L-arginine, in which is the amino group of the guanidine residue labelled with ^{15}N, resulted in the formation of MNIC which exhibited the doublet HFS in the absence of triplet structures. This gave unambiguous evidence that in animals NO is synthesized only from L-arginine. Experiments in which the oxygen isotope ^{17}O was utilized showed that HFS changed due to the hyperfine interaction with the nucleus of this isotope. It was inferred that atmospheric oxygen is involved in oxidation of the amino group in the guanidine residue of L-arginine to NO. Incorporation of ^{57}Fe into MNIC–MGD was used to trace the transformation of these complexes in mice. It was demonstrated that almost all of them are excreted with urine within one hour after administration. In contrast, hydrophobic MNIC–MGD are retained in different organs.

The EPR spectroscopic technique we proposed for the detection and quantitation of NO is now extensively used worldwide to study NO in biological systems. Moreover, it provided a basis for the development of EPR tomographic analysis in animal tissues at the laboratories of J Zweier (J Hopkins University, Baltimore, USA) and T Yoshimura (Institute for Technological Life Support, Yamagata, Japan).

In addition to the incorporation of NO into exogenous iron-containing traps, it can form in cells and tissues paramagnetic dinitrosyl iron complexes (DNIC) composed of endogenous iron and thiol (sulfur-containing) groups of proteins and low molecular weight compounds. We first found such complexes in yeast cells (1963) and thereafter in animal tissues (1967). They were identified by recording EPR signals from the cells and tissues characterized by axially-symmetric tensors of the g-factor with $g_\perp = 2.04$, $g_\parallel = 2.014$, $g_{av} = 2.03$ (2.03 signal). The nature of the centres responsible for these signals was elucidated after we found a paramagnetic compound the shape and parameters of whose EPR signal was identical with those of the 2.03 signal. This signal was recorded in a frozen DNIC solution with a thiol-containing amino acid, cysteine. Subsequent comparative physico-chemical analysis of these complexes and centres characterized by the 2.03 signal demonstrated the identical nature of the compounds of interest. A specific feature of DNIC synthesized in biological objects is the protein nature of their ligands, i.e. iron contained in these complexes coordinates with cysteine residues of their proteins. In living systems, a fraction of DNIC with low molecular weight thiol-containing ligands is as a rule insignificant.

Analysis of the electronic and spatial structures of the detected DNIC has demonstrated that in these low-spin complexes, as in MNIC with dithiocarbamate derivatives, the unpaired electron is largely localized in the d_z^2 orbital of the iron atom. The geometric structure of the complexes is either octahedron or square, with ligands in the cis-position at the square plane. The iron is in the Fe^+ state (electronic configuration d^7) while NO ligands are in the state NO^+.

Parallel to our studies, B Commoner and his group (USA) discovered and identified DNIC with thiol-containing ligands in rats developing hepatoma induced by various hepatocarcinogens (1965–1970). The complexes arose at the initial stages of tumour growth in the absence of apparent morphological changes in the liver. They disappeared at the later stages. These studies were interrupted until the 1990s but were restarted after the discovery of NO synthesis through the L-arginine pathway in animal tissues and cells (J Hibbs and R Bastian, USA). At present, endogenously produced DNIC complexes are known to occur in different types of cultured animal cells which synthesize nitric oxide.

The functional role of these complexes remains to be elucidated. There is every reason to believe that they may serve as storage depots or a transport form of NO, along with S-nitrosothiols.

Administration of such complexes obtained by chemical synthesis to animals (in the capacity of NO donors) results in different physiological effects. Specifically, they decrease platelet aggregation, reduce blood pressure, and dilate vessels. In other words, they can be utilized as basic materials to develop a new class of cardiovascular drugs. An interesting property of these materials is their ability to induce synthesis of stress proteins including the so-called heat-shock proteins which defend the organism against a variety of stressful factors.

To summarize, a new discipline, NO biology, has set in as a new field of natural science. NO studies yield to-day and will yield in the future new fundamental data which can be applied to practical medicine. The accomplishment of this task will require the concerted efforts of biologists, biochemists, physiologists, and biophysicists working in close collaboration with chemists and physicists.

Laser orthomolecular medical diagnostics

E V Stepanov, V A Milyaev, Yu G Selivanov

A promising field for the application of high-resolution laser molecular spectroscopy is an extremely sensitive analysis of gas exchange in biological objects. This method is applicable to the detection of microconcentrations of relatively light gaseous molecules in the human body formed as endogenous products of its vital activity. The data thus obtained may be used to evaluate health status and diagnose various disorders. When used for medical purposes, the method is usually referred to as orthomolecular diagnostics.

For several reasons, one of the most attractive areas in which to employ orthomolecular diagnostics is microanalysis of the composition of exhaled air. Respiration is the most effective mode of gas exchange between the human body and the environment. Thanks to the large area of the alveolar surface (over 100 m^2), human lungs provide a highly effective gas-exchange system which ensures the utilization of atmospheric oxygen and the disposal of final metabolic products. Apart from CO_2, the major metabolite, exhaled air contains about 600 other volatile compounds formed in the course of numerous biochemical reactions, which need to be released from the body. Both synthesis and transport of individual compounds are based on highly specific mechanisms. This allows them to be used as natural biological markers of the processes which occur in the human body. Moreover, the continuity and cyclicity of respiratory movements are important prerequisites for the continuous and long-lasting monitoring of these processes in close-to-real-time regime. The last but not the least advantage of orthomolecular diagnostics in clinical conditions is that it is a non-invasive method for obtaining valuable information, that is it gives the possibility to make measurements and examine a patient without invasion to studied organism.

The development of orthomolecular diagnostic techniques is directly dependent on the use of up-to-date physical methods for the detection of trace substances in both liquid and gaseous media. These methods must meet certain requirements before they can be applied to the microanalysis of gas exchange during respiration. To begin with, they must be sufficiently sensitive to ensure detection of trace endogenous products in a concentration range from 10 to 10^{-1} particles per billion volume (ppbv) with the time-of-analysis constant of $1-5$ s compatible with the interval between consecutive expirations. Direct detection of the compounds of interest in the exhaled air samples, without their previous concentration or enrichment, is preferred because these procedures may be a source of technical errors and hamper continuous monitoring. The method must be highly selective and insensitive to the presence of non-target constituents in the sample, such as the H_2O, O_2, N_2, and CO_2 predominant in the atmospheric air. At the same time, it is desirable that the method be universal and suitable for the detection of different chemical compounds.

A highly sensitive analysis of the gases contained in the exhaled air is feasible by various physical methods including mass spectrometry, gas chromatography, and optical (spectral) techniques. Each of them is appropriate for the optimal and rational solution of a well-defined range of analytical problems. Mass spectrometry and gas chromatography are as a rule used for the qualitative analysis of exhaled air and permit the detection and identification of moderately or very heavy trace volatile compounds ($50-300$ atomic mass units). However, the analysis takes too much time (from fractions to tens of minutes), and samples often need to be concentrated or enriched first. Optical methods are more sensitive, selective, and rapid when applied to the detection of relatively light molecules. Laser techniques, especially high-resolution molecular spectroscopy using tunable diode (semiconductor) lasers (TDL), appear to be of primary value for gas analysis.

The high analytical performance of TDL, regardless of the area of their application, is attributable to a variety of factors. These light sources now span a large portion of the spectrum, from the near-ultraviolet region to the far-infrared one ($0.3-40$ μm), due to variations in the chemical composition of semiconducting structures. Modulation of the width of the semiconductor gap and the resonator length allows for the gradual thermal tuning of the TDL generation frequency. It is therefore possible to cover a spectral range of $150-200$ cm^{-1} with a change in the sample temperature of $40-50$ K. Moreover, the electron tuning of the TDL frequency is achieved by applying an electric current, which leads to the Joule heating of the laser crystal and a change of carrier density in its active zone.

Spectral and noise characteristics of TDL depend on several factors including quantum noises, relaxation oscillations, fluctuations of carrier mobility, and competition of modes. The quantum constituent is determined by the optical power being generated and the resonator quality. Since semiconductor lasers are characterized by a specific relationship between the carrier density and the refraction coefficient of the active medium, the width of the TDL generation line due to quantum noises is somewhat greater than the value emerging from the Shawlow–Towens relation. For 0.5 mW lasers, it may be as large as $1-30$ MHz, given the Lorentz line contour. The existence of flicker noises $(1/f)$ which are usually attributed to fluctuations of carrier mobility and

technical noises may lead to an additional broadening of the generation line and a change in its shape. Amplitude noises have a 'white' quantum constituent, whose intensity is proportional to the cube of the power and lies in the range from 10^{-8} to 10^{-12} Hz^{-1}, and a flicker constituent predominant at frequencies of up to 1 MHz. Such noise characteristics make it possible to record changes in optical density with a spectral resolution of the order of 3×10^{-4} cm^{-1} and sensitivity as high as 10^{-7}, at an integration time of around 1 minute. The low inertia of the electron system in the semiconductor participating in the generation of optical radiation is worth special emphasis because it allows for the high-performance control of the TDL radiation frequency. Its modulation frequency can reach several hundreds of MHz.

The above properties of TDLs account for their unique spectroscopic and analytic characteristics and the high potential for practical application. These devices can be used to detect a large variety of molecules with spectra lying within the entire range accessible for this type of lasers. This makes it possible to select an optimal spectral range to record an individual compound and accomplish a specific analytical task taking into account atmospheric transparency windows and interference with the absorption spectra of gaseous impurities. At present, the highest sensitivity of TDL is attainable in the middle IR range where the fundamental rotational-vibrational absorption bands are located. The use of isolated absorption bands with integral sensitivity of the order of 10^{-18} cm mol^{-1} ensures detection of substances at relative concentrations around 10 particles per trillion volume (pptv). TDL operating at UV wavelengths would make it possible to further improve the sensitivity of the method by using electron transitions. The narrow generation line of TDL allows the fine rotational-vibrational structure of the molecular absorption spectra to be used and thus ensures enhanced sensitivity, precision, and selectivity of the analysis. This makes TDL especially useful for the detection of relatively light gas molecules exhibiting spectra of a well-defined resonance structure. Electronic control over the parameters of TDL radiation ensures rapid detection, with a high quality of analysis achieved using a rather simple functional design of measuring instruments. Indeed, given rational choice of spectral range, it is sometimes possible to detect several gases with the same laser. Moreover, the small size of TDLs is a prerequisite for their integration into multi-component analytical systems including fibre-optics-based ones.

The high analytical potential of TDLs described in previous paragraphs accounts for the wide scope of orthomolecular diagnostics, in which they can be used to analyse the exhaled air. This scope includes first of all the detection of light gas molecules known to be present in the exhaled air, viz. CO, CO_2, NO, NO_2, N_2O, NH_3, H_2O, H_2O_2, C_2H_4, C_2H_6, CH_2O, CH_4, CH_3OH, C_2H_5OH, CS_2, H_2S, C_5H_{12}, C_2H_6, CH_2OHS, etc. with sensitivity of their detection higher than 1 ppb. Second, the highly accurate detection of metabolites enriched in such isotopes as D, ^{13}C, ^{18}O, ^{15}N, and ^{35}S. Third, multi-component monitoring of all these molecules in the exhaled air during a long-lasting follow-up period and recording variations in their levels within one respiratory cycle in real time. All these analyses can be performed without previous accumulation and enrichment of the samples.

Techniques of the laser orthomolecular diagnostics have been elaborated during the last 10 years in the course of a

series of studies carried out by the Institute of General Physics (IOFAN) and the P N Lebedev Physics Institute (FIAN), Russian Academy of Sciences, jointly with a number of medical research institutions. TDLs operating in the middle IR range in which lead salts of the type PbSnTe, PbSSe, and PbEuSeTe are used as the laser material have been constructed at FIAN for the purpose. Until recently, diffusible laser homostructures have been extensively employed. They worked in the pulse regime at temperatures below 80 K. Five years ago, the technology for manufacturing double heterostructures (DHS) for lasers using molecular-beam epitaxy was implemented; such lasers ensure continuous generation at temperatures of up to 140 K and are characterized by operating currents as low as 1 A. Their radiative power amounts to $300-500$ μW, and the continuous frequency tuning region in the single-mode regimen is $6-8$ cm^{-1}. Today, such DHS lasers allow the spectral range between 4 and 12 μm to be covered.

The analytical methods using lasers described in the previous paragraph are being developed at IOFAN taking into consideration recent progress in research on molecular spectroscopy and spectral gas analysis. The studies include measurement of molecular spectra with high-resolution TDL and determination of the parameters of individual absorption bands, modeling rotational-vibrational spectra of molecules using molecular spectra databases, selection and optimization of spectral ranges for the solution of concrete analytical problems, and creation of laboratory prototypes of TDL gas analyzers. The latter task requires the development of software systems, specialized optico-mechanical units, and electronic devices to control the parameters of laser radiation and spectral data collection.

A laser-based analyzer of gaseous microcomponents in exhaled air is actually a highly sensitive IR spectrometer tuned to ensure high-resolution recording of transmission spectra of a gaseous medium in the selected spectral range. We detected spectra using a pulse-periodic regime of TDL current pumping. The typical duration of rectangular or oblique current pulses was $3-10$ ms at a frequency of $50-200$ Hz. In such a regime, scanning of generation frequency in each laser pulse occurs along the entire spectral region of interest (as a rule, $10-30$ cm^{-1} in length), due to periodically-repeated non-stationary heating of the laser crystal during a current pulse. The high reproducibility of laser frequency tuning between consecutive pulses is ensured by precise (up to within 0.001 K) stabilization of temperature in the cooling system of the laser and also by the good reproducibility of current pulse parameters. This facilitates accumulation of the signal to be detected and thus improves the signal/noise ratio. The spectral resolution of the analyzer depends on the width of the generation line and the stability of the tuning line. It equals 3×10^{-4} cm^{-1} or so.

The laser radiation passes through an analytical cell containing the gaseous mixture of interest and is then applied to a fast IR photoreceiver (CdHgTe or InSb). Transmission spectra are recorded using $8-12$ bit analog-digital processors with a high operating speed which ensure digitalization of a signal containing the spectrum of interest with a time resolution of 50 ns. The dynamic range of digital recording can be extended to $12-16$ bits by using a controllable swept-signal source. Typical transmission spectrum is shown in Fig. 1.

The concentration of the analysed substance is determined from the resonance absorption in the analytical line,
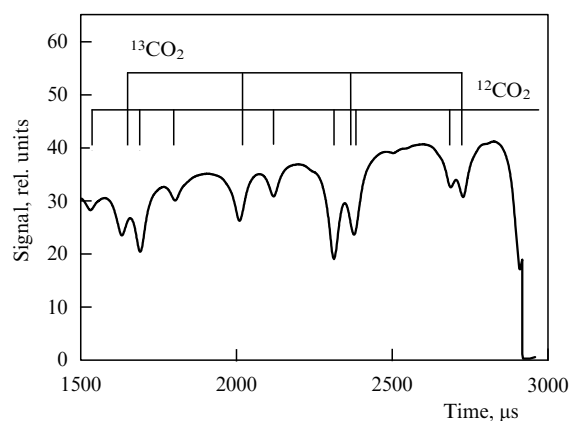

Figure 1. CO_2 transmission spectrum in the 2300 cm^{-1} region, recorded with TDL.

in accordance with the Bouguer – Lambert law, taking into account its contour parameters, which depend on the contribution of collisional and Doppler broadening. Very small absorption values (less than 0.1%) are measured using analog differentiation of the signal. The maximum measurement rate for the gas concentration is limited by the time necessary to scan the analytical absorption band. The speed of TDL frequency tuning in a pulse may range from 10^4 to 10^2 cm^{-1} s^{-1}. Therefore, the time of scanning an absorption band at atmospheric pressure may vary from tens of microseconds to several milliseconds. When the signal is pooled in the pulse-periodic generation regime of TDL, the measurement rate also depends on the pulse frequency. The operating speed can be increased by several orders of magnitude using a continuous generation regime of TDL and fast sweeping of the optical frequency. In this case, however, it is necessary to sacrifice a part of the spectroscopic information that could impair the precision and selectivity of the detection.

We used the described lasers in systems designed to quantify a variety of endogenous compounds in the exhaled air. Specifically, we developed methods and systems for the detection of such molecules as CO, NH_3, CH_4, C_2H_4, NO, N_2O and isotopic species of CO_2. These compounds regarded as biological markers of the processes which occur in the human body have different diagnostic value and specificity. The development of methods based on their detection in the exhaled air for the diagnostics of various disorders requires special in-depth investigations which are normally carried out jointly with medical research institutions. The most important results of such studies using laser orthomolecular diagnostics are presented below. They have been obtained in cooperation with the State Research Center 'Institute of Medico-Biological Problems', Russian Ministry of Health; M I Sechenov Moscow Medical Academy; Institute of Pulmonology, Russian Academy of Medical Sciences, and other institutions.

One biomarker is endogenous carbon monoxide (CO) formed in the body in the course of the enzymatically-catalyzed breakdown of heme-containing structures. This compound is buffered and transported from the site of origin to the lungs after reversible binding to myoglobin and haemoglobin. It competes with O_2 for common binding sites. The rate of endogenous CO disposal in the exhaled air is a function of its production rate and the efficiency of uptake by body buffering systems which in turn depends on the pH of
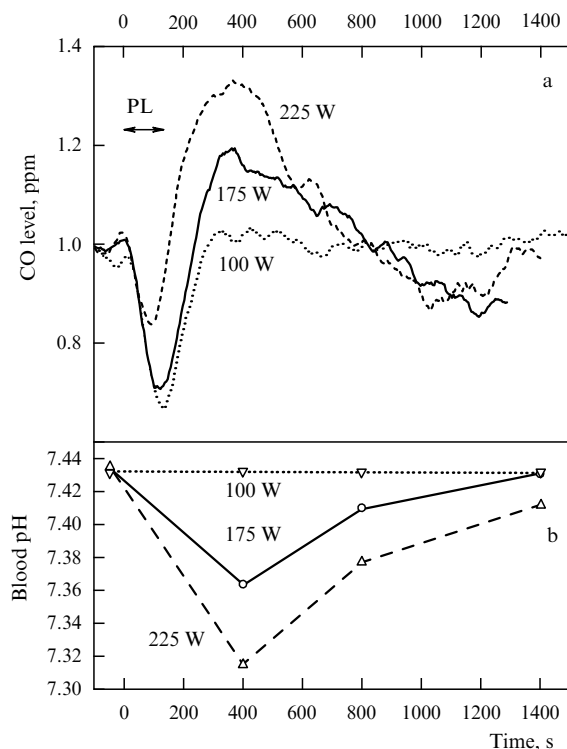
Figure 2. Dynamic of endogenous CO levels (a) and acid-base balance (pH) (b) in blood after physical exercise of different intensity (100, 175, and 225 W).

the medium, tissue saturation with oxygen, and the concentration of certain substances. A series of studies have revealed the basic patterns of the endogenous CO release from the human body in exhaled air. The studies were carried out using a laser-based analyzer allowing for CO detection with sensitivity of the order of 5 ppb and measurement time 5 s. We evaluated the dynamics of CO release in a number of physiological tests including physical exercises (Fig. 2), hyperventilation, breath holding, breathing air with abnormal oxygen (hypoxia and hyperoxia) or CO_2 (hypercapnia) levels. Also, we studied the effects of certain pharmaceutical products and dietary regimens, CO concentration in atmospheric air, and smoking on the release of endogenous CO. The CO content in the exhaled air was found to undergo circadian rhythms. Clinical investigations demonstrated that the endogenous CO levels in the exhaled air are significantly altered in various pathological conditions (hyperbilirubinemia, hepatic lesions, depressed activity of bone marrow, respiratory distress, and radiation injury). This finding provides a powerful tool for the diagnostics and treatment of these disorders. Monitoring CO release may be employed to characterize efficiency of oxygen transport to various tissues.

One more application of laser orthomolecular diagnostics is the detection of endogenous nitric oxide (NO) which plays an important role in a large number of biochemical processes in the human body. These molecules are involved in the transmission of nervous impulses, regulation of vascular tension, and the development of inflammation; also, they are powerful oxidants and have bacteriostatic potency. The laser NO analysis may be of value for the diagnostics and therapy of certain diseases complicated by inflammation. For example, NO detection in the exhaled air is useful in patients

with respiratory diseases (bronchial asthma, bronchitis). Measurement of NO in the gastric gas bubble is important for the diagnostics of gastro-intestinal disturbances (e.g. gastritis). NO levels in such clinical conditions were found to vary from 1 to 1,000 ppb and could be measured with a TDL based analyzer notwithstanding high water and CO_2 content in the air samples. This simple and non-invasive diagnostic technique provides a promising tool for both the rapid evaluation of the severity of a disease and the choice of the optimal medicinal therapy for its effective management.

Equally promising is the use of laser analysis for the accurate determination of the carbon isotopic ratio in the exhaled CO_2 in so-called breath tests in gastroenterology with the use of preparations labeled with stable isotope carbon-13. In these tests, a ^{13}C-labeled material was administered to a patient, and its metabolism in the target organ was followed by measuring the $^{13}CO_2$ that was first released into the circulation and then into the exhaled air. Changes in the $^{13}CO_2/^{12}CO_2$ ratio induced by the test conditions were used to characterize a metabolic process of interest and identify its abnormal features, if any. High-resolution laser spectroscopy turned out to be a simple technique for distinguishing between the absorption spectra of different isotopic species of CO_2 and highly precise (to the nearest 0.3‰) measurement of $^{13}CO_2$ relative concentration variations. The diagnostic efficiency of the laser-based analyzer developed in the course of our studies was evaluated in a clinical trial which involved patients with chronic gastritis and gastric ulcers complicated by infection with *Helicobacter pylori*. The evaluation was carried out using the so-called ^{13}C-urea breath test and ^{13}C-methacetin test for the assessment of hepatic function. Characteristic changes in the carbon isotopic ratio in the exhaled CO_2 observed in these tests are presented in Figs 3 and 4. In principle, laser diagnostics in combination with respiratory tests is equally applicable to the management of digestive disorders including those of the stomach, duodenum, small intestine, and pancreas.

Further improvement of the present diagnostic approach depends on the enhanced sensitivity and the reduced time of detection of microconcentrations of light molecules, the development of methods for recording other endogenous gaseous substances (H_2O_2, CS_2, CH_2O, CH_3OH, C_2H_6,
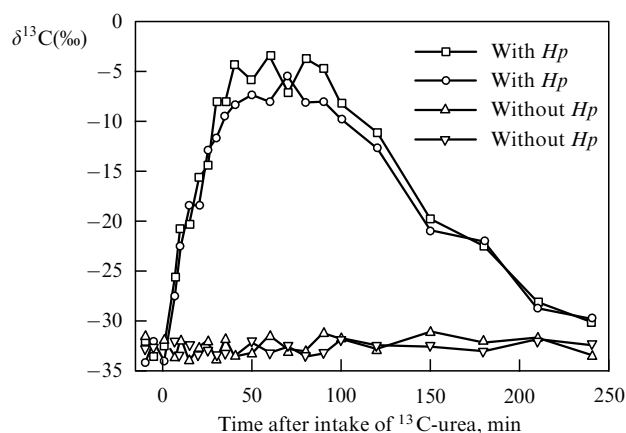

Figure 3. Dynamic of the carbon isotopic ratio in exhaled CO_2 after intake of an aqueous ^{13}C-urea solution (100 mg/100 ml) by patients with *Heliobacter pylori* infection (□, ○ — upper curves) and without it (△, ▽ — lower curves).
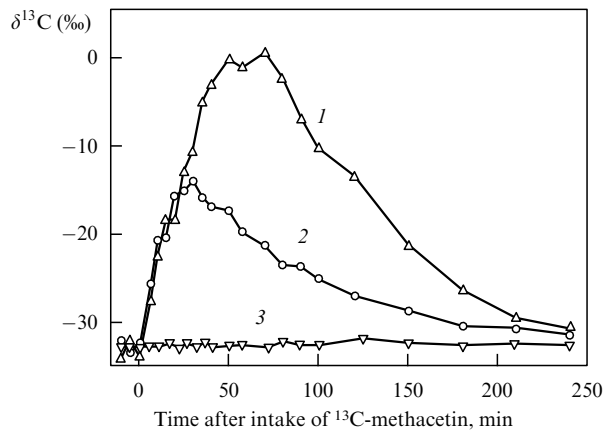
δ^{13}C (‰)

Figure 4. Dynamic of the carbon isotopic ratio in exhaled CO_2 during the carrying out of a ^{13}C-methacetin breath test: in the absence of liver pathology (*1*); with liver deficiencies of average seriousness (*2*); with developed cirrhosis of the liver (*3*).

C_2H_5OH, etc.), construction of multi-component analytical systems, and the broadening of the range of clinical problems that can be resolved using these methods.

Dissipative optical solitons

N N Rozanov

Spatial and temporal optical solitons, i.e. light beams or pulses for which the diffraction or dispersion-induced linear extension is compensated by nonlinear focusing, provide a striking example of self-organization of coherent radiation and formation of particle-like field structures. Their potential is equally important for optical data processing because solitons are natural units of information. Conservative solitons realized in transparent (with negligibly small energy dissipation) nonlinear optical media are better known than other varieties [1, 2]. It is significant that conservative solitons exhibit a continuous spectrum of basic characteristics (e.g., maximum intensity), which accounts for the drift of these characteristics under the influence of fluctuations. Another type of soliton, autosolitons or dissipative optical solitons (DOS's), was initially predicted for wide-aperture nonlinear interferometers excited by external radiation [3, 4] and for laser systems with saturable absorbers [5, 6]. DOS's are substantially different from conservative solitons, first of all in the discrete spectrum of major characteristics (the DOS energy balance is satisfied only for discrete values of maximal intensity). For this reason, the drift of soliton parameters under the effect of fluctuations is suppressed, and the unusual stability achieved may be of value for a number of applications. DOS's are formed under hard (threshold) conditions, and the loss of stability of uniform field distribution is not a necessary prerequisite for their existence. Unlike non-optical systems in which the mechanism of spatial coupling depends on diffusion [7], optical schemes are normally dominated by the diffraction mechanism of transverse linkage with characteristic diffractional field oscillations. The latter mechanism is conducive to a considerable broadening of the range of

DOS types. Theoretical and experimental studies that followed the publication of Refs [3–6] further increased the number of systems in which DOS's can be realized and their new varieties and properties revealed. The objective of the present paper is to summarize advances in this area of research (see also review [8] and monograph [9]).

Schemes with inertialess nonlinearity are primarily described by the equation for the slowly changing field E envelope (dimensionless variables are used):

$$\frac{\partial E}{\partial \varsigma} = (\delta + \mathrm{i})\Delta_{\mathrm{d}}E + f\big(|E|^2\big)E + E_i. \tag{1}$$

The evolutionary variable ς has the sense of time t for resonator schemes and the longitudinal coordinate z in resonatorless schemes, δ is the effective diffusion coefficient (rapid medium relaxation approximation), the Laplace operator Δ describes diffraction and dispersion and has the dimension $d = 1, 2, 3$ in the space of 'transverse coordinates' **r** (the latter variant is realized for a continuous nonlinear medium with frequency dissipation), f is a complex function of intensity $I = |E|^2$ which characterizes the nonlinearity of the medium including amplification and absorption, and E_i is the external radiation amplitude.

Nonlinear interferometers ($d = 1, 2$). An example is the Fabry–Perot wide-aperture interferometer filled with a nonlinear medium and excited by external radiation ($E_i \neq 0$). The shape of the DOS field is found analytically for nonlinearity of the threshold type and numerically for other types of nonlinearity. DOS's are characterized by local intensity overshooting (towards higher or lower values for 'bright' and 'dark' DOS's respectively), which contrasts with the constant intensity corresponding to the transversely-uniform distribution. External radiation determines both the frequency and the phase of radiation in the form of DOS's. DOS's can also exist in the absence of bistability of transversely-uniform states [10]. However, the greatest variety of DOS's occurs under bistability conditions. Such DOS's can be represented as the coexistence of two regimes, one corresponding to a branch of uniform states and undergoing excitation on a small part of the aperture of an interferometer and the other corresponding to one more state which is realized in the remaining aperture region (Fig. 1). Interpretation of DOS's as a bound state of switching waves is equally constructive [10]. It implies the discreteness of individual DOS spectra, with the DOS's differing in the width of intensity overshoot and the number of intensity
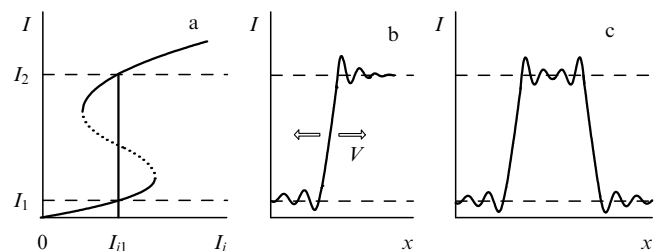
Figure 1. Variant of interpretation of dissipative solitons: (a) hysteresis dependence of output radiation intensity I on input radiation intensity I_i in the plane wave approximation; (b) intensity profile for a switching wave, x is the transverse coordinate, front velocity v is determined by intensity I_i; (c) intensity profile for a dissipative optical soliton.