The X is a subtelocentric chromosome of middle size (fig. 1, d). This description conforms perfectly with the one previously given by B. Kral from Tadjikistan[13]. Besides, karyotypes of *R. rattoides* were studied in India, Nepal and Afghanistan. They differ in the number of subtelocentric autosomes: 4 in India and Nepal, 7 in Afghanistan[14,15]. Such differences in chromosome morphology seem to be due to pericentric inversions.

A similarity in chromosome morphology and polymorphism by pericentric inversions in a number of homologous pairs of autosomes of the species of the subgenus *Rattus* mentioned above testifies to their common origin. Cytogenetic comparison between species of this subgenus substantiates the leading role of Robertsonian rearrangements (fusions-fissions) and pericentric inversions in the evolution of their karyotypes.

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Inhibition of tumor-induced angiogenesis and of tumor growth by activated lymphocytes

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Summary. Inhibition in angiogenesis (neo-vascularization) and of growth of a tumor piece graft in the anterior chamber of the eye of mice have been observed in the presence of activated syngeneic lymphocytes.

It has been shown that when a piece of a malignant tumor is placed in the cornea of a rabbit, it induces vaso-proliferation from limbal vessels towards the tumor graft[2]. This phenomenon is known as tumor-induced angiogenesis (TIA). Auerbach and his co-workers have shown how allogeneic lymphocytes can induce angiogenesis (LIA) at a s.c. site[3] and in the cornea of adult mice[4]. In this study, we observed that syngeneic lymphocytes, activated with a polyclonal stimulator like concanavalin A, were capable of inhibiting vasoproliferation and growth of the tumor graft when a piece of mesenteric lymph node containing activated lymphocytes was placed along with a tumor graft in the anterior chamber of eye of a mouse.

The anterior chamber of the eye of a mouse, instead of an intra-corneal pocket, was chosen as a site of grafting for convenience of placing the bigger mass of 2 grafts, and the site is known to favor growth of a graft[5] including foreign antigen-bearing implants. Moreover, we observed similar type of vascular reactions when the tumor graft was placed in the cornea or in the anterior eye chamber; only a difference in the degree of vaso-dilatation was noted during the first 3–4 days, slightly higher in the case of grafting into the cornea.

Male albino Swiss mice of 8–12 weeks of age were used throughout the study. They were obtained from Indian Institute of Experimental Medicine, Calcutta, and from colonies maintained in our Center with pellet food of Hindusthan Lever Ltd, Bombay, and water ad libitum.

Tumor pieces for grafting were obtained from rapidly growing tumors (diameter, 2 cm^2 or over) in Swiss mice,

Degree of different reactions and occurrence of tumor in the presence of different combinations of grafts in the anterior eye chamber of mice

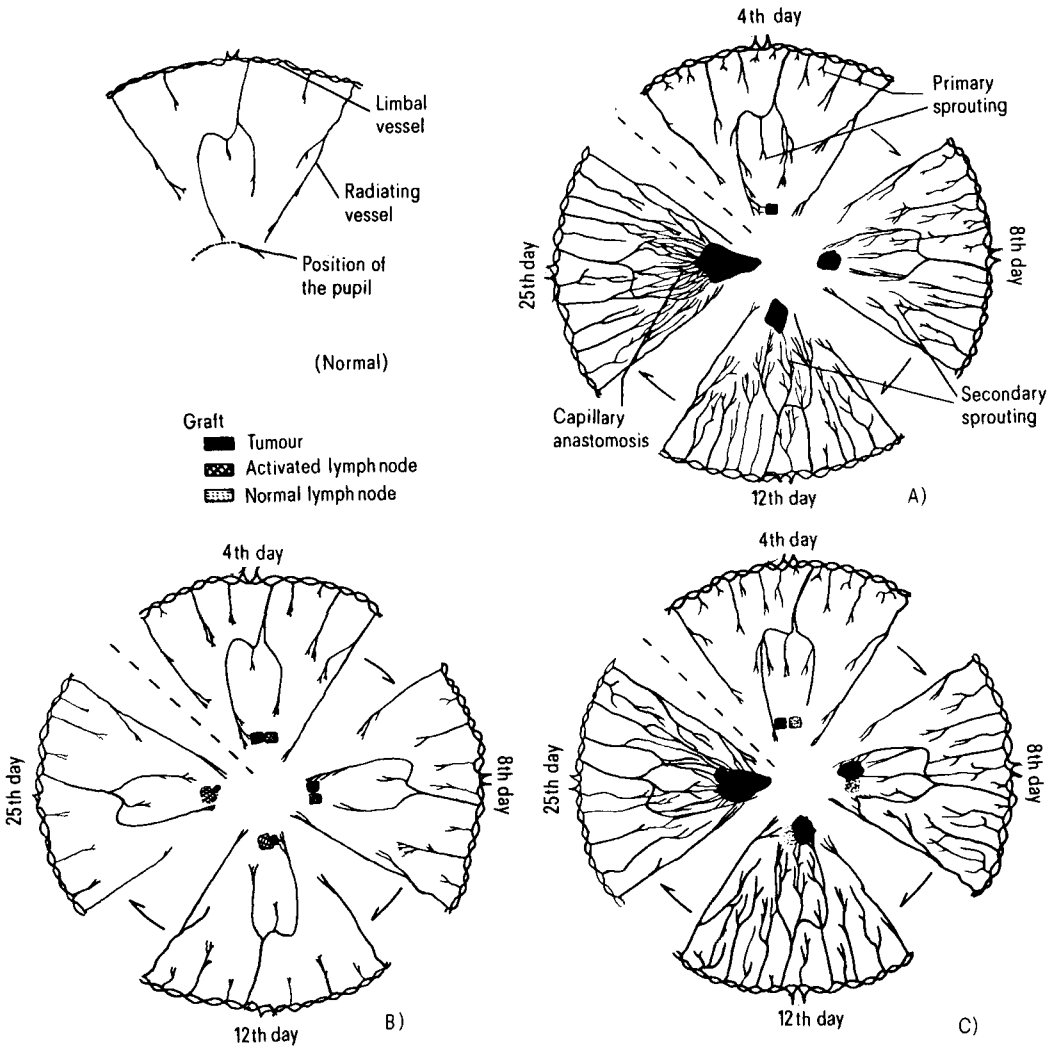
Group	Type of grafts	No. of animals	Degree of different reactions (percentage of cases with initial reaction)* Vasodilatation Days after grafting 4	8	12	25	Neo-vascular sprouting Primary 4	8	Secondary 12	25	Growth of grafts 4	8	12	25	Percentage of tumor occurrence
A	Tumor only	27	++ (88)	+++	++	+	++ (88)	++	++ (81)	+	+ (85)	++	++	++	70
B	Tumor + stimulated lymph node	25	+ (60)	±	±	−	+ (56)	±	− (84)	−	± (76)	±	±	−	12
C	Tumor + normal lymph node	21	++ (90)	+++	++	+	++ (80)	++	+ (71)	±	+ (90)	++	+	+	57

*The percentages (in parentheses) have been calculated in reference to the reaction on the 4th day in all cases except for secondary neo-vascular sprouting, where the reaction on the 12th day has been considered.

induced by injecting s.c. 0.5 mg of 20′ methylcholanthrene (Sigma Chemical Co., St. Louis) per animal. About 0.5 mm^3 sized tumor pieces were used for implantation.

Polyclonally stimulated lymph node pieces were obtained from mesenteric lymph node of Swiss mice injected 48 h earlier with 50 μg Con A (Sigma Chemical Co.) per animal. This dose of Con A was found optimal for lymphocytes to transform into blasts in vivo[6]. Non-stimulated lymph node pieces for control experiments were collected from normal Swiss mice. Lymph nodes were harvested aseptically in cold PBS and cut into pieces of approximately 0.5 mm^3 size. An incision of about 2 mm was made with a surgical blade over the cornea between the pupil and circular limbal vessels in an anesthetized mouse. The grafts were inserted into the anterior chamber of the eye with the help of a bent fine oral pipette and a small tip malleable spatula and positioned near to the pupil by applying pressure on the external surface of the cornea. No obvious post implantation infection was observed.

Grafts in 3 different combinations were placed into the anterior eye chamber of mice: A) a single tumor piece, B) a tumor piece plus a Con A stimulated lymph node piece and C) a tumor piece along with a normal lymph node piece. Reactions induced by the grafts have been enumerated in the table and diagrammatically represented in the figure. Neo-vascular reactions indicate the increase in diameter of circular limbal vessels and radiating corneal vessels, sprouting of new vessels and secondary sproutings from the primary loops. In the case of tumor plus activated lymph node, initial reactions of vasodilatation and primary sprouting of blood vessels occurred, but were feeble in comparison to those in the other 2 groups. Secondary sprouting of blood vessels from primary loops and radiating vessels and a notable growth of the tumor piece were not observed in this group. All these reactions were prominent in the other 2 groups – tumor alone and tumor plus non-stimulated (normal) lymph node. Control experiments by implanting a normal lymph node piece only and a piece of activated



Diagrammatic representation of changes in angiogenesis and growth of the tumor grafts in the anterior eye chamber of mice. Chronological changes in different groups are indicated by the segments arranged in a clockwise fashion. (Thickness of blood vessels indicates different degrees of vasodilatation. Different types of grafts are indicated by different shades as indexed in the middle of the figure.) Normal, showing arrangements of circular limbal and radiating corneal vessels in a normal eye without any graft. *A* Eye with tumor graft only – shows gradual thickening of blood vessels, primary sprouting of blood vessels on day 4, secondary sprouting on day 8 and capillary anastomosis on day 25 over the bulged out graft. *B* Eye with tumor graft plus stimulated lymph node piece showing feeble neo-vascular reactions (angiogenesis) and no tumor growth. *C* Eye with tumor graft with non-stimulated lymph node piece showing reactions similar to that of A.

lymph node alone were also performed and no obvious angiogenesis was observed in both the cases (data not presented).

Incidences of growth of the tumor piece graft into a bigger tumor which often bulged out of the limit of eye, have been registered in the table as percentages of occurrence of tumors. This index was lower in group B than in 2 other groups. Angiogenesis and tumor growth have been thought to depend on each other[7]. Possibly, this phenomenon may be due to lesser vasoproliferation causing inhibition of growth of the tumor in the case of grafting of the tumor plus a stimulated lymph node piece.

Several suggestions may be put forward to explain the inhibition of angiogenesis and then tumor growth. It has been shown that the phenomenon of angiogenesis is mediated by tumor-angiogenesis factor (TAF). released from the tumor graft[2]. Thus it seems possible the factor(s) released by the activated lymphocytes might neutralize TAF or affect the production of it. It is also conceivable that Con A stimulated lymphocytes mount a cell-mediated cytolytic reaction against the tumor cells and, in consequence, hinder the production of TAF and growth of the tumor graft. The later possibility might be supported with some evidences from previous work. Several authors have demonstrated the activation of cytotoxic functions in mouse thymocytes by lectins, particularly Con A. Although this substance is a polyclonal stimulator, it has been shown that Con A-mediated cytotoxicity is expressed through antigen-specific membrance receptors in unprimed[8-10] and primed lymphocytes[11,12] against a variety of targets, including tumor cells.

Activated lymphocytes were obtained from lymph node pieces from an animal injected earlier with Con A. We have shown elsewhere[6] that Con A is capable of inducing blast transformation in lymphocytes in vivo; differentiation of lymphocytes into blasts is considered as an index of activation. Preliminary experiments indicate that these in vivo transformed cells are capable of mounting cytotoxic reactions (to be published elsewhere). Modulation of tumor induced angiogenesis and growth of a tumor graft in the presence of polyclonally-activated lymphocytes suggests an interesting model for probing immune responses against neoplasms.

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Triiodothyronine and tetraiodothyronine in human semen prior to, and following, treatment with thyroid extracts

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Summary. The levels of thriiodothyronine and tetraiodothyronine were measured in blood and semen of normozoospermic, oligozoospermic, and azoospermic men, prior to, and following, treatment with thyroid extracts. The semen, hormones and sperm quality were not affected by treatment.

Some evidence indicates that in some animal species normal blood levels of thyroid hormones are essential for reproduction[1-3] and during a considerable period thyroid hormones were occasionally used in the treatment of infertile men and women[4-6]. Although this therapeutic approach has recently been abandoned we decided to carry out a study to determine whether the administration of thyroid extracts affects the levels of triiodothyronine (T_3) and tetraiodothyronine (T_4) in the semen and/or the quality of the sperm.

The subjects studied were 40 clinically euthyroid patients aged from 25 to 45 years. 5 were azoospermic, 24 oligozoospermic (sperm density, $1.0\text{-}50\times10^6$/ml; % motility, 45.6 ± 2.6 (SE); motility grade, 1.9 ± 0.1; % viability, 58.1 ± 2.6; % normal morphology, 25.9 ± 1.5) and 11 normozoospermic (sperm density, $>50\times10^6$/ml; % motility, 58.5 ± 3.1; motility grade, 2.6 ± 0.01; % viability, 64.5 ± 2.4; % normal morphology, 46.6 ± 3.3). The sperm count was made with a Neubauer camera. Motility was assessed according to Cockett et al.[7] and viability by the eosin-nigrosin test. Morphology was determined from smears stained by the Papanicolaou technique. Levels of T_4 and T_3 in blood and seminal plasma (obtained by centrifugation of semen at 15,000 rpm for 30 min) were determined by radioimmunoassay techniques.

In 8 volunteers (3 of them azoospermic and 5 of them oligozoospermic) treatment was instituted with thyroidin (0.1 g thyroidin (USP) has a biological activity of 0.04 mg T_3 and 0.16 mg T_4), given at a daily dose of 0.1 g for 4 weeks and then 0.2 g for an additional 2 weeks. Blood and semen samples were obtained for repeated determination of T_3 and T_4 levels and assessment of the andrological parameters after 4 weeks and again 2 weeks later.

The table presents the blood and semen levels of thyroid hormones in the untreated subjects. Although blood levels of T_3 and T_4 were within the normal range the values of T_3 tended to be lower in specimens from azoospermic subjects and those of T_4 were somewhat lower in specimens from both azoospermic and oligozoospermic subjects than they were in specimens from normozoospermic subjects. The