when there is sympathetic denervation, there is denervation hypersensitivity, and the pupil dilates. Cocaine (4–5% solution) blocks reuptake of norepinephrine in sympathetic nerve terminals innervating pupillary dilator muscles, and it causes pupillary dilation. In sympathetic denervation, norepinephrine is not pre-
sent, and dilation does not occur when cocaine is applied. Hydroxymethamphetamine is a sympathomimetic agent that releases norepinephrine; if the pupil fails to dilate when it is applied, the site of sympathetic denervation is postganglionic. Nonpharmacological tests of pupil function are not widely available, because special equipment is required and standardization is difficult. The edge-light pupil cycle time is a method of assessing parasympathetic innervation of the pupil. Light focused on the edge of the pupil stimulates the retina, which results in pupillary constriction. When the pupil constricts, the light is blocked and the stimulation to constrict is less strong and the pupil dilates. This oscillation or cycle mostly depends on the parasympathetic efferent limb of the pupillary light reflex. The cycle time is a method of assessing parasympathetic innervation of the pupil. Light focused on the edge of the pupil stimulates the retina, which results in pupillary constriction. When the pupil constricts, the light is blocked and the stimulation to constrict is less strong and the pupil dilates. This oscillation or cycle mostly depends on the parasympathetic efferent limb of the pupillary light reflex. The cycle time can be quantitated and is a sensitive measure of the parasympathetic function.

Lacrimation can be measured with the Schirmer’s test and tear osmolarity. In the Schirmer’s test, the wick end of a filter paper test strip is placed between the lower lid and the sclera. The length of wetting at 5 min is then measured. The sensitivity in detecting dry eyes is about 90%, and the specificity is about 85%. In measuring tear osmolarity, the sensitivity in detecting dry eyes is 76%, and the specificity is 84%.

Salivation may be tested by having patients chew a series of five gauze pads for 1 min each for a total of 5 min after the sublingual gutter is wiped dry. Pretest weight is subtracted from posttest weight to calculate saliva production, normally greater than 7.5 mL/5 min.

Clinical Comments. These tests are important in evaluating specific problems such as anisocoria or dry eyes, but they have more limited usefulness in generalized autonomic failure syndromes.

Tests of Gastrointestinal Autonomic Regulation. Anatomy and Physiology. The autonomic regulation of GI function is exerted on the local integrative system called the enteric nervous system, which consists of networks of nerves and plexuses embedded in the wall of the GI tract and integrated into local circuits for different operations such as contractile activity (peristalsis), absorption, secretion, and sphincter coordination. The sympathetic innervation causes contraction of the esophageal sphincters, slowing motility and contraction of the internal rectal sphincter. The parasympathetic innervation stimulates motility and relaxes the internal rectal sphincter. The external sphincters are innervated by the pudendal nerve and are under somatic, not autonomic, control. The afferent pathways synapse locally or in the ganglia, spinal cord, and more rostral portions of the autonomic nervous system. Central integration occurs in spinal centers and the central autonomic network, especially the nucleus of the tractus solitarius and the nucleus ambiguus.

Indications. Testing GI function may be indicated in patients with dysphagia, gastroparesis, chronic intestinal pseudo-obstruction, constipation, and fecal incontinence, especially if there is evidence of other autonomic dysfunction.

Normal Responses and Protocols. Videofluoroscopy and pharyngoesophageal motility studies help identify causes of dysphagia. GI motility, including gastric emptying times and colonic transit times, allows for identification of neurogenic disorders, but does not distinguish extrinsic autonomic disorders from intrinsic enteric nervous system disorders. Manometer or solid-state pressure transducers placed in different portions of the GI tract help localize sites of stasis. Sympathetic denervation may be identified by various neurochemical studies including the norepinephrine and epinephrine responses to edrophonium, which rise promptly after intravenous administration when there is normal postganglionic innervation. Parasympathetic denervation may be identified by the plasma pancreatic polypeptide response to sham feeding or hypoglycemia. Anorectal manometry helps identify causes of incontinence. Motor latencies of the pudendal nerve and electromyography of the anal sphincter are also helpful, but these assess somatic, not autonomic function.

Clinical Comments. In practice, it is difficult to distinguish autonomic regulation of GI function from nonautonomic regulation. In order to make this distinction, other parts of the autonomic system need to be tested as described above. Tests such as the heart rate variation with respiration and theValsalva ratio are easy, reliable, and readily accessible tests of vagus parasympathetic cardiac regulation and are sensitive to vagus parasympathetic GI regulation as well. In multiple system atrophy, the rectal sphincter is frequently denervated from degeneration of Onuf’s nucleus in the sacral spinal cord, and EMG of the rectal sphincter may be abnormal. However, this represents somatic, not autonomic, dysfunction.

Tests of Genitourinary Autonomic Regulation. Anatomy and Physiology. Sympathetic innervation of the GU system causes uterine contraction, ejaculation in males, bladder wall inhibition, detrusor and trigone muscle contraction, and urethral smooth
muscle contraction. Parasympathetic innervation causes genital vasodilation, erection in males, bladder wall contraction, detrusor and trigone muscle relaxation, and internal sphincter relaxation. The external sphincters are innervated by the pudendal nerve and are under somatic, not autonomic, control. The GU system’s afferent activity is transmitted by autonomic and somatic pathways. Central integration occurs in spinal centers and the central autonomic network.

**Indications.** Testing GU function may be indicated in patients with urinary dysfunction, such as incontinence and sexual dysfunction, especially if there is evidence of other autonomic dysfunction.

**Normal Responses and Protocols.** Urodynamic studies in incontinent patients assess overall bladder function—autonomic regulation is a component. Tests of male erectile function, such as nocturnal tumescence studies and penile rigidity studies, assess erectile function, which is largely controlled by vascular and autonomic function. If abnormal, injection of vasoactive agents such as papaverine into the corpus cavernosum helps differentiate vascular from nonvascular dysfunction—a poor response to injection means the cause is vascular. A method of electrophysiologic study of the smooth muscle of the corpus cavernosum by insertion of a concentric needle electrode through the skin and tunica albuginea has been introduced. Spike potentials are present when the penis is flaccid and attenuate when it becomes erect, perhaps due to cessation of smooth muscle activity, which maintains flaccidity by preventing vascular inflow. A variation of this technique has been described. The clinical utility of these tests is unclear. The bulbocavernosus reflex, an oligosynaptic and polysynaptic reflex contraction of the bulbocavernous muscles elicited by stimulation of sensory nerve such as the dorsal nerve of the penis, involves somatic, not autonomic, pathways. It has low sensitivity and specificity in male impotence. Similarly, sensory conduction in the dorsal nerve of the penis, pudendal nerve evoked potentials, motor latencies of the pudendal nerve, and routine and single-fiber EMG of the sphincters assess somatic, not autonomic function.

**Clinical Comments.** The evaluation of GU function is complex and beyond the scope of the current discussion. Most of the available tests do not isolate and assess autonomic as opposed to nonautonomic function. Therefore, in patients with GU dysfunction which may be caused by autonomic failure, the clinician often looks for evidence of non-GU autonomic failure and depends on the other tests of autonomic function.

**APPROACH TO THE PATIENT**

Clinical evaluation and understanding are paramount in the evaluation of autonomic dysfunction—the tests of autonomic function extend, not replace, this evaluation. Autonomic function tests are indirect, relatively imprecise, and fraught with difficulties: autonomic structures such as small unmyelinated nerve fibers which control many autonomic functions are inaccessible for direct neurophysiologic recording except by microneurographic techniques which are carried out only in specialized laboratories; numerous technical and physiologic variables must be controlled; autonomic regulation is slow, intricate, and complex; and the visceral physiological systems being regulated are themselves intricate and complex.

The purposes of objective testing are to detect and verify suspected autonomic dysfunction; quantify severity and extent of autonomic dysfunction for staging, monitoring, and treating; localize the abnormality to the central or peripheral levels; determine whether the sympathetic or parasympathetic or both systems are involved; and determine which physiologic organ systems such as parasympathetic cardiac (cardiovagal), sympathetic cardiovascular (adrenergic), sympathetic sudomotor (cholinergic), or other systems are involved.

Most laboratories perform a battery of tests to enhance reliability and probe various autonomic functions. A typical screening battery includes heart rate variation with breathing, Valsalva ratio and Valsalva maneuver analysis, orthostatic testing, and at least one of the available tests of thermoregulatory function. The analysis of heart rate variation with breathing is considered the best test of cardiovascular function, but duplicate testing with the Valsalva ratio is useful because of overlapping sensitivities. Testing GI, GU, and pupillary function are difficult for most neurophysiologists to do alone, and if performed at all are often done in collaboration with respective subspecialists. Medications with anticholinergic properties (such as antidepressants, antihistamines, and certain over-the-counter medications), with adrenergic antagonist action (such as beta blockers), with sympathomimetic properties, with parasympathomimetic properties, and with fluid-altering properties (such as diuretics or fludrocortisone) should be stopped—consultation with the patient’s primary physician may be necessary. Prior to testing, patients should abstain from alcohol, caffeine, and nicotine for at least 3 h and preferably for 12 h. Patients should be rested and relaxed. Compressive dressings such as elastic stockings should be removed. Testing patients with significant medical dis-
cases such as cardiac failure, atrial fibrillation, chronic obstructive lung disease, and Sicca syndrome is not reliable, since the end-organ of autonomic regulation is itself dysfunctional.

REFERENCES


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