Table 1. Parameters of intracellular motility in *P. tetraurelia*. Values are means ± standard errors. Abbreviation: N.A., not applicable to cyclosis.

Observation	Cells observed	Observations	Mean velocity during movement (μm/sec)	Mean displacement (μm)	Saltatory index
Saltatory motility					
Trichocysts	13	42	0.82 ± 0.08	3.0 ± 0.27	0.25 (32/127)
Mitochondria	8	29	1.2 ± 0.13	2.6 ± 0.41	0.37 (70/191)
Cellular cyclosis	12	142	2.6 ± 0.10	N.A.	N.A.

the two organelles could reflect independence in motility and could be based on different motility mechanisms or on different responses to a common force-generating mechanism.

The characteristics of saltatory motion of uninserted trichocysts are noteworthy. These movements are generally parallel to the cortex in this system, and individual saltations of 10 μm and more have been recorded. A trichocyst moves either tip first or body first—that is, with the long axis of the organelle parallel to the direction of movement (Fig. 2). A streamlined mode of movement is consistent: trichocysts have not been observed to saltate sideways. This characteristic of trichocyst saltatory motility is in marked contrast to cyclotic transport of trichocysts, in which the organelle is carried with random orientation of its long axis to the direction of cyclotic flow. If a change in direction is made between successive saltations, it is accomplished by a rotational reorientation of the trichocyst before the second jump. These activities give the observer the impression that the trichocyst is being "pulled" during its saltations.

The concept of saltatory motility of mitochondria and uninserted trichocysts has intriguing implications and gives added significance to the understanding of some of the cellular events of *P. tetraurelia*. Preliminary results (*10*) have indicated that two mutant cell lines which display a phenotype of mature, but uninserted, trichocysts (*11*) are incapable of transporting their trichocysts by saltatory motion. Observations of trichocyst replacement in the cortex (*12*) following massive trichocyst extrusion stimulated by electroshock (*13*) indicate that the motility shown by new trichocysts being transported to the cortex for insertion is saltatory. The parameters of trichocyst motility during cortical insertion (that is, velocity and displacement) are statistically indistinguishable from the trichocyst motility reported here (Table 1) in survey observations. Therefore, saltatory motion of trichocysts appears to be essential for their insertion into the cortex.

Perasso and Adoutte (*14*) found that the distribution of genetically marked mitochondria after transfer to an unmarked host cell is extremely rapid. Since many of the mitochondria in *P. tetraurelia* are located in the cortex (*6, 7*) and thus away from the cyclotic regions of the cell (*8*), it may be reasonable to suggest that mitochondrial saltatory motility could be a significant factor in the rapid intracellular mixing of mitochondria.

The observations described above suggest that subcortical saltatory motion may be involved in the distribution of uninserted trichocysts and of mitochondria in the noncyclotic, cortical regions of the cell. However, the apparent direct involvement of saltatory motion in transporting the uninserted trichocyst to the cortex for insertion and the genetic correlation between cortical insertion and saltatory motility of trichocysts, as mentioned above, suggest that saltatory motion is an important step in the functional development of the trichocyst. Saltation appears to be the type of motility displayed by the newly assembled trichocyst on its way from the site of assembly in the cytoplasm (*13*) to the cell surface for insertion. This would implicate saltatory motion in the development and maintenance of the normal cellular phenotype of *P. tetraurelia*: the insertion of approximately 4000 trichocysts in the cortex. In addition to paramecia, many other cell systems exhibit the use of saltatory motility in the intracellular transport and localization of various inclusions, such as the cortical localization of echinochrome granules in sea urchin eggs following fertilization, and the axoplasmic transport of materials such as neurotransmitters essential for the proper function of the neuron (*1, 15*). Saltatory motility would thus appear to be an important element in the process of intracellular morphogenesis in eukaryotic cells.

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References and Notes

1. L. I. Rebhun, *Int. Rev. Cytol.* **32**, 93 (1972).
2. D. Troyer *Nature (London)* **254**, 696 (1975); K. T. Edds, *J. Cell Biol.* **66**, 145 (1975).
3. E. A. Andrews, *Biol. Bull.* **108**, 121 (1955).
4. T. M. Sonneborn, *Methods Cell Physiol.* **4**, 241 (1970).
5. ———, *Symp. Int. Soc. Cell Biol.* **9**, 1 (1970); R. D. Allen, *J. Cell Biol.* **49**, 1 (1971).
6. A. Jurand and G. G. Selman, *The Anatomy of* Paramecium aurelia (Macmillan, London, 1969), pp. 13–28.
7. E. S. Horning, *Aust. J. Exp. Biol. Med. Sci.* **4**, 187 (1927).
8. L. Kúznicki and J. Sikora, *Acta Protozool.* **11**, 237 (1972); *ibid.*, p. 243; J. Sikora, *J. Cell Biol.* **70**, 399a (1976).
9. The saltatory index is defined as the number of particles observed to saltate in 1 minute divided by the total number of particles of the same type, potentially available for saltation in a particular region [R. Roisen, quoted in (*1*)].
10. K. Aufderheide, *J. Protozool.* **23**, 28A (1976).
11. S. Pollack, *ibid.* **21**, 352 (1974).
12. K. Aufderheide, paper presented at the Ciliate Genetics Conference, Madison, Wis., July 1976; *J. Protozool.*, in press.
13. A. Yusa, *J. Protozool.* **10**, 253 (1963).
14. R. Perasso and A. Adoutte, *J. Cell Sci.* **14**, 475 (1974).
15. P. Harris, *J. Cell Biol.* **70**, 112a (1976); M. Berlinrood, S. M. McGee-Russell, R. D. Allen, *J. Cell Sci.* **11**, 875 (1972); A. C. Breuer, C. N. Christian, M. Henkart, P. G. Nelson, *J. Cell Biol.* **65**, 562 (1975).
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Gonadotropin-Releasing Hormone in Milk

Abstract. *The hypothalamic hormone gonadotropin-releasing hormone (GnRH) has been found in milk of man, cow, and rat. Radioimmunoassays of acidified milk indicate concentrations of GnRH ranging between 0.1 and 3 nanograms per milliliter. Multistep extractions, followed by electrophoresis, reveal gonadotropin-releasing activity in the fraction that comigrates with the GnRH-marker. A second hypothalamic hormone, thyrotropin-releasing hormone, is present in milk at a much lower concentration. "Milk-GnRH" may influence the secretion of the gonadotropic hormones in neonates.*

The gonadotropin-releasing hormone (GnRH), which activates gonadal function by promoting gonadotropin secretion from the anterior pituitary, is present in minute amounts in the hypothalamus [approximately 100 ng and 5 ng in human and rat, respectively (*1*)]. Measurement of the peptide in the peripheral circulation has presented many difficulties (*2*) and only elaborate extrica-

Table 1. Scheme of extraction of GnRH from milk.

Step	Brief description	Recovery of 1 μc of [^{3}H]GnRH (%)
1	Lyophylized skim milk (4.5 liters) or 400 g of powdered milk was stirred with 3.5 liters of acetic acid (2*N*) in methanol for 48 hours at room temperature	100
2	Filtration through Whatman No. 1 filter paper, evaporation of solvent under vacuum, and extraction of residue with 200 ml of acetic acid in methanol	99
3	Filtration, evaporation, and extraction in methanol (200 ml)	
4	Filtration of suspension and evaporation. Residue redissolved in 250 ml of acetic acid (1*N*)	
5	Three extractions with ether (discarded); acetic acid phase evaporated	98
6	Residue redissolved in 400 ml of water and ultrafiltrated through Amicon UM 05 membrane*	
7	Residue (40 ml) lyophylized and redissolved in 10 ml of acetic acid in methanol	85
8	Electrophoresis of the material at *p*H 3.5 for 10 minutes at 10 volt/cm and 60 minutes at 60 volt/cm; extraction of area containing radioactive GnRH marker with acetic acid (1*N*) and removal of solvent by evaporation under reduced pressure	10

*Ultrafiltrate was processed like the residue through steps 7 and 8 and checked for TRH.

tions of plasma from the hypothalamo-hypophyseal portal system of the rat have yielded meaningful results: less than 30 pg/ml on diestrus and 115 ± 26 pg/ml on proestrus (*3*). We have sought GnRH in other biological fluids such as cerebrospinal fluid—which was found to be devoid of the hormone in normal human subjects—and in milk.

GnRH was first detected in pasteurized cows' milk previously acidified to *p*H 3. Amounts measured by radioimmunoassay ranged between 0.5 and 3 ng/ml. As serum proteins have been reported to interfere with GnRH radioimmunoassay (*2*), chemical identification of the GnRH-like material was attempted.

Gonadotropin-releasing hormone labeled with tritiated pyroglutamate (1 μc, New England Nuclear; 23.5 c/mmole) was added to powdered milk (Four Cows, Dutch Domo-Bedum, Beilen; 400 g, equivalent to 3 liters) or to pasteurized skim milk (Tnuva; 4.5 liters), and GnRH was extracted as shown in Table 1. The area of the electrophoresis paper encompassing the radioactive GnRH marker (Step 8) was extracted; its GnRH content was determined by radioimmunoassay and its gonadotropin-releasing activity assessed by bioassay in vitro. Radioimmunoassay of this electrophoretic fraction indicated the presence of 670 ng of GnRH. If one considers the 10 percent recovery of the labeled hormone (Table 1) and the amount of starting material (4.5 liters), this value corresponds to 1.5 ng of GnRH per milliliter of milk; powdered milk was found to contain a similar amount of GnRH. "Milk-GnRH" possesses the inherent biological property of the native hypothalamic hormone, that is, induction of the release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) (Table 2). Moreover, exposure to antiserum directed against the synthetic peptide (*4*) markedly reduces the biological activity of milk-GnRH, which denotes its immunological similarity to the native hormone. A second hypothalamic peptide, thyrotropin-releasing hormone (TRH), is present in milk at a much lower concentration (approximately 1/20 of that of GnRH), although the hypothalamus contains as much TRH as GnRH (*1*).

Table 2. Effect of milk-GnRH on gonadotropin secretion from rat pituitaries in vitro. Pituitaries from 12-day-old male rats were incubated for 90 minutes in 1 ml of Krebs-Ringer bicarbonate medium (*p*H 7.4) containing 1 mg of glucose per milliliter (*10*) and the agents listed. Hormone release was determined by bioassay (*11*).

Treatment	Hormone release (μg/ml)	
	LH	FSH
Control	0.42 ± 0.04*	0.42 ± 0.07
Synthetic GnRH (1 ng)	2.97 ± 0.15†	1.55 ± 0.06†
Milk-GnRH (1 ng)‡	3.75 ± 0.32†	2.76 ± 0.19†
Milk-GnRH plus antiserum§	1.42 ± 0.25‖	0.83 ± 0.22‖

*Mean ± standard error for 12 to 20 determinations of LH and six of FSH. †Significantly different ($P < .01$) from control (Student's *t*-test). ‡As determined by radioimmunoassay after electrophoretic separation. §Prior preincubation overnight with 20 μl of antiserum to GnRH. ‖Significantly different ($P < .01$) from milk-GnRH (Student's *t*-test).

Milk-GnRH may have a physiological role in inducing the elevated gonadotropin levels found in serums of human and rat neonates (*5*). When suckling of female pups is prevented, by separating them from their mothers for 3 hours, the concentration of LH in their serum is significantly lower than that of their suckling littermates (34.4 ± 2.9 as opposed to 125.0 ± 24.7 ng/ml; $P < .001$). This suggests that some of the milk-GnRH is absorbed from the gut of the immature rat in a biologically active form. The assumption that milk is the source of this gonadotropin-releasing activity is strengthened by the fact that in the rat, at least, the neonate pituitary is not yet functionally linked to the hypothalamic source of GnRH (*6*). Furthermore, the hypothalamus itself in this species undergoes sex-differentiation only during the first week of life, and milk-GnRH may be involved in the masculinization of the male hypothalamus by causing the release of testicular steroids which act on the brain (*7*). In the female rat, the ovary does not respond to gonadotropins during the first week of life (*8*).

The presence of considerable amounts of GnRH in milk raises not only the question of its role there but also of its origin. Somatostatin, a so-called hypothalamic hormone, has been found also in the pancreas and is assumed to be synthesized there (*9*). The high concentration of GnRH in milk, which greatly exceeds that in serum, implies either an active concentrating mechanism in the mammary gland or an additional, extrahypothalamic origin for this peptide.

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References and Notes

1. E. Okon and Y. Koch, *Nature (London)* **263**, 345 (1976); Y. Koch and T. Baram, *FEBS Lett.* **67**, 186 (1976).
2. L. E. Clemens, R. P. Kelch, M. Markovs, M. H. Westhoff, W. C. Dermody, *J. Clin. Endocrinol. Metab.* **41**, 1058 (1975).
3. D. K. Sarkar, S. A. Chiappa, G. Fink, *Nature (London)* **264**, 461 (1976).
4. Y. Koch, M. Wilchek, M. Fridkin, P. Chobsieng, U. Zor, H. R. Lindner, *Biochem. Biophys. Res. Commun.* **55**, 616 (1973).

5. J. S. D. Winter, C. Faiman, W. C. Hebron, A. V. Prasad, F. I. Reyes, *J. Clin. Endocrinol. Metab.* **40**, 545 (1975); K. D. Döhler and W. Wuttke, *Endocrinology* **97**, 898 (1975).
6. R. St. J. Glydon, *J. Anat. (London)* **91**, 237 (1957).
7. R. W. Goy, in *The Neurosciences, Second Study Program*, F. O. Schmidt, Ed. (Rockefeller Univ. Press, New York, 1970), pp. 196–206.
8. S. A. Lamprecht, F. Kohen, J. Ausher, U. Zor, H. R. Lindner, *J. Endocrinol* **68**, 343 (1976).
9. M. P. Dubois, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 1340 (1975).
10. Y. Koch, R. Meidan, P. Chobsieng, Z. Naor, *J. Reprod. Fertil.* **50**, 347 (1977).
11. The release of LH and FSH was determined by radioimmunoassay, with kits provided by the National Institute of Arthritis, Metabolism and Digestive Diseases Rat Pituitary Hormone Program. Results are expressed in terms of the RP-1 reference preparations.
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Arteriosclerosis: Is Stress-Induced Immune Suppression a Risk Factor?

Abstract. *Female Sprague-Dawley rats, purchased as retired breeders, developed arteriosclerosis that was accompanied by immune complex deposition in the arterial lesion and depressed immune responsiveness to T cell mitogens.*

Repeatedly bred female Sprague-Dawley rats develop a spontaneous arteriosclerotic condition characterized by medial necrosis and calcification, endothelial proliferation, thymus involution, and accelerated aging (*1*). These lesions, similar to those observed in humans, occur in 80 to 90 percent of the rats that have produced three or more litters (approximately ten pups per litter). The lesions begin in the lower abdominal aorta and are found throughout the body in many instances. Although hormones of the hypothalamic-pituitary-adrenal axis, diet, and other factors have been implicated in the pathogenesis of this disorder (*2*), an involvement of the immune system is also a possibility. Other researchers have investigated stress-induced alteration of the immune system (*3*), but this condition has not been linked to arteriosclerosis.

The animals used in these experiments were female Sprague-Dawley rats, purchased as retired breeders (*4*). Female virgin rats of similar ages, the same strain, and from the same supplier were used as controls. All the rats were fed standard rat chow prepared by ARS Sprague-Dawley laboratories prior to purchase, and in our laboratory they were fed Purina Rat Chow. The animals were given unrestricted access to food and water.

The lesions that developed in these rats appeared to be similar to those seen in serum sickness (*5*) and immune complex disorders (*6*). To characterize these lesions, we prepared frozen sections of aortas from diseased as well as control animals. The sections were stained with peroxidase-labeled rabbit antibody to rat immunoglobulin G (IgG) (*4*, *7*). This procedure demonstrated significant quantities of IgG in the medial layer of the vessel affected with the lesion (Fig. 1); no such deposits appeared in the controls. To determine if the IgG was directed against the constituents of the diseased vessel wall we conducted a hemagglutination assay with glutaraldehyde-fixed sheep red bloods cells (RBC) to which the antigen was covalently bound with *N*-ethyl-*N'*-(dimethylaminopropyl)-carbodiimide hydrochloride. Double diffusion tests in agar were also performed with a homogenized extract of diseased aorta being used as the antigen. Titers of the hemagglutination tests were the same for the serum from the diseased and control animals. All results of the diffusion tests were negative. These findings indicate that the antibody visible in the lesion was not directed against the arterial wall.

The IgG in the medial lesion probably represents deposits of an antigen-antibody complex similar to the immune complex deposits found in various tissues in a number of human conditions of

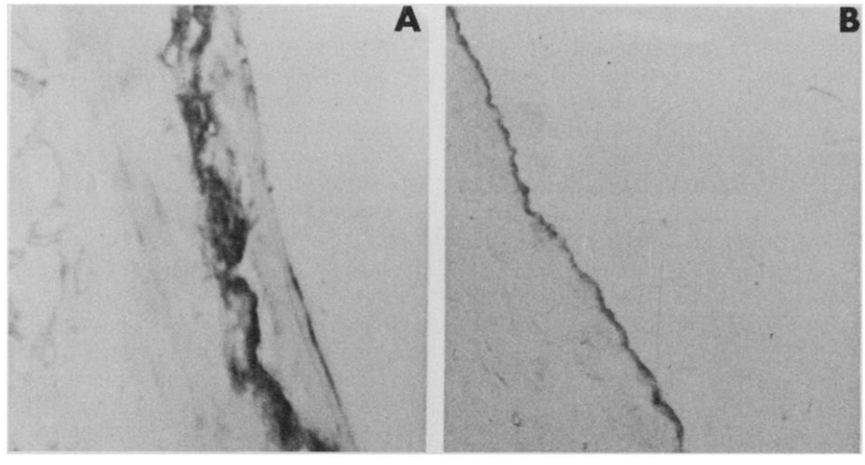

Fig. 1. (A) Immunoglobin G deposits in the medial layer of the abdominal aorta from the arteriosclerotic rat. (B) Normal control animal. (×160)

Table 1. Comparison of the response of spleen cells from arteriosclerotic and control animals to the mitogens PHA, Con A, and LPS, and the response of the spleen cells in the direct plaque-forming cell assay. Abbreviation: Amt., amount.

Amt. (μl/well)	Mitogens (count/min × 10^{-3})								Plaque-forming cells (plaques per 10^6 spleen cells)	
	PHA		Con A			LPS				
	Arteriosclerotic	Control	Amt. (μg/well)	Arteriosclerotic	Control	Amt. (μg/well)	Arteriosclerotic	Control	Arteriosclerotic	Control
0	6.9 ± 1.3	6.6 ± 0.9	0	4.3 ± 1.6	3.8 ± 0.8	0	13.5 ± 1.0	10.1 ± 0.5	219 ± 55	229 ± 67
2	13.6 ± 6.0	22.1 ± 2.4	0.5	8.2 ± 0.9*	74.2 ± 8.9	2	23.8 ± 1.1	17.1 ± 0.5		
4	13.5 ± 2.7*	34.5 ± 3.8	1	12.5 ± 1.3*	77.5 ± 6.1	4	25.0 ± 1.7	18.6 ± 1.0		
6	12.3 ± 2.5*	39.4 ± 3.9	2	12.3 ± 1.6*	59.5 ± 16.7	6	23.3 ± 1.1	16.0 ± 0.2		

*$P < .005$.