

The Effects of Tumor Sera on Cell Shape and Photosynthesis of *Euglena gracilis*

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Cells of *Euglena gracilis* treated with human sera show a marked change in cell shape: Fully elongated cells have nearly totally been transformed to disk-shaped cells. This serum-mediated contraction is followed by irreversible cytolysis. Disintegration of chloroplast membranes leads to decreased photosynthetic O₂ evolution. Sera from humans suffering from tumors reveal higher lytic activities than sera from individuals not suffering from tumors. Heating sera at 56 °C for 10 min or addition of EDTA destroyed or inhibited, respectively, the lytic activities completely. Polysaccharides transformed in polyanions by sulphatation like dextransulphates or heparin seem to protect *Euglena* against serum activities.

The effects described for human sera are believed to display the role of the complement pathway in the cytolysis of *Euglena gracilis*.

Introduction

A previous study [1] showed that roots of *Lepidium sativum* L. incubated in culture medium containing a defined quantity of human serum react in a characteristic manner. While sera from tumorfree individuals obviously inhibit root growth, sera obtained from humans suffering from tumor reveal only little inhibition activity, if any. The growth of roots in tumor sera is by all means comparable to the growth of roots incubated in a mineral culture medium.

For a causal analysis of this phenomenon the root, being composed of different tissues, turned out to be too complex a working model and therefore was considered unsuitable. So, for further experiments, we chose the green flagellate *Euglena gracilis*, a unicellular organism without cell wall. The unicellular algae of the Euglenophyceae are well known for their characteristic changes in cell shape called "euglenoid movement". Following a wide range of mechanical and chemical stimulants, the cells alter their shape in a way which varies from bending in one plane to a complicated shortening and twisting. A further advantage of *Euglena* is its capacity for light-dependent O₂-evolution, which we consider a sensitive indicator for the overall biological state of the cell.

Although human sera reveal effects on unicellular coccid green algae [2], it seems more advantageous to work with *Euglena* because of its pellicle: the characteristic surface membrane complex facilitates transport processes and cell-to-cell reactions in comparison to other plant cells with a more or less rigid cell wall.

The present paper describes the influence of human sera on cell shape and photosynthetic O₂-evolution of *Euglena gracilis*.

Materials and Methods

Human sera were obtained from Prof. Dr. Paulussen and Prof. Dr. W. Engel (Evangel. Hospital Cologne), Prof. Dr. W. Hoeffken (Radiol. Inst. AOK, Cologne), and from Drs. H. Doetsch and A. Baur, both physicians in Cologne. Without exception, the patients from whom the tumor sera were obtained were in a middle to advanced stage of disease. The tumors had been histologically confirmed as malignant. Spurr resin was supplied by Serva, Heidelberg, and the lyophilized hot-water extract from *Laminaria japonica* Ar. var. *ochotensis* was obtained from Dr. Ishiro Yamamoto, Dept. of Pathology, Kitasato University School of Hygienic Sciences, Sagamihara, Japan.

Cultures of *Euglena gracilis* (Algal Coll. Göttingen, SAG 1224-5/25) were grown at 30 °C in 300-ml flasks containing 150 ml of Cramer and Myers' medium [3] with 5% CO₂–95% air bubbled through the culture at a repetitive light-dark cycle

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(14 h light, 10 h dark). Light was supplied by a bank of 40 W cool-white/40 W warm-white (2:1) fluorescent tubes at an incident intensity of 300 ft-cd. Every two days, at the beginning of the light period the cells were spun down and resuspended at about 8×10^4 cells/ml in fresh sterile medium for maintenance of the culture, and an aliquot of the cells was transferred at about 4×10^5 cells/ml in 0.02 M KH_2PO_4 – Na_2HPO_4 buffer, pH 7.2, (henceforth: phosphate algae). Cells were counted with a Coulter Counter. After a further 48 h-culture of the phosphate algae at 30 °C under discontinuous illumination at 300 ft-cd and aeration without additional CO_2 , samples were removed for performance of the incubation experiments. For this, 0.15 ml tumor serum (*i.e.* serum from tumor-bearing humans) and control serum (*i.e.* serum from individuals not suffering from tumor), respectively, were added to 2.85 ml phosphate algae (final concentration 5%, v/v). The suspension were incubated at 30 °C under continuous illumination (300 ft-cd) without additional aeration. After incubation samples were taken for controlling cell shape and for measuring photosynthetic O_2 -evolution.

The percentage of disk-shaped cells were determined by light microscopy and counting the disk-shaped cells in a Zeiss-Thoma chamber. The measurement of O_2 -evolution was carried out by polarography with three electrodes, Pt, Ag/AgCl and Ag [4]. 1 ml of the algal suspension was directly pipetted on the two electrodes Pt and Ag/AgCl and illuminated by a Leitz projector: $600 \mu \text{ Einstein} \times \text{m}^{-2} \times \text{s}^{-1}$, $\lambda = 580\text{--}730 \text{ nm}$, for 30 s. O_2 -evolution recorded by an oscilloscope was registered by a polaroid camera.

For electron microscopy samples were fixed by addition of glutaraldehyde to the incubation medium to a final concentration of 5%. After 2 h at 4 °C the specimen was washed into 0.05 M phosphate buffer, pH 7.0, treated with 2% OsO_4 for 3 h at 4 °C, dehydrated with anhydrous ethanol, exchanged into propylene oxide and embedded in Spurr resin. Sections were cut with a DuPont diamond knife on a Reichert OmU4 ultramicrotome. After staining with uranyl acetate and lead citrate, the sections were observed and photographed in a Hitachi H 500 electron microscope.

Light micrographs were recorded on Agfa Pan 25 using an Olympus photomicroscope with Nomarski differential interference contrast optics.

Results

Euglena gracilis cells suspended in 2.85 ml of 0.02 M phosphate buffer, pH 7.2, and 1.15 ml of a tumor serum show after incubation at 30 °C under continuous illumination (300 ft-cds.) a marked change of their cell shape. The fully elongated cells, resembling a somewhat flattened cylinder (Fig. 1a), have nearly totally been transformed to spherical cells (Fig. 1b). Contracted, motionless cells seem to be more vacuolized than untreated ones. Only few cells can preserve their elongated shape combined with decreased motility. Substitution of tumor sera by sera from humans not suffering from tumor (control sera) results in cells, maintaining their elongated form during the incubation time. Only few cells become disk-shaped. Electron microscopy of sectioned disk-shaped cells confirms vacuolization mentioned above (Fig. 1c). The disk-shaped cells treated by tumor sera show the picture of an advanced cytolysis (Fig. 1c), compared to the intracellular organization of a cell incubated with control serum (Fig. 1d). The cytosol seems to be almost "empty" (Fig. 1e) and fragmented by vacuoles. The membrane systems are severely damaged, especially thylakoids and envelopes of chloroplasts. Only the structural integrity of the mitochondria still appears in good condition. Surprisingly, the pellicle of the contracted cells retain their basic and characteristic conformation (Fig. 1e). In the transverse section, the surface displays alternating ridges and grooves. The four microtubules belonging to the pellicle complex (two are located adjacent to the notch, one near the middle of the ridge and one microtubule in the region of the pellicular groove) are still present.

These striking changes in cell shape and intracellular organization of cells treated by tumor sera are accompanied by an inhibition of photosynthetic oxygen evolution (Fig. 2). In contrast to cells treated by control sera, cells which have been incubated in tumor sera don't reach their steady state within 30 s of illumination. The quantity of evolved oxygen can be calculated to about 30% of that released from control cells.

To see, whether the reported phenomena are specific for tumor sera, the above mentioned incubation experiments were carried out with 40 control and 40 tumor sera of different origin. The tumors had been confirmed histologically as malignant. In order to verify the statistical differ-

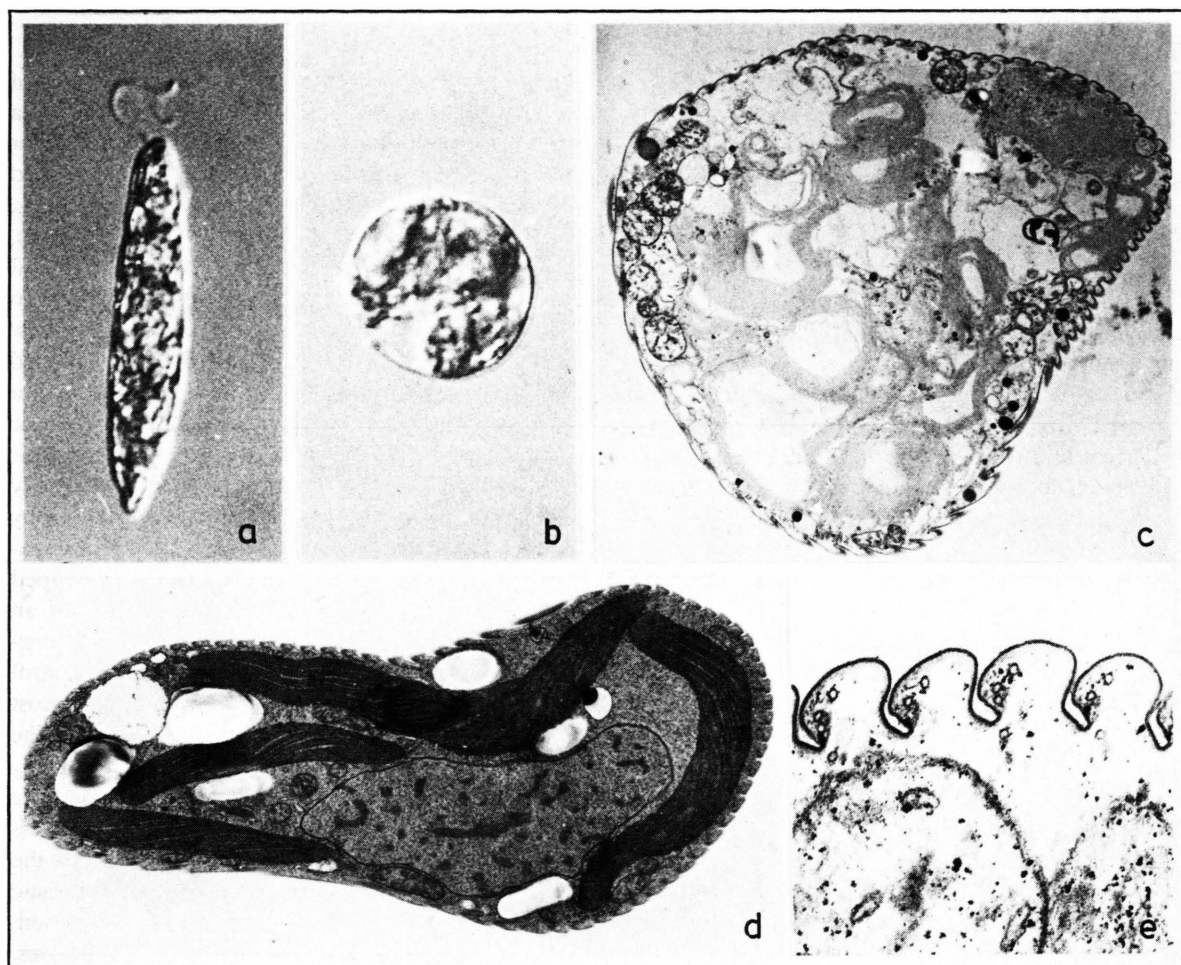


Fig. 1. Light and electron microscopy of *Euglena gracilis* treated with human serum. (1a and 1b) Light micrographs of cells treated with control serum and tumor serum, respectively; (1c and 1d) electron micrographs of sectioned cells treated with tumor serum and control serum, respectively; (1e) transvers section of pellicle after treatment with tumor serum. Incubation time: 20 h at 30 °C under continuous illumination (300 ft-cds.). 1a, 1b $\times 1400$; 1c $\times 7000$; 1d $\times 7000$; 1e $\times 60000$.

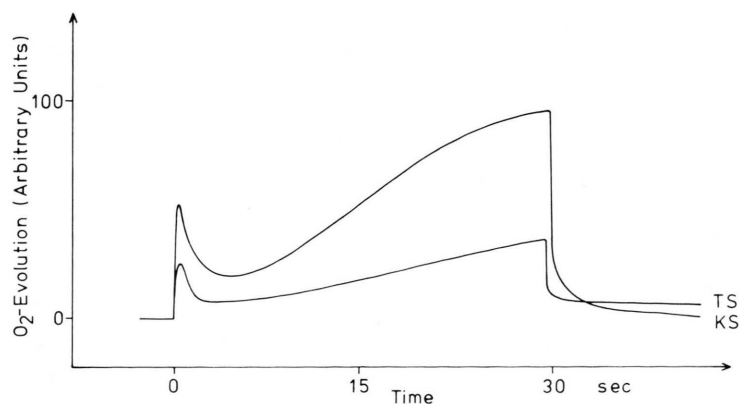


Fig. 2. Time course of photosynthetic O₂ evolution of *Euglena gracilis* cells treated with tumor sera (TS) and control sera (KS). O₂ evolution was measured polarographically in a three electrode system as described in Materials and Methods. Light ($\lambda = 580-750$ nm, 600μ Einstein \cdot m⁻² \cdot s⁻¹) was given for 30 s. The curves were measured at the same sensitivity.

ences between cell shape in tumor sera and control sera, 20 different control sera were chosen at random and compared with 20 tumor sera representing different species of tumor (Fig. 3). With respect to the difference between the oxygen evolution of both cell types, contracted and elongated, 20 different other control sera were chosen at random and compared with 20 tumor sera (Fig. 4). In both cases, the differences of the average number of disk-shaped cells and oxygen evolution values between *Euglena* suspensions incubated in tumor and control sera are highly significant ($p < 0.01$).

The cytolytic activities of sera are dependent on the serum concentration in the incubation medium. As shown in Fig. 5, where the percentage of disk-shaped cells is plotted against serum concentration, the formation of spheric cells is saturated at 5% tumor serum, while the same concentration of control serum yields only about 30% spheric cells.

No activities at all can be seen at concentrations below 2%.

The lytic activity of sera can be demonstrated only in the pH range of 6.8–8.0.

As compared with the lytic effects of sera in 0.02 M phosphate buffer, sera in other buffer systems like HEPES or TRIS show only little activities and even none when mixed with the Cramer-Myers-medium (see Materials and Methods) for *Euglena*. Heating sera at 56 °C for 10 min or addition of EDTA to the incubation medium (final conc. 10 mM EDTA) destroyed or inhibited, respectively, the lytic activities completely.

Polysaccharides transformed into polyanions by sulphatisation like dextranulphates and heparin also inhibit the cytolytic property of the sera (Table 1). Interestingly, a hot-water extract from the brown alga *Laminaria japonica* also protects *Euglena* cells against the fatal attack of sera. Non-

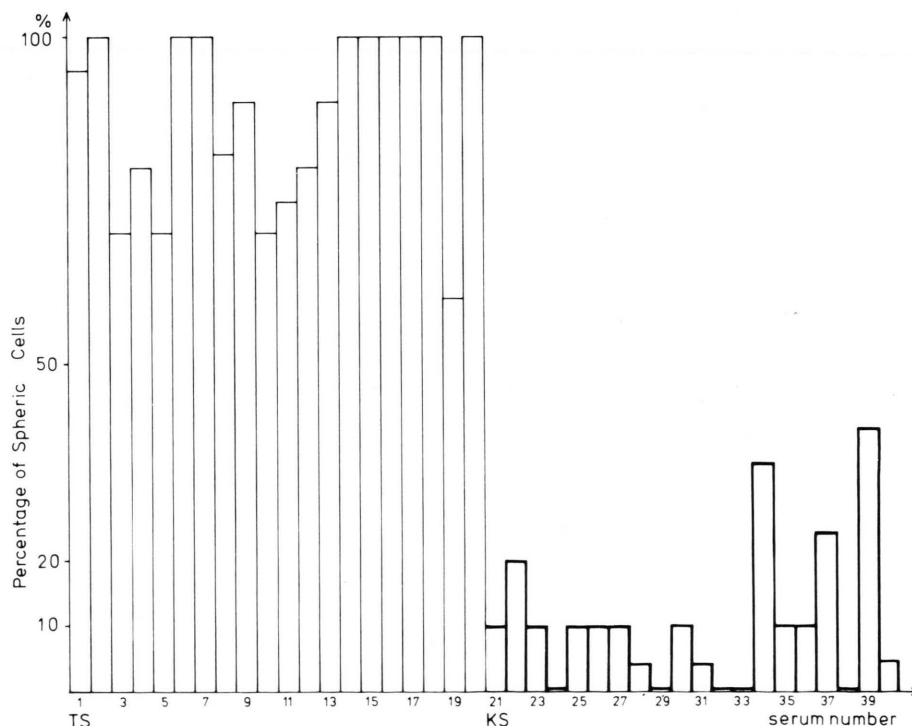


Fig. 3. Shape changes in *Euglena gracilis* in response to treatment with human tumor sera (TS 1–20) and human control sera (KS 21–40). Details of incubation see for Materials and Methods. 100% spheric cells = all cells in the incubate are completely contracted. Tumor types: 1, 7 mamma; 2, 10 bladder; 3, 16 rectum; 18 colon; 6 intestine; 5 prostata; 19 uterus; 17 scapula; 8, 20 oesophagus; 9, 13 lungs; 14, 15 melanom; 11 stomach; 12 tonsil; 4 brain. Control sera: humans not suffering from tumor; among this group individuals with other diseases (23 liver; 40 rheuma; 34 appendicitis; 25 rectum polyp).

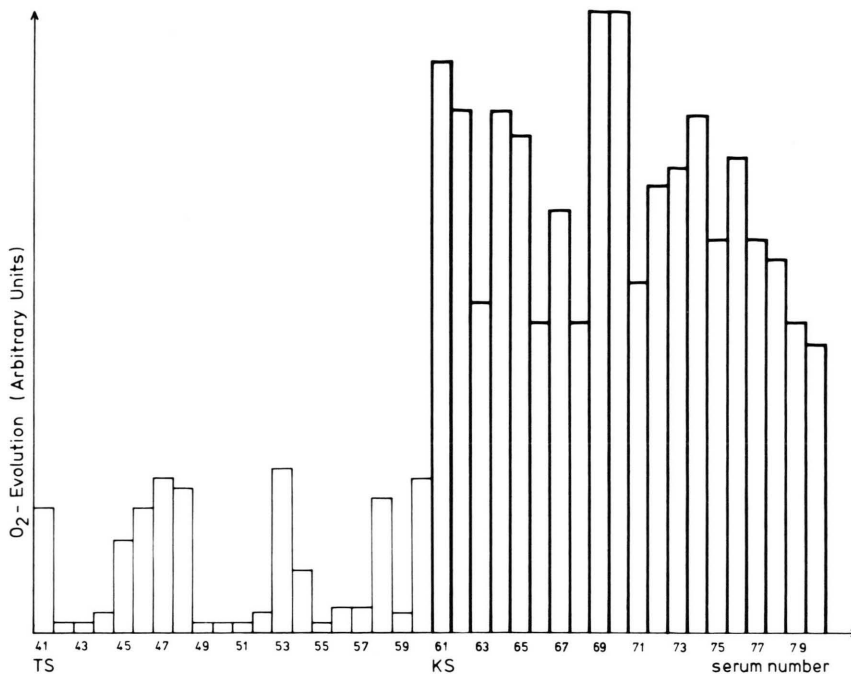


Fig. 4. O₂ evolution of whole *Euglena* cells treated with tumor sera (TS 41–60) and control sera (KS 61–80) at the end of an illumination period of 30 s ($600 \mu \text{ Einstein} \cdot \text{m}^{-2} \text{s}^{-1}$, $\lambda = 580\text{--}750 \text{ nm}$). Evolved O₂ was measured polarographically in a three electrode system as described in Materials and Methods. Tumor types: 41 rectum; 47 colon; 42, 52 sigma; 57 abdomen; 60 mamma; 51, 58 bronchia; 59 peritoneum; 43 oesophagus; 53 lungs; 44, 46, 55 bladder; 45, 56 stomach; 48 mal. lymphom; 50 pancreas; 49 tonsils; 54 unknown prim. tumor. Control sera: humans not suffering from tumors; among this group individuals with other diseases (63 appendicitis; 76 Morbus Crohn; 77 Dysplasie).

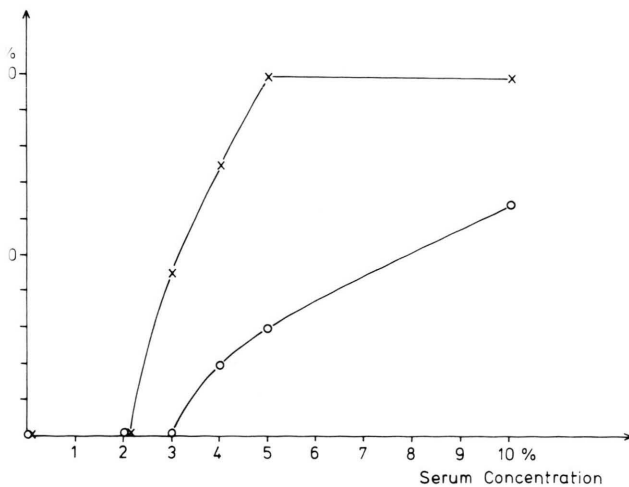


Fig. 5. Effect of the concentration of human serum on formation of spheric cells of *Euglena gracilis*. Assay conditions are described in materials and methods. (○—○) control serum, (×—×) tumor serum (mamma).

sulphated polysaccharides with or without carboxylic residues do not reveal any protective effect.

Discussion

The present work suggests that in general human sera exhibit cytolytic properties when incubated with the green flagellate *Euglena gracilis*. These cytolytic activities are apparently more pronounced in tumor sera than in sera either from humans being healthy or at least not suffering from malignant tumors. The serum-mediated contraction of cells is followed by irreversible lysis. The degradation of cell structures includes in particular the endoplasmic reticulum, nuclei, and chloroplasts. Disintegration of thylakoids consequently leads to decrease in photosynthetic oxygen evolution. As a result of ultrastructural studies of contracted cells, the microtubules located under the ridges of the pellicle seem

Table I. The influence of sulphated polysaccharides on the serum-mediated contraction and cytolysis of *Euglena gracilis*. Each of the 3 ml-incubation volume is composed of 2.85 ml 0.02 M phosphate buffer with *Euglena*, 0.15 ml tumor serum, and 3 mg of the polysaccharide to be tested. Incubation time: 20 h at 30 °C under continuous illumination (300 ft-cds) without additional aeration.

Carbohydrates added to the incubation medium:	Cell shape	O ₂ -Evolution	Motility
Dextranulphate (5×10^3 MG)	elongated	no inhibition	motile
Dextranulphate (5×10^5 MG)	elongated	no inhibition	motile
Heparin (168.4 units/mg)	elongated	no inhibition	motile
Hot-water extract from <i>Laminaria japonica</i>	elongated	no inhibition	motile
Agarose	disk-shaped	inhibited	motionless
Na-alginate	disk-shaped	inhibited	motionless
Galactose	disk-shaped	inhibited	motionless
Galacturonic acid	disk-shaped	inhibited	motionless
Control test without polysacch.	disk-shaped	inhibited	motionless

to be resistant against serum activities. This is in accordance to the reported insensibility of the euglenoid microtubule system to colchicine and triton X-100 [7]. Therefore a disassembly of these microtubules, belonging to the repeating units of pellicle complexes, cannot account for the extreme change of cell shape.

The serum-mediated contraction of the cells resembles in somewhat an extreme form of transformation of cell shape of *Euglena* called "euglenoid movement" [5]. This movement, characteristic for Euglenophyceae, is regulated by the intracellular calcium ion concentration [6]. After increasing the Ca²⁺ concentration in the cytosol either supported by the Ca²⁺ ionophore A23187 or by treating cells with caffeine (efflux of Ca²⁺ ions out of the ER), cells contract very soon. This contraction is a reversible process. The serum-mediated contraction of *Euglena* could be caused also by an uncontrolled influx of Ca²⁺ ions into the cells. The Ca²⁺-concentration in the incubation medium is about 1.5×10^{-4} M due to the relatively high Ca²⁺ content of the serum. This concentration would be sufficient to contract *Euglena*, provided that Ca²⁺ ions could penetrate the pellicle [6]. We believe that there exists a system in the serum making the pellicle of *Euglena* permeable for ions like calcium. The most likely candidate responsible for a lytic effect of sera is the complement system [8–10]. Complement activation on a target membrane like the pellicle of *Euglena* leads

to assembly of a protein complex, causing formation of a trans-membrane pore [11–13]. The concentration of free Ca²⁺ in the cytosol could be changed quickly by the loss of controlled transport functions of the pellicle [14, 15]. The idea of a probable role of the complement system in the serum-mediated *Euglena* reaction is supported by the heat sensitivity of the phenomenon, for the lytic activity of the sera is destroyed by heating sera at 56 °C for 10 min. This may be due to the heat sensitivity of some of the complement fractions and the lability of certain enzymes formed from these fractions during the activation of complement.

In contrast to the well-known activation of the alternative complement pathway by zymosan [16, 17] and polysaccharide sulphates [18], we find in our experiments an inhibiting effect upon the lytic activity of sera. After preliminary experiments polysaccharide sulphates seem to decorate the cell surface of *Euglena* resulting in an effective protection of the cell against serum attack. In this case the inhibiting property of a non-dialyzable fraction of a hot-water extract from *Laminaria japonica* is of great interest. According to the experiments of Yamamoto *et al.* [19] the fraction mentioned above is mainly composed of carbohydrates with ester sulphate. Hot-water extracts from brown algae are well known to markedly inhibit the growth of sarcoma-180 cells subcutaneously implanted into mice [20–26].

It is noteworthy that all tumor sera tested reveal higher lytic activities than control sera. There are some indications that sera from humans suffering from rheumatic disease exhibit also increased cytolytic effects on *Euglena* cells.

It will be of utmost importance to establish the relationship of the serum-mediated *Euglena* reaction to the serum factor(s) like the complement system. Experiments are now in progress which, we hope, will give more information concerning this problem.

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