J. Pharm. Pharmacol. 1994, 46: 221–225
Received June 7, 1993

Adaptogenic and Cardioprotective Action of *Galleria mellonella* Extract in Rats and Frogs

A. K. RACHKOV*, N. A. SPIRIDONOV AND M. N. KONDRASHOVA

*Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences, Pushchino, Moscow Region 142292, Russia, and * Ryazan Agricultural Institute, Kostychev St 1, Ryazan 390040, Russia*

Abstract—Pharmacological and metabolic effects of *Galleria mellonella* larvae extract used in Russian folk medicine to treat cardiovascular and senile diseases were studied. It was shown that the extract possesses adaptogenic, cardiotropic, cardioprotective, and hypocoagulant properties. The extract possesses low toxicity and does not cause significant changes in biochemical parameters in the blood serum of laboratory animals. Increase in catecholamine content in the heart and aortic tissues and their decrease in adrenal glands are unfavourable effects of high doses of the extract.

The great wax moth *Galleria mellonella* L. (*Lepidoptera, Pyralidae*) is one of the few creatures evolutionally adapted to live in a beehive. The name of the insect reflects its unique ability to consume and digest beeswax. Along with beeswax, the natural diet of the insect consists of beebread, honey, and other products from the honeybee. An ethanol extract of the wax moth larvae has been used in Russian folk medicine as a geriatric remedy and for treating tuberculosis (Spiridonov et al 1993). Early records of such use were found by Mukhin (1961) in a folk medicine manuscript dated back to the seventeenth century (The Manuscript Department of the State Historical Museum, Moscow).

The first experimental studies of the insect were carried out at the end of the last century by Metchnikov, who discovered the ability of *Galleria mellonella* digestive enzymes to dissolve wax capsules of *Mycobacterium tuberculosis* (Metchnikov 1899). The antituberculosic action of digestive lipases and haemolymph of the insect was subsequently studied in detail by Metalnikov (1920, 1935), Mankiewicz (1949, 1952) and Kuzniecow & Wojciechowski (1950).

The therapeutic properties of the ethanol extract of *G. mellonella* larvae were investigated clinically by Mukhin, who particularly noted its beneficial effect on the cardiovascular system (private communication). The extract has been successfully used as the main part of a complex preparation, Vita drops, for treatment of atherosclerosis, stenocardia, myocardial infarction and other cardiovascular diseases (Mukhin 1961).

We have described previously the general chemical composition of the ethanol extract from the larvae and some of its active components (Spiridonov et al 1992a). Stimulating effects of the extract on the oxidative metabolism in cardiac and aortic tissues (Rachkov et al 1993), and on growth and morphological differentiation of cells in culture (Spiridonov et al 1984, 1992b) were revealed. It was shown that the biological activity of the extract depends on the insect's peculiar diet of honeybee products (Spiridonov et al 1992b).

In the present paper we describe adaptogenic and cardioprotective properties of the extract, as well as its effects on blood coagulation and some biochemical parameters of laboratory animals.

Correspondence: N. A. Spiridonov, Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences, Pushchino, Moscow Region 142292, Russia.

Materials and Methods

G. mellonella were collected in the Moscow Region. The larvae were reared in laboratory conditions on their natural diet (empty honeycombs containing beebread and the remainder of honey). Extraction of biologically active substances from larvae was carried out with 40% ethanol, in accordance with the method employed in folk medicine (Spiridonov et al 1984). The extract was stored in the dark at 4°C and used within a year.

Investigation of pharmacological and metabolic effects of the extract was carried out on white unbred rats, 180–200 g, and frogs, 30–35 g. The extract was administered orally or intraperitoneally; control animals received the corresponding quantities of 40% ethanol. Organs, tissues and blood for pharmacological and biochemical investigation were taken under ether anaesthesia after 2 h from the last extract administration.

Adaptogenic properties of the extract were studied by testing for the maximal duration of rat swimming with a load of 20 g at room temperature (21°C).

The activity of the blood coagulation system was measured with a haemocoagulometer GKGM-4-02.

Investigation of the extract effect on stability of the heart muscle towards a toxic action of strophanthin-K was carried out on prepared frog heart (The State Pharmacopoeia of the USSR 1968).

Isolation of glycogen from tissues, and its acidic hydrolysis were conducted as described by Johnson & Fusaro (1966). Determination of the hydrolysed glycogen was carried out by reaction with *o*-toluidine (Hyvarinen & Nikkala 1962).

Adrenaline and noradrenaline in rat tissues were determined by a differential fluorescence method (Ossinskaya 1957) adapted for cardiovascular tissues.

The following biochemical parameters were measured in rat blood serum: bilirubin (Iendrassi & Cleghorn 1937), alkaline phosphatase (Kolb & Kamishnikov 1976), aspartate aminotransferase and alanine aminotransferase (Reitman & Frankel 1957).

Toxicity of *G. mellonella* extract was estimated in white

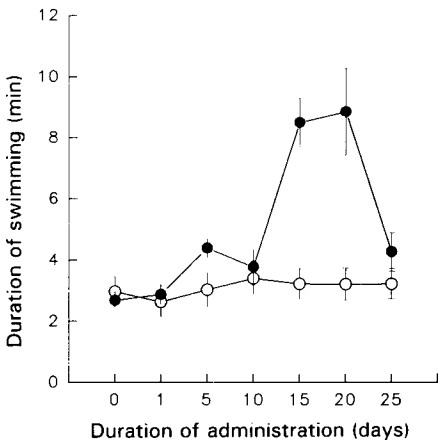

FIG. 1. The effect of *G. mellonella* extract (100 μL/rat day^{-1}, p.o.) on duration of rat swimming with a load of 20 g (n = 10). ○ Control animals, ● animals treated with the extract.

unbred rats, 160–180 g, using intraperitoneal administration. Toxicity of commercial *Eleuterococcus* extract, a widely used adaptogenic pharmaceutical preparation, was determined in parallel experiments under the same conditions.

Statistical analysis of data was performed using Student's *t*-test. Data are expressed as means ± s.d.

Results

Effect on physical working capacity

The effect of daily administration of the extract (100 μL/rat) on maximal duration of rat swimming is presented in Fig. 1. Swimming time reached its maximum on the 15th and the 20th days of the experiment.

Cardioprotective action

Daily oral administration of the extract (50 μL/rat) resulted in a marked increase in the relative heart weight between the 30th and 90th days of the experiment with a maximum on the 60th day (65% above control values). An increase in the liver was apparent on days 60 and 90 of the extract administration (Fig. 2).

A decrease in the relative kidney weight was accompanied by an increase in urine excretion and decrease in protein content in the urine (Table 1). In combination with the increased heart weight, these changes may indicate the cardiogenically conditioned diuresis and decrease in kidney hydration.

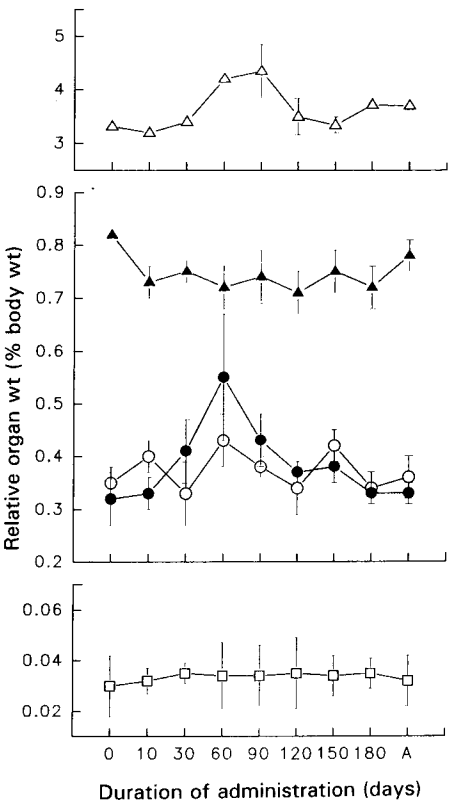

FIG. 2. The effect of *G. mellonella* extract (50 μL/rat day^{-1}, p.o.) on the relative weight of rat organs (n = 6). △ Liver, ▲ kidney, ● heart, ○ spleen, □ adrenal glands, A after cessation of treatment (15 days).

Similar changes in the rat organ weights were observed in the experiment involving intraperitoneal administration of the extract (Fig. 3). The most marked effect of the extract was the increase in the relative heart weight which developed on the 30th day and reached its maximum (65% above the control) on days 60–90 of the experiment. In this case enhanced values of the heart weight persisted during the whole period of the administration. Cyclic changes in the weight of the spleen were also observed. Development of the first maximum in the spleen weight coincided with the maximal increase in the heart weight. Decrease in the kidney weight was more pronounced (20% below control), and correlated well with the increase in the heart weight. A feebly expressed increase in the liver weight was also observed. No statistically significant changes in the weight of the adrenal glands were observed in any experiments.

Table 1. The effect of *G. mellonella* extract (50 μL/rat day^{-1}, p.o.) on rat kidney function.

Duration of the extract administration (days)	Urine excretion (mL)	Urine density	Protein content in the urine (mg mL^{-1})
Control	3·3 ± 0·5	1·02 ± 0·05	6·1 ± 0·4
30	3·8 ± 0·2	1·03 ± 0·05	5·4 ± 0·4
60	3·7 ± 0·3	1·04 ± 0·03	5·7 ± 0·2
90	3·6 ± 0·4	1·02 ± 0·03	5·8 ± 0·2
180	3·3 ± 0·3	1·03 ± 0·04	5·9 ± 0·1
After cessation of treatment (15 days)	3·4 ± 0·4	1·04 ± 0·03	6·0 ± 0·2

n = 6.

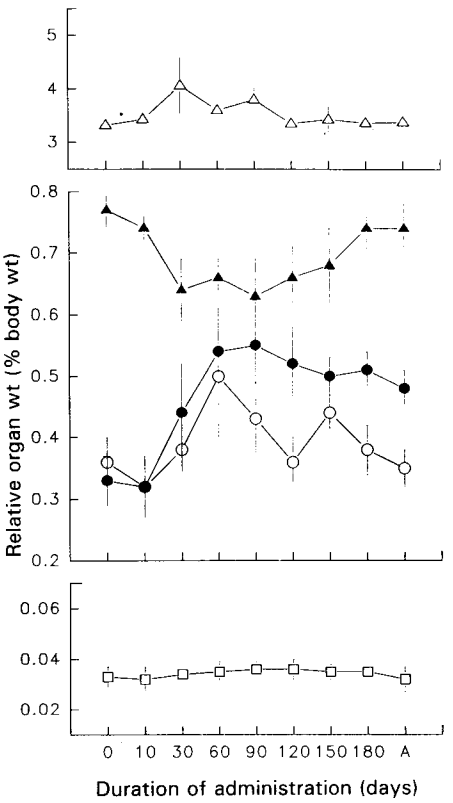

FIG. 3. The effect of *G. mellonella* extract (50 μL/rat day^{-1}, i.p.) on the relative weight of rat organs (n = 6). △ Liver, ▲ kidney, ● heart, ○ spleen, □ adrenal glands, A after cessation of treatment (15 days).

The effect of the extract on glycogen content in rat tissues is presented in Table 2. It is seen that a 5-day administration of the extract (200 μL/rat) induced a 2-fold increase in glycogen content in the myocardium. Glycogen content increased 5-fold on day 10, and 8-fold on day 15 of the experiment. A slight decrease in the glycogen content was observed on days 20–25 of the experiment, although significant excess over control values persisted. Seven days after stopping the extract administration, a 6-fold excess of the glycogen content in the heart over control was observed.

Further studies on the cardioprotective effect of the extract were carried out by using a functional test for the resistance of frog heart muscle towards the toxic action of strophanthin-K. The extract was administered to frogs orally (50 μL/frog day^{-1}), for 7 and 14 days, then the contractility time of the prepared frog heart after injection of strophanthin-K was determined (Table 3). Administration of the extract increased the duration of contractility after strophanthin-K injection, as compared with control frogs which received no extract.

Effect on blood coagulation

The data on the extract effect on blood coagulation are presented in Table 4. Two main parameters were determined: the reaction time (R) which is the first catecholamine-dependent stage of blood coagulation, and the clotting time (K) which is characterized by thromboplastin accumulation and clot formation. The extract displayed marked hypocoagulating action. The reaction time deteriorated considerably, while the clotting time was little affected. A noticeable increase in duration of blood coagulation was observed after a single extract administration (100 μL/rat). After two weeks of extract administration, the coagulation time increased almost 3-fold. On the 30th day of the experiment the reaction time decreased and some signs of hypercoagulation appeared.

Effect on catecholamine content in tissues

The effect of 30-day extract administration (10 and 100 μL/rat) on adrenaline and noradrenaline content in rat tissues is shown in Table 5. An increase of catecholamine content in cardiac and aortic tissues accompanied by a catecholamine content decrease in adrenal glands, which is characteristic of stress, was found in both cases. The effect was more pronounced in animals which received 100 μL day^{-1} of the extract, but reducing the dose to 10 μL day^{-1} did not result in a proportional decrease of the effect.

Metabolic effects

The extract effects (50 μL/rat day^{-1}) on biochemical parameters in blood serum are presented in Fig. 4. An increase in the aspartate aminotransferase activity was observed on the 30th day of extract administration. However, at the next determination (the 60th day) almost a complete normalization of this parameter was observed. A second small rise in the aspartate aminotransferase activity was registered on days 120–150 of the experiment. Insignificant increases in the alanine aminotransferase activity was observed on day 30 of the experiment. Small changes in the alkaline phosphatase activity and bilirubin content were also found. Variations of the investigated parameters were within the limits of normal physiological values and disappeared after stopping administration of the extract.

Table 2. The effect of *G. mellonella* extract (200 μL/rat day^{-1}, p.o.) on glycogen content in rat tissues.

Duration of the extract administration (days)	Glycogen content (μM glucose equiv. (g tissue)$^{-1}$)		
	Myocardium	Liver	Skeletal muscle
Control	294 ± 70	2647 ± 448	167 ± 25
5	594 ± 211	766 ± 170*	85 ± 14*
10	1685 ± 558*	11880 ± 2670*	516 ± 15*
15	2484 ± 121*	3845 ± 825	423 ± 29*
20	2052 ± 243*	8013 ± 1966*	67 ± 7*
25	1490 ± 704	2052 ± 534	55 ± 1
After cessation of treatment (7 days)	2074 ± 146*	886 ± 79*	61 ± 5*

n = 6, * $P < 0.05$.

Table 3. The effect of *G. mellonella* extract (50 μL/frog day^{-1}, p.o.) on the duration of frog heart contractility time after strophanthin-K injection.

Duration of the extract administration (days)	Duration of heart work after strophanthin-K injection (min)
Control	21·5±2·8
7	34·0±4·3*
Control	9·3±0·2
14	29·1±3·6*

n=8–10, *$P<0·05$.

Table 4. The effect of *G. mellonella* extract (100 μL/rat day^{-1}, p.o.) on blood coagulability in rats.

Duration of the extract administration (days)	Thromboelastogram parameters (min)	
	R	K
Control	2·25±0·22	1·07±0·09
1	3·90±0·56*	1·33±0·17
14	6·73±0·62*	1·97±0·34*
30	1·34±0·29*	1·64±0·42
After cessation of treatment (7 days)	2·85±0·40	1·95±0·02*

R = reaction time, K = clotting time, n=6, *$P<0·05$.

Table 5. The effect of *G. mellonella* extract (30 days, p.o.) on catecholamine content in rat tissues.

Tissue	Dose (μL/100 g)	Adrenaline (μM kg^{-1})	Noradrenaline (μM kg^{-1})
Heart	Control	1·3±0·2	3·7±0·6
	10	2·1±0·4	3·3±0·3
	100	5·8±1·0*	1·9±1·0
Aorta	Control	3·6±0·6	5·8±0·9
	10	4·6±0·3	8·6±0·9*
	100	8·0±3·1	12·9±2·6*
Adrenal glands	Control	1113±90	1031±173
	10	924±79	620±148
	100	724±150*	313±54*

n=6–12, *$P<0·05$.

Toxicity

G. mellonella extract possesses exceptionally low acute toxicity (LD50=9·6 mL/200 g) in comparison with its therapeutic dose. The toxicity of *G. mellonella* extract was lower than that of commercial adaptogenic *Eleuterococcus* extract (LD50=6·6 mL/200 g) determined in parallel experiments.

Discussion

In folk medicine, *G. mellonella* extract is administered orally, in small doses and by prolonged courses; for example, a quantity of the extract in the daily dose of the complex Vita drops was about 10–20 μL, and its course was for up to 2–3 months. In the present work substantially higher doses of the extract (50–200 μL/rat day^{-1}) were selected, since folk medicine doses seemed to be extremely low at the first stage of our experimental study.

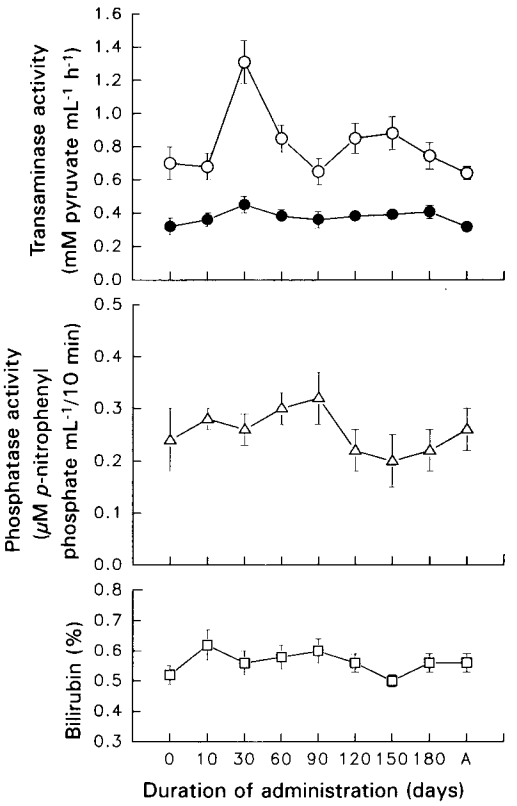

FIG. 4. The effect of *G. mellonella* extract (50 μL/rat day^{-1}, p.o.) on biochemical parameters in rat blood serum (n=6). ○ Aspartate aminotransferase, ● alanine aminotransferase, △ alkaline phosphatase, □ bilirubin, A after cessation of treatment (15 days).

According to a private communication from Mukhin, the cardioprotective action is the main therapeutic effect of the extract. It may be suggested that the cardioprotective action of the extract is largely due to its ability to adapt the organism to physical work. This notion is consistent with earlier data on the extract-induced stimulation of oxidative processes in the heart and aorta (Rachkov et al 1993). The observed increase in the relative heart weight and glycogen content of the heart is evidence of intensification of the anabolic processes in this organ under the action of the extract. Redistribution of the glycogen from liver to the myocardium was also observed (Table 2), which is characteristic of animals adapted to physical work and hypoxia (Arshawsky 1982; Kosenko et al 1983).

Deterioration of the catecholamine-dependent phase of blood coagulation suggests a moderation of catecholamine reactions and an increase in the cholinergic state of animals under the action of the extract, which is favourable for the heart. This is characteristic for trained and adapted individuals which have a higher blood acetylcholine level in a quiet state, than untrained ones. Circulating acetylcholine plays the role of a local hormone, economizing an oxidative and energetic processes (Burn 1961; Arshawsky 1982; Shostakovskaya et al 1986; Kondrashova & Doliba 1989).

Accumulation of catecholamines in tissues and catecholamine-dependent hypercoagulation characteristic of overdose of the extract are evidently opposite to its action in the therapeutic doses. The data on catecholamine content decrease in the heart after single administration (100 μL/rat) confirm this supposition (Stefankiv, unpublished data).

Changes in catecholamine content in the adrenal glands, cardiac and aortic tissues after administration of the extract for 30 days, was accompanied by a decrease in the glycogen content in the liver and skeletal muscle and indications of hypercoagulations characteristic of hyperadrenalaemia (Zubairov & Popova 1976), observed at the same period, allowed us to suggest that an increase in the catecholamine content in tissues may be an unfavourable effect of high doses of the extract.

Long-term administration of the extract in moderate doses did not lead to unfavourable biochemical changes in the blood of laboratory animals. The observed moderate activation of transaminases may be regarded as an indication of activation of intracellular energy metabolism. A more pronounced increase in the aspartate aminotransferase activity as compared with that of alanine aminotransferase, which is primarily associated with cytosol, suggests that the extract activates mainly mitochondrial processes. Intensification of transaminase reactions in the mitochondrial processes leads to an increase in the mitochondrial energy production (Kondrashova et al 1988; Kondrashova 1989, 1991).

Thus, the extract from the great wax moth used in Russian folk medicine exerts a diverse biostimulating influence in the mammalian organism. The extract combines adaptogenic, cardiotropic, cardioprotective and hypocoagulant properties. Even at high doses of the extract the cardiotropic action was strongly expressed in 2-3-week courses without toxic side-effects. Although some unfavourable effects were observed in the following weeks, our data indicate extremely low toxicity of this preparation.

References

Arshawsky, I. A. (1982) Physiological Mechanisms and Regularities of Individual Development. Maximova, E.V. (ed.) Nauka, Moscow, pp 190–221 (In Russian)

Burn, J. N. (1961) Functions of Chemical Transmitters of Autonomic Nervous System. In: Koshtoyants, H. S. (ed.) Izdatelstvo Inostrannoi Literaturi, Moscow, pp 9–59 (In Russian, translation from: Burn, J. N. (1956) Functions of Autonomic Transmitters, Baltimore)

Iendrassi, K. L., Cleghorn, R. A. (1937) Photometrische Bilirubinbestimmung. Biochem. Ztsch. 289: 1–14

Johnson, J. A., Fusaro, R. M. (1966) The quantitative enzymatic determination of animal liver glycogen. Analyt. Biochem. 15: 140–149

Hyvarinen, A., Nikkala, E. (1962) Specific determination of blood glucose with *o*-toluidine. Clin. Chim. Acta 7: 140–143

Kolb, V. G., Kamishnikov, I. S. (1976) Clinica Biochemistry. Belaruss, Minsk, pp 89–95 (In Russian)

Kondrashova, M. N. (1989) Structure-kinetic organization of tricarboxylic acid cycle at active work of mitochondria. Biofizika 34: 450–458 (In Russian)

Kondrashova, M. N. (1991) Interaction of transamination and oxidation of carboxylic acids in different functional states of tissues. Biochimia 56: 388–405 (In Russian)

Kondrashova, M. N., Doliba, N. M. (1989) Polarographic observation of substrate-level phosphorylation and its stimulation by acetylcholine. FEBS Lett. 243: 153–155

Kondrashova, M. N., Grigorenko, E. V., Kosenko, E. A. (1988) Rapid cycle of substrate oxidation under activation of energy metabolism. Abstracts of the 5th Eur. Bioenerg. Conf., Aberystwyth, p. 297

Kosenko, E. A., Kaminsky, Y. G., Kondrashova, M. N. (1983) Energy metabolism adaptation to hypoxia in liver and muscle of rabbit. Biochimia 48: 17–22 (In Russian)

Kuzniecow, A., Wojciechowski, E. (1950) The effect of extracts from *Galleria mellonella* larvae on the growth of tuberculosis bacilli. Med. Dosw. Microbiol. 2: 246–249 (In Polish)

Mankiewicz, E. (1949) The lipidolytic enzymes of larvae of *Galleria mellonella*. Can. J. Res. 27: 195–201

Mankiewicz, E. (1952) The action of lipidolytic enzymes of larvae of *Galleria mellonella* on virulent *Mycobacterium tuberculosis*. Can. J. Med. Sci. 30: 106–112

Metalnikov, S. I. (1920) L'immunite naturelle et acquise ches la chenille de *Galleria mellonella*. Ann. Inst. Pasteur 34: 888–909

Metalnikov, S. I. (1935) Les facteurs de l'immunite dans la tuberculose. La Presse Medicale 48: 962–966

Metchnikov, I. I. (1899) Letters to O.N.Metchnikova. In: Belkin, R. I., Vygodchikov, G. V. (eds) Academicheskoe Sobranie Sochinenii. Vol. 10, Gos. Izd. Med. Lit., Moscow, pp 452–453 (In Russian)

Mukhin, S. A. (1961) On Some Problems of Homoeopathic Treatment of Heart Diseases. Rybak, V. I. (ed.) Mosc. Homoeopath. Soc., Moscow, pp 15–40 (In Russian)

Ossinskaya, V. O. (1957) A study of adrenalin and noradrenalin metabolism in animal tissues. Biochimia 22: 537–545 (In Russian)

Rachkov, A. K., Spiridonov, N. A., Mukhin, S. A., Kondrashova, M. N. (1993) Stimulation of the tissue oxidative metabolism of rat tissues with *Galleria mellonella* preparation. In: Vasina, N. M. (ed.). On Homoeopathic Treatment of Cardiac Diseases and mechanism of Action of Homoeopathic Drugs, Homoeopathic Soc., Moscow, pp 61–64 (In Russian)

Reitman, S., Frankel, S. (1957) A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am. J. Clin. Pathol. 28: 56–63

Shostakovskaya, I. V., Doliba, N. M., Gordy, S. K., Kondrashova, M. N. (1986) Activation of α-ketoglutarate oxidation in rat liver mitochondria with acetylcholine. Ukr. Biochim. Zh. 58(5): 54–61 (In Russian)

Spiridonov, N. A., Kostenko, M. A., Volkova, S. P., Pogorelov, A. G., Kondrashova, M. N. (1984) Influence of biologically active substances from *Galleria mellonella* larvae on neurons of *Lymnaea stagnalis* in culture. Comp. Biochem. Physiol. (C)78: 207–210

Spiridonov, N. A., Kashparova, E. V., Baskunov, B. P. (1992a) On chemical composition of the biologically active preparation from *Galleria mellonella*. Comp. Biochem. Physiol. (C) 102: 199–203

Spiridonov, N. A., Arkhipov, V. V., Narimanov, A. A., Shabalina, S. A., Zverkova, L. A., Shvirst, E. M., Kondrashova, M. N. (1992b) Effect of *Galleria mellonella* larvae preparation and honeybee products on growth of cell cultures. Comp. Biochem. Physiol. (C) 102: 205–208

Spiridonov, N. A., Rachkov, A. K., Kondrashova, M. N. (1993) Cardiovascular preparation from the wax moth. Pchelovodstvo 4: 5–8 (In Russian)

The State Pharmacopoeia of the USSR (1968) Medicina, Moscow, pp 918–920 (In Russian)

Zubairov, D. M., Popova, L. G. (1976) New evidence for the activation of factor XII by epinephrine. Thromb. Res. 8: 587–597