from the incubation mixture and transferred into 100 µl of 10% TCA for assaying the cGMP level (for standardization of the assay conditions, 7.5 µl of ACh or A23187 solution respectively were later added). Immediately after that, ACh or A23187 solution was added. After 0.5, 1, 2, 3, 4 min, or 5 min if A23187 was used, samples (volume 60 µl) were taken and transferred into 100 µl of 10% TCA; they were then homogenized and the precipitated protein separated by centrifugation. The supernatant was used to measure cGMP concentration and the pellet was assayed for protein. Radioimmunoassay kits for cGMP assay were from New England Nuclear. For obtaining the exact values of cGMP concentration the reagent blank was subtracted. The data represent the means of triplicate incubations, each of which was assayed for cGMP in duplicate.

Results and discussion. The specific binding of [^{3}H]QNB to the chick blastoderm homogenate was $19(\pm 5)\cdot 10^{-15}$ moles/mg of protein. Calculating the amount of bound [^{3}H]QNB with respect to the cell number, we found that the number of muscarinic receptors is $1.4(\pm 0.4)\cdot 10^4$ per gastrulating chick embryo cell. Unfortunately, the detailed binding analysis of muscarinic receptors in the early chick embryo using a Scatchard plot is not feasible because of the difficulty of preparing the necessary amount of blastoderms.

To examine biochemically the mechanism of ACh action, we studied the ACh responses on dissociated embryo cells in vitro. The studies were based on the observation that ACh acting at muscarinic receptor sites raises tissue levels of cGMP. This effect is blocked by atropine, and requires the presence of calcium in the medium[9-11]. The data obtained for the chick embryo cells are summarized in the table.

ACh increased the cGMP level in dissociated chick embryo cells about two-fold; it reached a maximum between 1 and 3 min after stimulation. This increase was Ca^{2+} dependent; in Ca^{2+}-free Ringer solution the cGMP level, in consequence of ACh stimulation, decreased markedly. The calcium ionophore A23187 caused an increase in cGMP level within 3–5 min in the presence of extracellular calcium.

From the observation that atropine blocked the ACh-stimulated cGMP increase it could be concluded that ACh really acted at muscarinic receptor sites. The receptor stimulation experiments in the medium without Ca^{2+}, and the experiments with calcium ionophore A23187, well support the idea that Ca^{2+} is the muscarinic second messenger[12].

It may be suggested that the muscarinic ACh-receptor is one of the cholinergic characteristics whose presence is indicated by acetylcholinesterase and choline acetyltransferase activity in the pregastrulating and gastrulating chick embryo[13,14]. At the same time, it is interesting that in the spatial distribution of acetylcholinesterase activity differences become clearly apparent during the gastrulation[13,14].

Our observation gives an insight into the problem of how ACh is involved in the early embryogenesis. ACh may control or modify the ionic, especially Ca^{2+}, transport via muscarinic receptors. The stimulation of a receptor can open not only its closely associated ionic channel but also trigger the release of Ca^{2+} through phosphatidylinositol turnover, from stores located inside the cell[15-17]. The regulation of cell cytoskeleton, metabolism and division are all closely connected with Ca^{2+}. The increased cGMP level implies the formation of diacylglycerol which is known as an activator of protein kinase C.

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Position of the Y-chromosome at somatic metaphase in patients with chronic myelogenous leukemia (CML)

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Summary. A random distribution of the Y-chromosome at somatic metaphase was found in 50 patients with Ph' positive chronic myelogenous leukemia (CML). Thus, it is concluded that the 'positive' of the Y-chromosome at somatic metaphase does not appear to influence the loss from bone marrow cells.
Key words. Chronic myelogenous leukemia (CML); Ph'-chromosome; Y-chromosome.

It has been generally accepted that the distribution of human chromosomes at somatic metaphase is not random. For example, acrocentric chromosomes (13–15, 21 and 22) tend to lie closer to each other. The partial concordance between interphase and metaphase supports the idea that chromosome distribution data reflect the organization of the nucleus at interphase[1]. Information on the position of metaphase chromosomes may provide some important clues to the understanding of euploidy, aneuploidy and non-disjunction. Therefore, the loss of the Y-chromosome in a hematopoietic cell line from patients with chronic myelogenous leukemia (CML) deserves particular attention with respect to its position at metaphase. Could the loss of

Position of Y-chromosome at somatic metaphase

Parameters	Location of the Y-chromosome*										
	1	2	3	4	5	6	7	8	9	10	Total
CML:											
Frequency	1	33	44	70	78	90	75	66	43	37	537
Percent	0.186	6.145	8.190	13.035	14.520	16.759	13.966	12.290	8.009	6.890	100
Control:											
Percent	0.452	3.060	8.590	12.050	14.040	16.740	15.670	13.650	7.960	7.790	100
Expected:											
Percent	0.319	4.602	8.390	12.542	14.280	16.749	14.818	12.970	7.984	7.340	100

* for detailed description of these locations (1–10), see Verma et al.[4].

Y-chromosome from metaphase be due to its position in hematopoietic cells? Therefore, we examined the position of the Y-chromosome at metaphase in bone marrow cells from patients with CML and compared it with normal controls.

Materials and methods. 50 male patients whose clinical diagnosis was CML were studied. All had the Ph'-chromosome with the 9q; 22q translocation as determined by QFQ and RFA techniques[2,3]. The chromosome preparations were made from bone marrow aspirates and QFQ cells were photographed on tri-X film (Kodak) using a Zeiss photomicroscope II. At least 20 cells from each individual were initially photographed. Recording of the location of the Y-chromosome was performed directly by enlarging the metaphase onto a circle or square as previously described[4]. The square and circle were divided into 10 equal parts (1–10) at a distance of 1 cm.

The following criteria were introduced to identify the location of the Y as either peripheral or non-peripheral. If the Y-chromosome fell in area No. 10 and there was no other chromosome located further from it, then Y was described as being peripheral. The location of the Y-chromosome was also recorded when it was not on the periphery. Only complete metaphases with 46 chromosomes and those which fitted into the square or circle were included. The logic behind this approach has been described elsewhere[4]. A total of 537 metaphases were included. The control data were taken from an earlier report[4].

Results and discussion. There is a subgroup of patients with Ph'-positive CML (8–10% of male cases) who have been described as having a chromosome constitution of 45 chromosomes[5]. The missing chromosome was the Y, unequivocally identified by fluorescence banding techniques[6]. The mechanism underlying this selective loss, however, remains a mystery. The loss of the Y-chromosome is a phenomenon that appears to be secondary to the induction of the Ph' but may be closely related to it. Nevertheless, the missing Y-chromosome in marrow cells has been postulated to have significance in the prognosis of CML[7]. Does the position of the Y-chromosome and metaphase play any role in loss from somatic cells? The distribution of Y-chromosome at metaphase is recorded (table). The normal distribution of the Y-chromosome was noted when compared to the control value. There was no significant difference from the expected value. Therefore, it is concluded that the position of the Y-chromosome in hematopoietic cells in patients with CML at somatic metaphase is random. The missing Y-chromosome in bone marrow cells may be a normal aging phenomenon. Nevertheless, there is a strong suggestion that a missing Y is a reflection of a basic defect occurring in elderly males in whom the marrow is affected by a hematologic disorder. Thus, the position of the Y-chromosome does not appear to influence loss from bone marrow cells.

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Hematocrit and hemoglobin concentration in four chromosomal species and some isolated populations of actively speciating subterranean mole rats in Israel

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Summary. Hematocrit (HCT) and hemoglobin (Hb) concentration were measured in four chromosomal species and some peripherally semi-isolated and isolated populations of the mole rat superspecies *Spalax ehrenbergi* in Israel. HCT was 52.0, 51.4, 50.9, and 47.8%, and Hb was 16.0, 16.6, 16.3, and 14.7 g/100 ml for 2n = 52, 58, 54, and 60, respectively. The species 2n = 60, which lives in arid habitats, had lower HCT and Hb than the other three species. HCT decreased as aridity increased between the species and within the species 2n = 60. Changes in HCT probably reflect clinal changes in both soil permeability to gases and ambient temperature.
Key words. Blood; aridity; habitat selection; fossoriality.

The fossorial mole rat superspecies *Spalax ehrenbergi* is in the final stages of an active process of speciation and has produced four chromosomal subspecies as it invaded more arid habitats, in the order of 2n = 52, 58, 54, and 60[1,2]. The species 2n = 60 extends furthest into arid regions, where it forms peripherally semi-isolated and isolated border populations[3] and, because of