each pumping sites were activated when two molecules of K^+ attached to a pumping site.

In general, an increase of the electrogenic Na^+ pump current would be expected in the following cases: 1) an increase of the rate of Na^+ extrusion with a constant Na/K coupling ratio, 2) an increase of the Na/K coupling ratio with a constant rate of Na^+ extrusion, and 3) an increase in both the rate of Na^+ extrusion and the Na/K coupling ratio in each pumping site. According to the present experimental result, it seems reasonable to assume that adrenaline increases neither the total number of pumping sites nor the Na/K coupling ratio. Thus, it appears that adrenaline increases the rate of Na^+ extrusion by increasing the overall affinity of the pumping site to the extracellular K^+ at a fixed concentration. In other words, adrenaline increases the number of activated pumping sites per unit time at a fixed extracellular K^+ concentration.

1 Acknowledgments. The authors thank to Prof. K. Kuba for critical comments and suggestions in the preparation of the manuscript. - This work was supported by a grant (257037) from the Ministry of Education of Japan.
2 S. Nishi and K. Koketsu, J. Neurophysiol. *31*, 717 (1968).
3 M. Nakamura and K. Koketsu, Life Sci. *11*, 1165 (1972).
4 T. Akasu and K. Koketsu, Experientia *32*, 57 (1976).
5 T. Akasu and K. Koketsu, Jap. J. Physiol. *26*, 289 (1976).
6 K. Koketsu and Y. Ohta, Life Sci. *19*, 1009 (1976).
7 T. Akasu, Y. Ohta and K. Koketsu, Jap. Heart J. *18*, 860 (1977).
8 J. Dudel and W. Trautwein, Experientia *12*, 396 (1956).
9 W. Trautwein and R.F. Schmidt, Pflügers Archiv *271*, 715 (1960).
10 J.A. Graham and J.F. Lamb, J. Physiol., Lond. *197*, 479 (1968).
11 G.W. Beeler, Jr, and H. Reuter, J. Physiol., Lond. *207*, 165 (1970).
12 L.R. Thomas, Jr, J. gen. Physiol. *43*, 1193 (1960).

Inhibition of new blood vessel formation in mice by systemic administration of human rib cartilage extract[1]

M. Kamiński, Grażyna Kamińska and S. Majewski

Department of Histology and Embryology; and Department of Transplantology, Institute of Biostructure, Medical School, Chałubińskiego 5, 02-004 Warsaw (Poland), 29 August 1977

Summary. 1 M HCl-guanidine extract of human funnel chest rib cartilage administered i.v. to mice decreased specifically vasoproliferation induced by intradermal injection of allogeneic murine lymphocytes.

New blood vessel formation may be induced by tumor-angiogenesis factor (TAF) isolated from tumor cells[2], or by substance released from lymphocytes engaged in a local graft-versus-host (GVH) reaction[3]. Vasoproliferation induced by these angiogeneic factors could be inhibited by local application of cartilage fragments[4], extract[5] or isolated chondrocytes[6]. The present work was aimed to test whether the systemic (i.v.) administration of xenogeneic cartilage extract to mice could inhibit vasoproliferation induced in the course of a local GVH reaction evoked by intradermal injection of allogeneic lymphocytes.

Materials and methods. Cartilage extract was made from 42 g of costal rib cartilage removed in the course of funnel chest correcting operation performed in 11- and 13-year-old boys. Viability of chondrocytes estimated by neutral red staining was nearly 100%. Cartilage was cut into pieces and extracted with 1 M HCl-guanidine in 0.02 M phosphate buffer (pH 5.8) for 48 h at room temperature. Extract was dialyzed against distilled water at 4 °C and then centrifuged and lyophilized. About 120 mg of crude extract containing 30% of protein as estimated by the Lowry method was obtained.

In funnel chest cartilage, some focal degenerative changes could be observed[7], but we used this type of cartilage because of its excellent viability when compared with normal costal rib cartilage obtainable after autopsy.

As recipients, 8–10-week-old Swiss mice, irradiated with a single dose of 700 R (59 R/min in air) to depress their immunological response, were used. 2 h after irradiation, 1 group of recipients was injected i.v. with a single dose of 7.5 mg of cartilage extract per mouse, containing 2.5 mg of protein, dissolved in 0.2 ml of TC 199. 2 other groups were injected i.v. with 2.5 mg of either human plasma albumin (Biomed, Warsaw) or egg-white lysozyme (Reanal, Budapest) dissolved in 0.2 ml of TC 199 to check the effect of systemic administration of xenogeneic proteins on angiogenesis. Control group was injected i.v. with 0.2 ml of TC 199 only.

Vasoproliferation was induced on the following day in all irradiated and i.v. injected animals using the lymphocyte-induced-angiogenesis assay[3]. In this assay, an intradermal injection of allogeneic lymphocytes evokes a local GVH reaction resulting in pronounced angiogenesis. The intensity of the reaction, i.e. number of newly formed blood vessels, depends on the number of cells injected and reaches its peak on the 3rd day post injection. Swiss mouse recipients were injected intradermally with CFW mouse splenocytes obtained by disruption of donor spleens in a loosely fitted glass homogenizer. Each of the recipients was given 6 injections onto both flanks, consisting of 4×10^6 viable cells suspended in TC 199 in a total volume of 0.1 ml per single injection. Additional group of control mice was given 2×10^6 splenocytes to test the dose effect. 3 days later, angiogeneic response was evaluated at the inner surface of the recipient skin using the criteria of Sidky and Auerbach[3]. All extra blood vessels connected with the injection site and contrasting with the background vasculature due to their tortuosity and divarications were counted. Counting was done by a person who did not know the coding pattern of the recipients.

To prove the specific effect of cartilage extract on endothelial cell proliferation, a group of nonirradiated Swiss mice were injected i.v. with the extract in previously-used doses. Control mice were given albumin or TC 199 alone. Mitotic

Recipients injected i.v.	Mean number of blood vessels ± SD	No. of animals*
Cartilage extract	30.76 ± 7.82	12
Albumin	36.32 ± 8.55	6
Lysozyme	36.72 ± 8.00	12
TC 199 control	38.47 ± 7.80	12
TC 199 control**	29.08 ± 6.69	6

* 6 injections of splenocytes per single recipient;
** mice injected with 2×10^6 splenocytes.

indices of small intestine crypts were calculated 5 h after injection to check whether any inhibitory effect on mitoses could be generally observed.

Results. The table shows the number of newly formed blood vessels observed in preirradiated Swiss mice on the 3rd day after single intradermal injection of 4×10^6 CFW splenocytes.

The mean number of blood vessels in recipients injected with cartilage extract was significantly lower from those in the other groups at $p < 0.05$, as estimated by Student's t-test. This number was comparable with that evoked in the control group by injection with half of splenocyte dose, i.e. 2×10^6 cells. Mitotic indices in intestinal crypts in all groups were not different at $p < 0.05$ and ranged from 3.94 to 4.06.

Discussion. The results show that human funnel chest cartilage extract, when administered systematically (i.v.), inhibits vasoproliferation in mice, induced in the course of a local GVH reaction. The extract decreases the angiogeneic response induced by intradermal injection of 4×10^6 allogeneic splenocytes to a level obtained by giving a dose of 2×10^6 spleen cells to control animals. The effect is specific, since other xenogeneic proteins do not decrease angiogenesis. Target cell seems to be endothelial cell, because mitotic indices of other cells like those in intestinal crypts are not affected, which rather excludes a nonspecific toxic effect of the extract on cell divisions.

It was suggested that small cationic proteins of protease inhibiting properties are responsible for cartilage effect on endothelial cell proliferation[8,9]. The cartilage is a very rich source of lysozyme[10,11], a cationic protein of mol. wt of the same range as inhibitory substance from cartilage[5]. A lysozyme was shown to inhibit some proteolytic enzymes[12], but an other study[13] did not confirm that finding. In our study the lysozyme from egg-white did not affect angiogenesis.

Further purification of human cartilage extract seems to be justified in view of possible application of endothelial cell growth inhibitor to block angiogenesis in tumors or in other pathological conditions, where hyperproliferation of blood vessels is observed.

1 Acknowledgments. This work was supported by grant No. 10.5. from the Polish Academy of Sciences. We wish to acknowledge the valuable help of Dr A. Piasek in cartilage extract preparation.

2 J. Folkman, E. Merler, C. Abernathy and G. Williams, J. exp. Med. *133*, 275 (1971).
3 Y.A. Sidky and R. Auerbach, J. exp. Med. *141*, 1084 (1975).
4 H. Brem and J. Folkman, J. exp. Med. *141*, 427 (1975).
5 R. Langer, H. Brem, K. Falterman, M. Klein and J. Folkman, Science *193*, 70 (1976).
6 M. Kamiński, G. Kamińska, M. Jakóbisiak and W. Brzeziński, Nature *268*, 238 (1977).
7 W. Tischer and G. Leutert, Beitr. Orthop. *15*, 65 (1968).
8 R. Eisenstein, K.E. Kuettner, C. Neopolitan, L.W. Soble and N. Sorgente, Am. J. Path. *81*, 337 (1975).
9 N. Sorgente, K.E. Kuettner, L.W. Soble and R.Eisenstein, Lab. Invest. *32*, 217 (1975).
10 K.E. Kuettner, H.L. Guenther, R.D. Ray and G.F. Schumacher, Calcif. Tiss. Res. *1*, 298 (1968).
11 K.E. Kuettner, R. Eisenstein and N. Sorgente, Clin. Orthop. *112*, 316 (1975).
12 S. Sakamoto, M. Sakamoto, P. Goldhaber and M.J. Glimcher, Calcif. Tiss. Res. *14*, 291 (1974).
13 K.E. Kuettner, J. Hiti, R. Eisenstein and E. Harper, Biochem. biophys. Res. Commun. *72*, 40 (1976).

Electrical resistivity of labellar taste hairs of male and female blowflies, *Phormia regina* (Meig.)[1]

P. Pietra, A.M. Angioy, A. Liscia and R. Crnjar

Institute of General Physiology, University of Cagliari, viale Poetto 1, I-09100 Cagliari (Italy), 20 August 1977

Summary. The electrical resistivity of labellar taste hairs is higher in males than in females of *Phormia regina,* thus indicating a different ion diffusion and different interaction probabilities between stimulating agents and chemosensory dendrites in males as compared to females. This could account for differences in food intake between males and females.

The blowfly, *Phormia regina,* and various other species of insects, show different survival capabilities in the two sexes[2]. Besides, it has been noticed in *Protophormia terraenovae* R.D. that both mortality and the percentage of inactive labellar taste hairs change with the same time course as a function of age[3]. The percentage of inactive taste hairs of *Phormia* also varies with sex, being lower in females[4]. In addition, females consume more sugar and protein than males (Greenberg and Stoffolano, personal communication), and this coincides with the higher body weight and O_2-consumption of females[5]. On the basis of these facts, it seemed of interest to us to investigate whether different properties of labellar taste hairs, specifically electrical resistivity, existed in the two sexes. The electrical resistivity of the hairs was taken into account to estimate the diffusion of ions from the external environment to the dendrite, bearing in mind that the stimulating effectiveness of the ions present in the external environment is obviously related to their diffusion properties. In this respect, the mucopolysaccharidic layer which separates, at the labellar hair tip, the external environment from the dendrite[6,7], may be the important barrier.

To evaluate their electrical resistivity, the hairs were bathed in 2 groups of equiconductive solutions: the former consisted of alkaline chlorides, the latter of alkaline-earth chlorides, all of them exerting a stimulating action. The former group comprized a 0.4 M NaCl solution and KCl and LiCl solutions equiconductive with the NaCl one; the latter a 0.4 M $MgCl_2$ solution, and $CaCl_2$ and $BaCl_2$ solutions equiconductive with the $MgCl_2$ one. The equiconductive solutions were experimentally prepared by adding adequate amounts of the given salts and by measuring their conductivity by means of a Kohlrausch bridge. The resistivity measurements were performed on labellar taste hairs of *Phormia* (6–8 days old) according to the method described by Stürckow[8]. Only the first 2–3 long rostral aboral labellar hairs, following the classification of Den Otter[9], were taken into account. Either group of equiconductive solutions was applied to each hair in a random sequence. The results in table 1 show that the resistivity of labellar taste hairs bathed in NaCl, KCl or LiCl equiconductive solutions is higher in males than in females. The differences were statistically significant.

The same results were obtained by bathing the hairs in