4.3 at the anodal to 6.8 at the cathodal end. The mol.wt separation of the second dimension gel was from about 90,000 dalton at the top to about 10,000 dalton at the bottom. Under these conditions a new spot appeared consistently in the electrophoretograms of operated animals; by comparison of several in vitro translations this was shown to be an all-or-none response. The spot corresponds to a protein of an apparent mol.wt of 70 kdalton and an isoelectric pH of about 5.80. The general pattern of the in vitro translated proteins does not seem to be qualitatively influenced by the operation. Differences at the level of lower mol.wt proteins, such as those concerning the spot having the coordinate 14 kD-6.6 isolectric pH and 26 kD-6.0 isolectric pH, were only quantitative and not so reproducible. An identical map, including the new spot, was obtained when hypertrophic growth of the heart was induced by daily treatment with 1.0 mg/kg of thyroxine for 7 days (data not shown).

The new polypeptide induced by a stimulus causing hypertrophy does not appear to be produced in a large amount by the heart. In fact, it is not detectable in the map either of soluble or membrane-bound proteins extracted from hypertrophic and control hearts 7 days after operation. This was tested by direct analysis of the 100,000 × g × 2 h supernatant and of the pellet obtained thereafter, by two-dimensional gel electrophoresis followed by staining with Coomassie blue (data not shown). Similarly no differences could be found when the hearts were perfused in vitro (Langendorff apparatus)[8] for 60 min in the presence of [^{35}S] methionine and the protein extracted and analyzed by autoradiography, suggesting that under these conditions the protein is not produced at a sufficiently high rate to be visualized after methionine incorporation by this method.

Thus, hypertrophic growth of the rat heart resulting form aortic constriction or from treatment with thyroid hormone induces in vitro the synthesis of a protein species which is not obtained with hearts of normal animals. This may depend on specific gene derepression or specific activation of untranslated messenger RNA classes[9]. The new protein, which is not detectable before 7 days after operation or thyroxine treatment, seems to be a constant correlate of the hypertrophic growth of the heart, since it is induced by stimuli acting with completely different mechanisms. Apart from the 70 kdalton spot, the spectrum of in vitro translated heart proteins does not seem to change qualitatively after stenosis or thyroxine treatment. The large protein accumulation occurring during heart hypertrophic growth thus appears mostly to be sustained by an increased rate of production of the proteins normally synthesized within the heart. This can be based on a generalized increase in the rate of synthesis of all classes of messenger RNA[10] or/and on a more efficient utilization of the existing ones by means of activation of the translation process. We suggested previously[2] that this latter mechanism may be involved in rat heart enlargement by aortic stenosis, where we showed the activation of a factor that behaves like the eucaryotic initiation factor 2 (eIF-2).

Our further studies are directed toward establishing whether the new protein has a role in the development and maintenance of the hypertrophic state of the heart.

1 Sanford, C. F., Griffin, E. E., and Wildenthal, K., Circulation Res. *43* (1978) 688.
2 Mezzetti, G., Ferrari, S., Davalli, P., Battini, R., and Corti, A. J., Mol. cell. Cardiol. *15* (1983) 629.
3 Gordon, J. I., Smith, D. P., Andy, R., Alpers, D. H., Shonfeld, G., and Strauss, A. W., J. biol. Chem. *257* (1982) 971.
4 Aviv, H., and Leder, P., Proc. natn. Acad. Sci. USA *69* (1972) 1408.
5 Pelham, R. B., and Jackson, R. J., Eur. J. Biochem. *67* (1976) 247.
6 O'Farrell, P. H., J. biol. Chem. *250* (1975) 4007.
7 Bonner, W., and Laskey, R., Eur. J. Biochem. *40* (1974) 83.
8 Langendorff, O., Pflügers Arch. *61* (1895) 291.
9 Jackson, R. J., in: Protein Biosynthesis in Eukaryotes, p. 363. Ed. R. Pérez-Bercoff. Plenum Press, New York, London 1982.
10 Zähringer, J., and Klaubert, A., J. molec. cell. Cardiol. *14* (1982) 559.

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Mating type-specific antibodies in the ciliate *Euplotes raikovi*[1]

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Summary. Three different types of gamones, each representing a different mating type of *Euplotes raikovi,* were used to elicit the production of antisera in mice. Each type of antiserum proved to be capable of preventing the gamone activity in correlation with the different specificities of the mating type.

Key words. Mating type; conjugation; gamone-antibodies; ciliates, *Euplotes.*

Conjugation in ciliates is a developmental process normally triggered by genetically controlled differences in the mating type products between conspecific cells[3]. These products are usually designated as mating type substances if they remain attached to the ciliary membranes, as in *Paramecium,* or as gamones if they are released into the extracellular environment, as in different species of *Blepharisma* and *Euplotes*[4]. Here we deal with gamones released by different mating types of *E. raikovi.* In practice, the gamone of cells of a given mating type diffuses into the environment where it can reach cells of another mating type and promote their conjugation.

Until recently the nature of the gamones was known only in *B. japonicum,* a species that has been considered as showing a dual mating type system[5]. The gamone of cells of mating type I is a glycoprotein of approximately 20 kD, whereas the gamone of cells of mating type II consists of a tryptophan derivative[5].

Gamones have now been identified in *E. raikovi* which, unlike *B. japonicum,* shows a high polymorphism of the locus for the mating type. For instance, seven different *mat* alleles have been identified by cross-breeding four of the 12 different mating types that were originally isolated after sampling a very restricted natural population[6,7]. The gamone released by cells of strain 13 was first isolated and characterized as a heat-stable glycoprotein of 12 kD, capable of promoting mate pair formation at a concentration of 0.036 ng/ml[8]. Subsequently, isolation of gamones was undertaken from other wild strains as well as from their descendant clones, which were distinguished from one another by the mating type. The new gamones characterized so far have proved to be glycoproteins similar in mol.wt and other significant chemical characteristics to the glycoprotein representing the gamone of strain 13[7].

To inquire into the nature of the different specificities within a

family of glycoproteins which appear to be similar in function and structure, we started with an immunological approach to the gamone system of *E. raikovi*. Our initial aim was to elicit antisera that were specific for each of the different gamones which we could obtain in reasonably large amounts. Here we report our success in obtaining these antisera, which are the first ones capable of interfering with ciliate conjugation mating type-specifically.

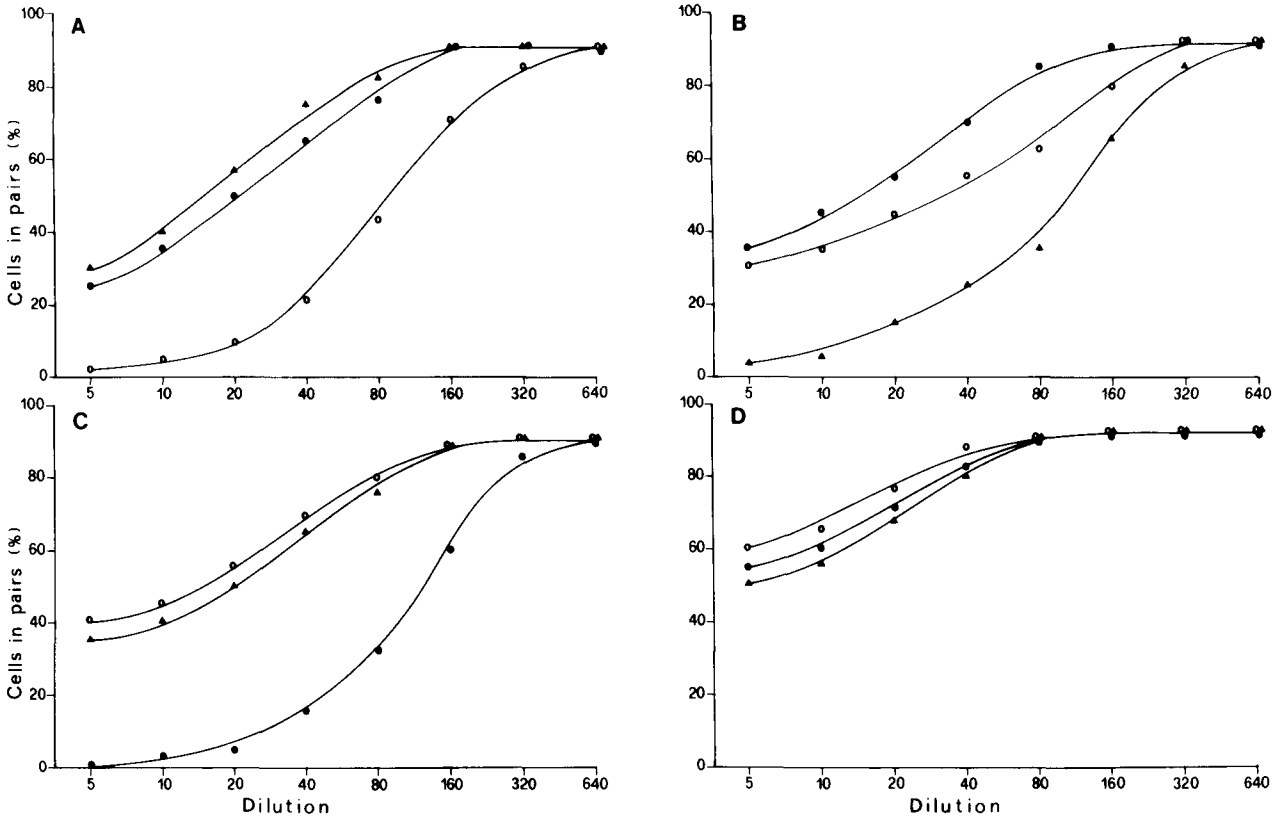
Materials and methods. Strains and gamones. The strains used were Nos 4, 13, 19, and 27, representatives of different interbreeding mating types. All of them were derived from single individuals collected from their natural surroundings, and were fed on the green alga *Dunaliella tertiolecta* under a cycle of 12 h of light and 12 h of darkness, at 24 ± 1 °C. Strains 13, 19, and 27 were sources of gamones. Strain 4 provided standard tester cells for evaluating gamone activity. The three different types of gamones were isolated and purified according to either a protocol that involves concentration of the cell supernatants by ultrafiltration[8], or to a new method based on the use of Sep-pak C 18 Cartridges (Millipore)[9].

Immunization. Each of the three different types of gamone was brought to a concentration of 200 µg/ml in phosphate-buffered saline (PBS) at pH 7.2 and injected (500 µl) into the retro-orbital plexus of BALB/C female mice of 6 weeks of age. The control was a littermate injected with an identical volume of PBS. Booster injections were made into the retro-orbital plexus and i.p. at 15 and 18 days after priming, respectively. The boosters consisted of 250 µl injections of the original buffered gamone preparations for the immunized mice and of PBS for the control. Blood was withdrawn from the jugular vein 4 days after the boosters. Titers of the three types of immune sera were measured by ELISA.

ELISA. The immunoenzymatic assays for measuring the antiserum titers were performed according to the following protocol. 1) Gamones were adsorbed onto a 96-well plate (50 µl/well at a concentration of 10 µg/ml) and incubated overnight at 4 °C; 2) the wells were rinsed and filled with PBS added to 1% bovine serum albumin and incubated for 1 h at 37 °C; 3) the wells were rinsed with PBS, filled with serially diluted immune sera, and incubated for 1 h at 37 °C; 4) the wells were rinsed with PBS, filled with a peroxidase labeled antiserum against murine immunoglobulins and incubated for 1 h at 37 °C; 5) the wells were rinsed with PBS and filled with peroxidase substratum; 6) the reaction was blocked with 4 N H_2SO_4 and the wells containing the antibody-enzyme conjugates were read at 492 nm. The concentration of antigamone immunoglobulins was determined from a standard curve obtained by an ELISA modified at steps 1 and 3. At step 1 an antiserum antimurine immunoglobulin (Dako) was adsorbed onto wells and at step 3 wells were filled by serial dilutions of a standard preparation of murine immunoglobulins (Serotec). In all three types of immune sera, antibodies were detected up to dilutions of 1:320, and their concentrations ranged from 1 to 2 µg/ml. No significant differences were shown by any immune serum in the reactions with the three different types of gamones.

Bioassay. Immune sera were serially diluted with distilled water and 50 ng of one gamone to a final volume of 50 µl. After incubation for 1 h at 37 °C, the mixtures of immune serum and gamone were added to 1 ml samples of 10^4 tester cells at room temperature and after 3 h cells in pairs were counted.

Results and discussion. In the figure, plots A–C show that each of the three types of immune sera obtained against the gamones of *E. raikovi* are quite specific in blocking the activity of the gamone at which they were directed. Tester cells failed to form pairs



Residual activities of the gamones of strains 13 (○), 19 (▲), and 27 (●) after being incubated with different types of serially diluted immune sera. In A, the immune serum was that elicited against the gamone of strain 13; in B, it was that against the gamone of strain 19; in C, it was that against the gamone of strain 27. In D, the gamones were preincubated with a serially diluted serum obtained from nonimmunized, control mice.

either completely (plot C), or almost completely (plots A and B), after being suspended with a gamone preincubated with the corresponding 5-fold diluted immune serum. There was no prevention of conjugation only when tester cells were suspended with a gamone preincubated with the corresponding 640-fold diluted antiserum, and then maximal mating reactions were observed. However, a certain degree of shared antigenicity characterizes the different types of immune sera. Tester cells did not perform maximal mating reactions until they were suspended with a gamone preincubated with a 160-fold diluted immune serum elicited against another type of gamone. Cells in pairs were uniformly found to be approximately 20–30% lower than the respective control (shown in plot D of the figure).

Therefore, it is probable that the antisera raised against the different types of gamones, each of which distinguishes a different mating type, are directed not only at gamone-specific epitopes, but also at other immunogenic determinants common to all gamones. This possibility is substantiated by the results of immunoenzymatic assays (reported in the section on materials and methods) where the intensity of the reaction of each type of antiserum with the corresponding gamone was virtually identical to that shown by the same antiserum in cross-reactions with other types of gamones. It might be also possible that the cross-reactivity between the antibodies contained in an immune serum and non-corresponding gamones is, to some extent, the result of a differential binding at the gamone-specific epitopes. At least some of the epitopes might have slight variations in the amino acid sequences.

Until now, efforts to elicit mating type-specific antibodies in ciliates have all been concentrated on *Paramecium* which, however, does not appear to be an encouraging system for this purpose. The mating type substances of *Paramecium* consist, in fact, of proteins which are intrinsic in the membranes of a small number of cilia and coexist with other powerful immunogens such as immobilization antigens[10]. Therefore, apart from a partial, preliminary success by Hiwatashi[11] in obtaining mating type-specific differences between two types of antisera in *P. caudatum*, the other antisera that have been produced are effective in blocking cell mating but lack mating type specificity[12]. On the other hand it appears to be readily possible to define the antigenic properties of the gamones of *E. raikovi*. The knowledge of such properties is important for tracing both the activity of these mating signals in target cells and for providing insights into their molecular details. This should help towards a better understanding of basic mechanisms in cell-cell recognition of ciliate conjugation.

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2 To whom correspondence should be addressed.
3 Sonneborn, T. M., Proc. natn. Acad. Sci. USA *23* (1937) 378.
4 Miyake, A., in: Biochemistry and Physiology of Protozoa, vol. 4, p. 125. Eds M. Levandowsky and S. H. Hutner. Academic Press, New York 1981.
5 Miyake, A., in: Sexual Interactions in Eukaryotic Microbes, p. 95. Eds D. H. O'Day and P. A. Horgen. Academic Press, New York 1981.
6 Miceli, C., Luporini, P., and Bracchi, P., Acta protozool. *20* (1981) 215.
7 Luporini, P., and Miceli, C., Protistologica *20* (1984) in press.
8 Miceli, C., Concetti, A., and Luporini, P., Exp. Cell Res. *149* (1983) 593.
9 Miceli, C., Raffioni, S., Concetti, A., and Luporini, P., manuscript in preparation.
10 Hiwatashi, K., in: Sexual Interactions in Eukaryotic Microbes, p. 351. Eds D. H. O'Day and P. A. Horgen. Academic Press, New York 1981.
11 Hiwatashi, K., in: Progress in Protozoology, Proc. IVth Int. Congr. Protozool., p. 185. Eds P. De Puytorac and J. Grain. Clermont-Ferrand 1983.
12 Barnett, A., and Steer, E., J. Protozool. *27* (1980) 103.

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Epicatechin can cause the seedling growth inhibitor, nagilactone E, to induce growth stimulation

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Summary. A possible new role for the flavonoid (−)-epicatechin (**II**) is described. It has no growth effects on its own, but when it is added to lettuce and rice seeds together with the known seedling growth inhibitor nagilactone E (**I**), the growth inhibitor activity of **I** can cease and growth stimulation can be observed.
Key words. Flavonoids; growth inhibition; seedling growth; lettuce seedling; rice seedling.

We recently investigated the chemistry of several species of *Podocarpus*[1]. These species are known for their strong resistance to insect attack and their ability to control other plant species in their surroundings[2]. During the course of our bioassay-directed isolation of chemical components it was noticed that there was a discrepancy in the strength of plant growth inhibition. The norditerpenoid dilactone nagilactone E (**I**) was isolated as the major component of a *P. nagi* root bark extract and was found to be responsible for the observed plant growth inhibitory activity[3]. However, the activity of the crude extract of *P. nagi* was less active in seed germination and growth tests than could be explained when the amount of **I** present in the crude extract and strength of the activity of pure **I** was considered. This led to the examination of other components of the crude extract and their effect, when combined with **I**, in seedling growth studies.

The growth bioassays were carried out according to the method of Kamikawa et al.[4], employing seeds of lettuce (*Lactuca sativa*, L., cv Grand Rapids) and rice (*Oryza sativa*, L., cv Norin 20). Lettuce and rice seeds were placed on two layers of filter paper in a 9-cm petri dish containing 4 ml of the test solution. The lettuce seeds were allowed to germinate and grow under continuous fluorescent light (3000 lux at plant level) at 25 °C. Rice seeds were allowed to germinate and grow in the dark at 30 °C. After 5 days the lengths of the lettuce hypocotyl, or the rice shoot, and their roots were measured and an average was taken of 30 seedlings from three petri dishes. One of the major components of the crude mixture was isolated and identified as the known flavonoid (−)-epicatechin (**II**)[5]. The test compounds **I** and **II** showed no visible effects on the germination of both plants except at the highest concentration of **I** used, where 100 µg/ml of **I** resulted in 80% and 50% germination inhibition of lettuce and rice, respectively. Growth effects can be seen in tables 1 and 2.

When **II** was tested alone it elicited little change in the growth of lettuce or rice seedlings. Only at 100 µg/ml does it cause any