increase of the intracellular free calcium concentration[11]. Reed[12] suggested a requirement of calcium for plant cytokinesis and reported irregular cell plates with calcium deficiency. This ion seems to play a role in the preservation of the plasma membrane and cell wall as well as in cell wall ATPase activation[13]. Paul and Goff[14] have suggested that caffeine and calcium deficiency interfere with some aspects of membrane recognition and fusion, and it has been confirmed that caffeine interferes with cytokinesis by releasing calcium and magnesium from those sites which are required for regular building of the new cell plate[3]. Adenosine[15], and treatments which increase the cellular ATP level[4] decrease the efficiency of caffeine in inhibiting higher plant cytokinesis, and it has been postulated that caffeine can block cell plate formation by the inhibition of a certain ATPase activity[4] required for cell membrane fusion[16,17].

Hepler[18] suggests that endoplasmic reticulum might regulate the ionic conditions that allow vesicle fusion to occur. Our results show that vanadate itself interferes with plant cytokinesis, thus producing binucleate cells. The role of vanadate in the inhibition of the endoplasmic reticulum calcium-pump[6] induces us to think that vanadate may alter the control of cellular free calcium concentration through the endoplasmic reticulum.

Considering the suggested interference of caffeine with some ATPase activity required for membrane fusion[4,5,19], and the inhibitory effect of vanadate on phosphatase activities[5,9], our results showing inhibition of cell plate formation by vanadate and an additive effect of caffeine and vanadate are fully compatible with the data published. Vanadate and caffeine appear to affect plant cytokinesis at different levels, which confirms their additive effect.

1 Frey-Wyssling, A., Lopez-Sáez, J. F., and Mühlethaler, K., J. Ultrastruct. Res. *10* (1964) 422.
2 Risueño, M. C., Giménez-Martin, G., and Lopez-Sáez, J. F., Exp. Cell Res. *49* (1968) 136.
3 Becerra, J., and Lopez-Sáez, J. F., Exp. Cell Res. *111* (1978) 301.
4 Lopez-Sáez, J. F., Mingo, R., and González-Fernandez, A., Eur. J. Cell Biol. *27* (1982) 185.
5 Dux, L., and Martonosi, A., J. biol. Chem. *258* (1983) 2599.
6 Pick, U., J. biol. Chem. *257* (1982) 6111.
7 Gallagher, S. R., and Leonard, R. T., Pl. Physiol. *70* (1982) 1335.
8 O'Neill, S. D., and Spanswick, R. M., Pl. Physiol. *75* (1984) 586.
9 Binari, L. L., and Racusen, R. H., Pl. Physiol. *71* (1983) 594.
10 Tjio, J. H., and Levan, A., Ann. Est. Exp. Aula Dei *2* (1950) 21.
11 Plattner, H., Cell Biol. int. Rep. *5* (1981) 435.
12 Reed, H. S., Ann. Bot. *21* (1907) 501.
13 Jones, R. G. W., and Lunt, D. R., Bot. Rev. *33* (1967) 407.
14 Paul, D. C., and Goff, D. W., Exp. Cell Res. *78* (1973) 399.
15 González-Fernandez, A., and Lopez-Sáez, J. F., Envir. exp. Bot. *20* (1980) 455.
16 Plattner, H., Reichel, K., and Matt, H., Nature *267* (1977) 702.
17 Poste, G., and Allison, A. C., J. theor. Biol. *32* (1971) 165.
18 Hepler, P. K., Protoplasma *111* (1982) 121.
19 Becerra, J., and Lopez-Sáez, J. F., in: The Role of Calcium in Biological Systems, p. 241. Eds L. J. Anghileri and A. M. Tuffet-Anghileri. CRC Press, Boca Raton 1982.

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Acetylcholine receptors in the gastrulating chick embryo

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Summary. Muscarinic acetylcholine receptors are present in the gastrulating chick embryo.
Key words. Chick embryo; gastrulation; muscarinic acetylcholine receptors; cGMP.

Studies on the embryos of various organisms have indicated the possible regulatory role of acetylcholine (ACh) in the early stages of development[1-4]. However, the mechanism of ACh action in the early embryogenesis has not been investigated yet. The observation that atropine, a muscarinic antagonist, prevents the normal process of chick gastrulation[3], implies the existence of muscarinic ACh-receptors in gastrulating chick embryos. Using the [^{3}H]QNB binding assay and biochemical study of the ACh responses in vitro, we demonstrate here the presence of muscarinic ACh-receptors in the gastrulating chick embryo.

Materials and methods. Binding assay. White Leghorn embryos (area pellucida only), at the stage of development 4 HH (Hamburger and Hamilton)[5], were dissociated in Ca-Mg free Ringer solution with 0.2 mM EDTA, and the number of cells counted. Cells were homogenized in 0.05 M sodium-potassium phosphate buffer, pH 7.4, using a conical glass-to-glass ice-cooled microhomogenizer. Protein was determined by the method of Lowry et al.[6]. The binding assay was performed according to Yamamura and Snyder with slight variations[7]. The incubation volume of each sample was 1 ml, containing 200 µl of the homogenate. The specific binding of [^{3}H]QNB (Amersham, sp. act. 32 Ci/mmol) was determined from the difference of binding in the absence and presence of 10 µM atropine. The [^{3}H]QNB concentration used was 2 nM; this was assumed to be a saturating concentration, as shown by a previous study of muscarinic receptors in chick limb buds[8]. The incubation was terminated by rapid filtering of the suspension through a glass fiber filter (GF/B). The filters were washed four times with 5 ml of the ice-cold sodium-potassium buffer, and immersed in 10 ml dioxane-naphthalene scintillation mixture. Radioactivity was counted after 24 h in a liquid scintillation counter ('RackBeta', LKB).

cGMP assay. In each experiment, about 30–40 chick embryos (area pellucida only) at stage 4 were dissociated in 1 ml Ca-Mg-free Ringer solution with 0.2 mM EDTA. The dissociated embryo cells were preincubated at 37°C for 1 h in 180 µl Ringer bicarbonate buffer (pH 7.4). The cells were further incubated for 5 min in the presence of 0.1 mM IBMX and in the presence or absence of 1 mM atropine. A control sample (52.5 µl) was taken

Effects of various agents on levels of cGMP in dissociated chick embryo cells at stage 4 (HH)

Conditions	Percent of control ± SE
Acetylcholine (500 µM)	224 ± 43
Acetylcholine (500 µM) + atropine (1 mM)	120 ± 10
Acetylcholine (500 µM) in Ca^{2+}-free medium	30 ± 15
A23187 (10 µg/ml)	170 ± 30

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.

1 Buznikov, G.A., Kost, A.N., Kucherova, N.F., Mndzoyan, A.L., Suvorov, N.N., and Berdysheva, L.V., J. Embryol. exp. Morph. *23* (1970) 549.
2 Gustafson, T., and Toneby, M., Exp. Cell Res. *62* (1970) 102.
3 Penner, P.L., Acetylcholinesterase and cell movement in the epiblast of the primitive streak chick embryo. Doctoral thesis. New York University, New York 1977.
4 Soliman, S., Comp. Biochem. Physiol. *74 C* (1983) 397.
5 Hamburger, V., and Hamilton, H.L., J. Morph. *88* (1951) 49.
6 Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J., J. biol. Chem. *193* (1951) 256.
7 Yamamura, H.I., and Snyder, S.H., Proc. natn. Acad. Sci. USA *71* (1974) 1725.
8 Schmidt, H., Histochemistry *71* (1981) 89.
9 Goldberg, N.D., and Haddox, M.K., A. Rev. Biochem. *46* (1977) 823.
10 Bartfai, T., Study, R.E., and Greengard, P., in: Cholinergic Mechanisms and Psychopharmacology, p. 285. Ed. D.E. Jenden. Plenum Press, New York 1977.
11 Ohsako, S., and Deguchi, T., Biochem. biophys. Res. Commun. *122* (1984) 333.
12 Schultz, G., Hardman, J.G., Schultz, K., Baird, C.E., and Sutherland, E.W., Proc. natn. Acad. Sci. USA *70* (1973) 3889.
13 Drews, U., Kussähter, E., and Usadel, K., Histochemie *8* (1967) 65.
14 Laasberg, T., and Neuman, T., W. Roux's Arch. devl. Biol. *194* (1985) 306.
15 Michell, R.H., in: Cellular Receptors for Hormones and Neurotransmitters, p. 353. Eds D. Schulster and A. Levitzki. John Wiley & Sons, London 1980.
16 Berridge, M.J., and Irvine, R.F., Nature *312* (1984) 315.
17 Burgess, G.M., Godfrey, P.P., McKinney, J.S., Berridge, M.J., Irvine, R.F., and Putney, J.W. Jr, Nature *309* (1984) 63.

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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