both classes of ribonuclease H occur in metazoa and in kinetoplastida suggests that their occurrence is a general feature of eukaryotes. The kinetoplastids diverged very early in evolution form the other eukaryotes[20]; this can be taken as an indication that the class I and class II ribonuclease H appeared very early in eukaryotic evolution.

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Mitogenic activity of selenoorganic compounds in human peripheral blood leukocytes

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Summary. A variety of organoselenium compounds were originally described as antiinflammatory, antioxidant or glutathione-peroxidase-like agents, and as inhibitors of prostaglandin and leukotriene synthesis. Recently, the compounds have also been found to be inducers of interferon gamma and tumor necrosis factor in human peripheral blood leukocytes (PBL). We evaluated the effects of bis [2-(N-phenylcarboxamido)phenyl] diselenide and Ebselen®; 2-phenyl-1,2-benzisoselenazol-3(2H)one, on the incorporation of tritiated thymidine into the DNA of PBL cultured in vitro. Both compounds were mitogenic and this effect was correlated with the expression of interleukin 2 receptor in T-lymphocytes. Therefore, we suggest that the selenoorganic compounds may induce mitogenic cytokines.
Key words. Selenoorganic compounds; Ebselen®; human leukocytes; mitogenic activity; IL-2 receptor expression.

The organoselenium compounds have many different biological activities in vitro and in vivo. Originally they were described as glutathione-peroxidase-like agents, antioxidants and inhibitors of prostaglandins and leukotrienes[1–5]. Ebselen®; 2-phenyl-1,2-benzisoselenazol-3(2H)one is a potential antiinflammatory drug of a new generation. The toxicity of Ebselen in mice, rats, pigs and man is low because in this structure Se is not bioavailable[4,5].

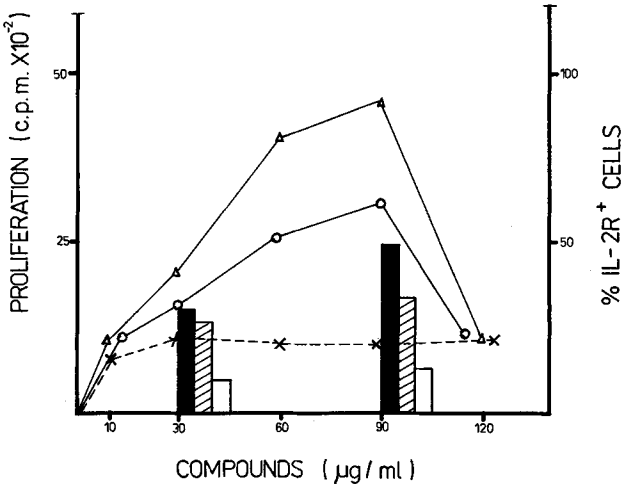
Recent studies conducted in our laboratory have provided evidence that the selenoorganic compounds are inducers of cytokines including interferon gamma (IFN-γ) and tumor necrosis factor (TNF) in human peripheral blood leukocytes (PBL). Therefore, the compounds may be also regarded as biological response modifiers[6].

Here, we describe the activation of human lymphoid cells to proliferate brought about by Ebselen and bis [2-(N-phenyl-carboxamido)phenyl] diselenide (diselenide 1). This activity was correlated with the expression of receptors for interleukin-2 (IL-2R).

Materials and methods

The selenoorganic compounds were synthesized at the Institute of Organic and Physical Chemistry, Technical University, Wrocław[7,8]. The stock solutions (20 mg/ml) of the compounds were prepared in dimethyl sulfoxide. The compounds were chemically pure and they were free of lipopolysaccharides.

The buffy coats of leukocytes were obtained from the Wrocław Regional Blood Transfusion Center. The erythrocytes from the suspension of leukocytes were removed be repeated lysis with 0.83% ammonium chloride according to Cantell et al.[9]. The separated PBL were



Relationship between mitogenic activity and IL-2 receptor expression by selenoorganic compounds in PBL. *A* Cells incubated with: (o) Ebselen, (Δ) diselenide **1** or (x) without the compounds, for 72 h at 37 °C. Proliferation was measured directly by incorporation of ^{3}H-TdR into DNA. *B* Two doses (30 μg/ml and 90 μg/ml) of each compound were analyzed for the IL-2R expression. Percent of IL-2R positive cells was determined with a Leitz fluorescence microscope. ▨ Ebselen, ■ deselenide **1** and □ controls without the drugs.

brought to a concentration of $3-4\times10^6$ cells/ml in RPMI 1640 supplemented with 10% fetal calf serum, 2 mM glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin. Cells were seeded in triplicate 0.1-ml cultures in 96-well flat-bottomed Nunc® tissue culture plates. The cultures were incubated for 72 h at 37 °C in an atmosphere of 5% CO_2 in air with or without the selenoorganic compounds, which were added as suspensions of fine crystals in the complete RPMI 1640 medium (0.1 ml/well). To assess proliferation the cells were pulsed with tritiated thymidine (^{3}H-TdR), 0.8 μCi/well, during the last 12 h. Methyl-^{3}H-TdR (Amersham), activity 42 Ci/mmol, was used in the experiments.

The appearance of IL-2 receptors in the PBL cultures was determined by an indirect immunofluorescence assay[10,11]. PBL were cultured with or without various concentrations of selenoorganic compounds, as described above, for 72 h at 37 °C. Suspensions of 1×10^7 cells/ml were prepared and after three cycles of washing in phosphate buffered saline (PBS) they were treated with 100 μl of murine monoclonal antibody detecting IL-2R (OKT® 26a, Ortho Diagnostic Systems, GmbH). After incubation at 4 °C for 30 min the cells were washed twice with PBS and incubated with diluted 1:10 Ortho® FITC-GAM reagent (fluorescein isothiocyanate conjugate-goat anti-mouse IgG) in an ice-water bath for 30 min. Finally the cells were washed three times by centrifugation and the percentage of the fluorescing cells was determined with a Leitz fluorescence microscope.

Results and discussion

The selenoorganic compounds used at concentrations of 10–90 μg/ml activated the human lymphoid cells to proliferation. The decline of the incorporation of ^{3}H-TdR at a concentration of 120 μg/ml of the compounds suggested that this was a toxic dose. The mitogenic activity was accompanied by IL-2 receptor expression, as shown in the figure. The optimal doses for the maximal expression of both activities were 60–90 μg/ml. More precise determination of the effective mitogenic dose of the compounds was difficult because they are insoluble in water. Diselenide 1 was found to be consistently more active than Ebselen. It has been observed by others[4,5] that Ebselen is unstable in vivo and it is metabolized to diselenides. Therefore, diselenide 1 may be regarded as an active metabolite of Ebselen[6].

As many as 11 different batches of PBL were used in the experiments because of the variation in the properties of cells from individual human blood donors. The sera were pretested and only batches with very low spontaneous mitogenic activity for PBL were used in the experiments. Phytohemagglutinin (Pharmacia, Sweden) at concentrations of 1–5 μg/ml was always used as a positive, mitogenic control. On the other hand the negative controls were sodium selenite and bis [3-carboxypropyl] diselenide which, at the nontoxic concentrations, did not stimulate the ^{3}H-TdR incorporation into PBL DNA (data not shown).

Almost all known inducers of interferon gamma, such as phytohemagglutinin, concanavalin A, staphylococcal enterotoxin A, lipopolysaccharides, calcium ionophore, $ZnCl_2$ and others may stimulate normal lymphoid cells to proliferate and to produce various cytokines[12–15]. For this reason the mitogenic action of the compounds was to be expected[6]. However, it remains to be established whether it is a direct action of the compounds on leukocytes or results from an initial induction of such cytokines as IFN-γ or TNF[6].

It is of interest that the described activities of the selenoorganic compounds resemble the immunomodulating actions of a new organotellerium compound, AS-101, which is an analogue of the antitumor agent cisplatin[16]. Preliminary studies showed that AS-101 may be useful in the treatment of clinical conditions involving immunosuppression[16]. It appears that the described organoselenium compounds have an immunostimulating activity similar to that of AS-101, with a considerably lower toxicity.

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Relationship between lectin-affinity granules in anuran embryos and formation of primordial germ cells

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Summary. Embryos of the anuran, *Rana nigromaculata*, contained granules with a specific affinity for Con A and GS-I. Larvae derived from embryos injected with these lectins had a noticeable reduction in both the number and size of primordial germ cells (PGCs). This observation suggests that the granules are somehow involved in the formation of PGCs.
Key words. Lectins; granules; anuran embryos; primordial germ cells.

In anuran embryos there is a specific portion (germ plasm) of the cytoplasm of some cells that contributes to the formation of primordial germ cells (PGCs), as conclusively demonstrated by Blackler [1], who confirmed the initial observation by Bounoure [2]. However, it is not possible to detect the germ plasm, or cells that contain this cytoplasm, throughout the duration of embryogenesis in all anuran species. In some species, the cells with germ plasm (presumptive PGCs = pPGCs) can barely be seen in embryos from the neurula to the hatching stage. *Rana nigromaculata*, the Japanese pond frog, is one such species [3]. Recently, I reported that a certain fraction of granules derived from embryos at the tail-bud stage contributes significantly to the proliferation of PGCs [4]. However, the particular granules that play a role in the proliferation of PGCs have not been characterized. If it were possible to purify the effective granules, they would be very helpful in our attempts to understand the mechanism of formation of PGCs.

In the present study, lectins are used to identify specific granules in sectioned preparations. Such granules might correspond to the PAS-positive granules (granules stainable with periodic acid-Schiff stain) that are found in specific locations during embryogenesis of some species in which pPGCs are not detectable [3]. PAS-positive granules appear to be a valid candidate for such a function, as judged from their size and behavior. The effects of lectins on the formation of PGCs were then examined. Based on the results, a discussion is presented as to whether lectins are available that might help in the purification of granules related to the formation of PGCs.

Materials and methods

Eggs of the Japanese pond frog, *Rana nigromaculata*, were artificially inseminated by routine methods in our laboratory. Room temperature was not specifically regulated (15–22 °C). Two experiments were performed: the detection of materials with an affinity for lectins; and the effects of lectins on the formation of PGCs.

In the first experiment, embryos at the tail-bud stage were fixed in cold (– 20 °C) Gendre's solution for two days and dehydrated in absolute ethanol (changed three times a week) at the same temperature to prevent the embryos from skrinking. These samples were cut into 6-μm serial paraplast sections, which were then reacted with ten lectins, each conjugated with rhodamine: BPA (*Bauhinia purpurea* agglutinin), Con A (*Concanavalia ensiformis*, jack bean, agglutinin), DBA (*Dolichos biflorus*, horse gram, agglutinin), GS-I (*Griffonia simplicifolia* I agglutinin), GS-II (*Griffonia simplicifolia* II agglutinin), MPA (*Maclura pomifera*, osage orange, agglutinin), PNA (*Arachis hypogaea*, peanut, agglutinin), SBA (*Glycine max*, soy bean, agglutinin), UEA (*Ulex europaeus*, grose, agglutinin) and WGA (*Triticum vulgaris*, wheat germ, agglutinin) (EY Laboratory, Inc., San Mateo, CA). Each lectin was used at a concentration of 0.2 mg/ml in a suitable buffer as indicated by the supplier. The sections were incubated with lectin for 30 min at 25 °C.