

PART IV
Eleutherococcus Extract:
Prospective Trends in Application

GENERAL METHODOLOGICAL PRINCIPLES OF THE USE OF
ELEUTHEROCOCCUS EXTRACT FOR DECREASING THE
EFFECTS OF EMBRYOTOXIC FACTORS

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According to the published data, the incidence of developmental anomalies has drastically increased in large cities over the past 10-20 years (Veselov et al., 1976). The growing application of senobiotics, i.e. drugs, food additives, alcohol, residual pesticides, and so on many of which appear toxic for sexual cells and human embryos, is considered to one of the causes of this increase (Sanotsky and Salnikova, 1979). Among the other causes is the action on man of antropogenic environmental factors (electric and magnetic fields, vibration, ionizing radiation, etc.). All these causes may be supplemented with the actions of powerful psychological loads during which the products of stressor reactions may be toxic for sexual cells and embryos.

The resistens (adaptogens) which non-specifically increase the total body's resistance can be used for making sexual cells more stable to toxic factors of the environment and human internal medium. Among them are drugs obtained from *Eleutherococcus senticosus*.

The purpose of this article is to consider the possibilities of the use of resistens and eleutherococcus, in particular for increasing the resistance of sexual cells and embryos to embryotoxic factors, and to suggest a complex of models, methods and procedures for treating the data necessary for primary testing the possibilities of the use of eleutherococcus extract for protection against embryotoxic factors or their combinations.

**1. DAMAGING FACTORS AND
PROTECTIVE ACTION OF RESISTENS**

All factors of the environment and human internal medium that act on sexual cells and embryos might be classified according to numerous indicators. The first level of classification covers physical and chemical factors. Ionizing radiation, the flux of sound frequency energy (infra and ultra included), temperature exposures, mechanical exposures, including such specific patters as vibration, as well as hyper- and hypobaria may be referred to physical factors.

Light exposures stand by themselves. In principle, human sexual cells and embryos are protected from light. However such operations as obtaining and storage in cryogenic banks of human spermatozoa, artificial insemination and embryo cultivation until definite stages *in vitro* may be associated with light exposure as side effect. Laboratory study of sexual cells and embryos is made, as a rule, with the use of light research methods beginning from traditional optic microscopy. Therefore the different aspects of the photochemical reactions of the objects indicated are of great interest.

Normally all physical factors are external with regard to the human body, with the exception of temperature exposures (high temperature of the body itself can be the source of excess heat).

Chemical factors can be divided into exogenous and endogenous. The source of exogenous chemical factors is the environment that of endogenous factors is the body itself.

All exogenous chemical compounds can be divided, in turn, into those made by man (1) for the influence on its own body and (2) those whose action on the body is quite incidental. The first action on the body is quite incidental. The first group includes drugs, food additives, cosmetic products, alcoholic drinks, and narcotics, the second group- residual pesticides contained by foods, toxic substances of industry and agriculture and of environmental pollution.

The greatest hazard is apparently created by chemical compounds belonging to the first group. Among them is the notorious thalidomide, which caused the appearance in the world of the population of abnormal children. Some salicylates (aspirin), alkaloids (caffeine, nicotine), tranquilizers (meprobamate, chlorpromazine, reserpine), antihistaminic drugs (meklizine, cyclizine), antibiotics (penicillin, chloramphenicol), and many others are referred to the substances with established teratogenic hazard (at any rate for laboratory animals) (Murphy, 1965; Wilson, 1973).

A number of toxic substances including carcinogens were discovered among food additives (Rosival et al., 1982). It may be assumed that part of these substances will display embryotoxicity. In some cases embryotoxic substances occur in foods under the action of treatment or storage: for instance, it was described that extracts from gamma-irradiated potato tubers produce a mutagenic effect on sexual cells of male mice (Kopylov et al., 1972).

The substances belonging to the second group are also a source of the increased hazard for sexual cells and embryos. For instance, pesticides (Sanotsky, 1965), volatile components of latexes (Gasparyan et al., 1975), and other compounds produce an inhibitory action on spermatozoa. In certain cases this action is supplemented by the effects of physical factors, such as electric fields of industrial frequency (Andrienko et al., 1977), and so on.

Endogenous chemical compounds that can act on sexual cells and embryos represent first of all the products of stressor reactions in terms of the prevalence. This group also comprises any products of the organism's pathological processes, including conditions marked by the excess output of any compounds inherent in the

body in health, e.g. steroid hormones, prostaglandins (Leonov et al., 1979).

Hypoxia is a special case. Oxygen starvation may be a consequence of both oxygen deficiency in the environment and definite abnormalities of the body. Therefore, this factor can be regarded as exogenous or endogenous depending on the situation

In real situations, exo- and endogenous factors are combined, forming complex combinations of embryotoxins. The real causes sons of hazard are commonly unknown.

Factor may act secretly as is the case with suddenly increased an uncontrolled ionizing radiation. In other cases, the factor itself is used evidently and on purpose. However, the embryotoxic properties of the given substance or physical exposure are unknown as it happens with some drugs, food additives and pesticides. In certain cases, the embryotoxic action results from the combination of factors, each of which alone does not carry any hazard for sexual cells and embryos. Our abilities to predict such situations are generally approximating zero.

A wide range of actions on sexual cells and embryos, numerous sites of application of these exposures to the indicated objects give rise to a great diversity of the manifestations teratogenicity itself (Murphy, 1965; Wilson, 1973; Dyban, 1976).

It is possible to set task aimed at increasing the resistance of sexual cells and embryos to different damaging factors. A great number of embryotoxic agents and manifestations of their actions determine the choice of agents which should possess a broad range of the protective action and the ability to increase non-specifically the body's resistance. This property is exhibited, in particular, by resistens (adaptogens) of the natural and synthetic origin: extracts obtained from eleutherococcus, ginseng, *Schizandra*, as well as dibazol, mival and many others.

An essential feature of the adaptogens is their ability to protect the body against numerous damaging factors of the environment and human internal medium, including those that present potential embryotoxic hazard. This ability is of paramount importance, even if it is linked with the protection may be expanded to embryos while remembering that there is a definite specificity.

Extracts obtained from eleutherococcus and other adaptogens can increase the resistance of the integral bodies to physical and chemical factors,

which are hazardous for both sexual cells and embryos. The question arises whether eleutherococcus extract can exert a protective action with regard to these objects. There are not so many articles that answer this question; nevertheless they point to great possibilities of eleutherococcus extract in this field.

First, the ability of eleutherococcus extract to produce a beneficial effect on sexual cells and embryogenesis of agricultural animals and poultry is of great scientific and practical importance (Lyapustina, 1980).

According to these data, in experimental bulls fed eleutherococcus the volume of ejaculate increased by 21.4% versus control, the concentration of spermatozoa rose by 10-35% (depending on the time of estimation). The same patterns were noted for ejaculate of cocks. The number of eggs laid by hens fed eleutherococcus for a month increased by 133%. There was a noticeable increase in the proportion of inseminated cows among those, which were fed ground root of eleutherococcus. Eleutherococcus extract was found to stimulate mink fecundity.

Second, there are examples illustrating the ability of eleutherococcus extract to produce a protective action in the presence of embryotoxic factors of different origin. Chebotar and Gordeichuk (1981) made experiments on 166 mongrel female rats to examine the effect of eleutherococcus on embryotoxic and teratogenic activity of sodium salicylate, chloridine and thioguanine applied in threshold and approximating doses. Eleutherococcus reduced embryotoxic activity of sodium salicylate. It had a protective effect in the pre- and postimplantation periods of embryotoxic chloridine action. The embryotoxic effect of thioguanine that was administered together with eleutherococcus remained unchanged. It is assumed that eleutherococcus promotes the reduction of the embryotoxic effect of a number of teratogens whose action is based on the mechanism of inhibition of respiration and oxidative phosphorylation.

Bolkhovitinova and Zvereva (1981) successfully used eleutherococcus extract in conjunction with other prophylactic agents for the antenatal prophylaxis of fetal hypotrophy. In this case, the incidence of acute infections and

exacerbation's of chronic infections, complication of pregnancy, delivery and postnatal period was found to be reduced. Eleutherococcus exerted a regulatory action on the fetal growth and development.

The mechanism of this action of eleutherococcus is unknown, but for comprehending its essence of substantial interest are the data according to which the total amount of eleutherosides obtained from the plant root enhances protein synthesis in the early stages of the embryogenesis of the amphibian (Voropaev, 1971). To understand the action mechanism, it is also advisable to know that triterpene glycosides obtained from the eleutherococcus leaves have a cytotoxic action, while individual eleutherosides B, B₁ and E obtained from the root have no influence on the early stages of the embryogenesis of the urchin (Anisimov et al., 1973).

Thus, eleutherococcus extract (and possibly individual eleutherosides) can be used for stimulation embryogenesis in health and for increasing the resistance of sexual cells and embryos to embryotoxic factors of exogenous and possibly of endogenous origin.

2. PRINCIPLES OF ESTABLISHING THE ADVISABILITY OF THE USE OF ELEUTHEROCOCCUS EXTRACT FOR DECREASING THE ACTION OF EMBRYOTOXIC FACTORS

There is evidently a great number of embryotoxic factors and their combinations. In many cases these are a complex and undefined composition. It is also evident that in each particular case, an individual estimation of the possibility of the use of eleutherococcus extract is required. To solve this problem, it is desirable to develop a system of primary testing of the action of eleutherococcus with regard to numerous embryotoxic factors. Such a system has been developed by the authors of this article.

As test objects the system includes sexual cells and embryos of the urchins (*Strongylocentrotus intermedius*) and loach (*Misgurnus fossilis*), which are widely used for model studies in embryology and whose embryogenesis mechanism has been studied well (Buznikov and Podmarev, 1975; Kostomarov, 1975; Neifakh and Timofeeva, 1977; Korzh, 1981).

Traditional research methods used in embryology are visual estimations of the status of

sexual cells and embryos on the basis of various techniques of optic microscopy. In this case, the purpose of research is to establish the agreement between the morphology and time of appearance of definite developmental stages and the same indicators accepted in the literature as reference (Buznikov and Podmarev, 1975; Kostomarova, 1975). This work is to be performed by a skillful researcher, and should be oriented for his possibilities. Technologically it cannot be applied to the large-scale studies.

The tasks of estimation of the embryotoxicity of a great number of physical and chemical factors and selection of the appropriate resistens require for their solution methods characterized by high informative value: they allow the use of automatic devices and marked on the whole by high effectiveness. In our opinion, there are at any rate six groups of processes in the vital activity of gametes and embryos, which make it possible to apply the modern physical techniques of the control over the status of these objects during embryogenesis*:

- 1) morphological changes (general description of the form, geometric characteristics),
- 2) generalized cell reaction to external exposures,
- 3) processes of energetics and biosynthesis,
- 4) structural status of some subcellular structures and biopolymers,
- 5) permeability of gametes and embryos for test and marker substances** and their morphological distribution,
- 6) mobility.

Let us consider in brief the importance of these indicators and some measurement methods.

The modern instrumental methods of analysis allow morphometry automation. Three approaches to such automation may be described:

- 1) measurement of the integral optic indicators—light diffusion, absorption and object fluorescence on the whole;
- 2) measurement of the same indicators during object scanning by light sound whose dimensions are many times less than the object size;
- 3) television morphometry analysis.

All other indicators of the vital activity (with the exception of mobility) indicated in items 2-5 of this part should be in automated systems of biological control measure by fluorescent-absorption analysis methods.

The internal photofluorescence of cell proteins is one of the most sensitive indicators of the cell generalized response to external exposures: it is closely related to the cell physiological status, to exposures to different chemical compounds, ionizing radiation, heating, etc. (Barenboim et al., 1969; Chernogryadskaya et al., 1978).

The status of flavine mononucleotides and pyridine nucleotides involved in electron transport respiration is an important characteristic of the cell energy level. The fluorescence of these components reflects their status (oxidation-reduction) fairly well and serves as a reliable indicator of the cell energy (Yudenfrend, 1965).

Variation in the membrane status, mitochondrial potential, the cell content of DNA, the electric potential on the external membrane are usually recorded in relative units, with the use of appropriate fluorescent probes (Vladimirov and Dobretsov, 1980). In certain cases the test substances may play the role of such probes.

Mobility is one of the most integral indicators of the physiological and functional status of the cells capable of moving. Among gametes and embryos, mobility is displayed by spermatozoa and embryos of marine invertebrates, fish and other organisms at a definite developmental stage.

The movement of spermatozoa has been studied most comprehensively. As a rule, the mean rate and the time of the mobility preservation were measured. Different authors applied high-speed microphotography (Lukin and Leonov, 1974), the combination of a slit chamber with bacterial capillaries (Rombe and Spiridonova, 1972), spectroscopy of optic combination (Arefyev et al., 1978) and other techniques.

In our opinion, the optimal methods for studying the action of damaging agents and resistens on mobility are the following three methods: the method of track characteristics, spectroscopy of optical combination and television analysis. The method of track characteristics can be effected by photographic recording of the tracks in observing the object movement under microscope under dark-field illumination.

To study the mobility of biological objects and spermatozoa spectroscopy of optical combination (SOC) has been intensively used in the past 10-15 years. The method is based on the displacement of the frequency of the light diffused on a moving object (Kammins, 1976).

In 1967, the spectra of internal beats of the light diffused on the rabbit sperm were measured for the first time (Berge et al., 1967) which showed the presence of widening due to the cell mobility.

The television speed analyzers are based on the different principles of analysis and comparison of the television signals in time (USA patent, 1975).

The main advantage of these methods lies in the possibility of visual observation over a moving object at the display, of image fixation on a video tape recorder or electron memory, and of the objective quantitative evaluation of the speed.

To measure the speed of the movement of spermatozoa, it is advisable to combine all three methods: track characteristics and television analysis which are the most effective for understanding the process essence, construction of a model, introduction in diagnosis of track characteristics, and the SOC for large-scale experiments, plotting of the concentration dependencies, etc.

Study of the movement of migratory embryos, e.g. of the urchin at the stage of the medium blastula, deserves special attention. It seems to us that the speed and track characteristics of migratory embryos as quantitative indicators of their vital activity in health and under exposure to different external factors have not been employed by anybody.

To raise the efficiency of the experiments, we used the methods for experiment planning (Hicks, 1967; Finni, 1970) and the theory of pattern recognition for analysis of the data obtained (Gorelik and Skripkin, 1974; Too and Gonsales, 1978).

Thus, there is a real possibility to use the eleutherococcus extract for decreasing the effects of embryotoxic factors. Experimental studies carried out in our laboratory have demonstrated that the availability of the system of preliminary assessment of eleutherococcus extract for reducing the action of various

damaging factors on sexual cells and embryos makes it possible to search rapidly, economically and efficiently for new approaches to the application of eleutherococcus for solving this fairly pressing problem. The subsequent screening should be performed in this case by traditional methods of pharmacology and toxicology on animals.

It may be assumed that such screening methods will make it possible to widen considerably the field of eleutherococcus extract application.

As far as the health status of the population groups exposed to potentially hazardous environmental physical and chemical factors or high psychological loads is concerned, eleutherococcus extract and similar drugs may play a role of the prophylactic protective agents reducing the possibility of damage to sexual cells and embryos.

It is not excluded that eleutherococcus extract and the drugs with a similar biological activity are peculiar "vitamins" that increase the body's resistance. They should be used prophylactically by the people who are interested in maximal preservation of their gametes and embryos whatever the status of the environment, especially as the embryotoxicity is often insidious in character.

When used clinically, these drugs may in principle increase the resistance of the body in different pathologies of pregnancy and of spermatogenesis. It seems to us that they may play an essential role in artificial insemination of women: at the stages of cryogenic storage of the sperm, activation of the sperm because of insufficient rate and lifespan, artificial inoculation, etc.

As for the agriculture, the preparations manufactured on the basis of eleutherococcus hold promise for cryogenic storage of the semen from highly pedigreed sires, in addition to the application for raising the fecundity of agricultural animals and poultry.

In fish husbandry, the same preparations may promote a more rapid growth of embryos at high temperatures, the survival of young fish, interspecific hybridization. This has been confirmed by the work performed in collaboration with the authors of this article with reference to the action of eleutherococcus extract on the development of salmon embryos.

Of interest is the potential role of such resistens in increasing the sperm resistance in conservation of the genetic material of rate and dying off animals.

Such a conservation is envisaged by a special international program (Veprintsev and Rott, 1980).

To solve the above-indicated problems, the tests that will follow the screening tests may be performed with the use of such models as the spermatozoa obtained from different agricultural animals (bull, sheep), rare animals and man in health and disease. Deep cooling, an imitation of cryogenic storage, should be also added in this case to the number of damaging factors.

Female sexual cells and the process of their fertilization *in vitro* should be also grouped with the models important for medicine.

In subsequent tests, it is also desirable to use fish embryos that are of great value for industrial fish husbandry in thermal waters. These works may shed light on one of the most important trends in the use of eleutherococcus extract, which has been already demonstrated as promising.

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