The 2 outer electron-dense lines have an average thickness of 60–70 Å. The total length of the intercellular contact increased with the incubation period and after 48 h the cell contacts covered almost the entire membrane area.

If we consider the coupling ratio (V_2/V_1) as a convenient index of the junctional membrane permeability, its increase as a function of the PHA incubation period suggests a concomitant increase in the low-resistance structures responsable for the intercellular permeability. Since in our preparations the areas of close cellular contact broadened with the incubation time, and the only specialized junctional structures which appeared were identified as gap junctions, we have inferred that these structures are identical with the low-resistance pathways in stimulated lymphocytes. Our identification of the contacts as gap junctions is based on: a) the thickness of the junctional complex (200 Å), which is greater than that of the zonula occludens (about 140–150 Å); b) the periodicity of the electron-dense particles of the median line of the junctional profile, which corresponds to the centre-to-centre spacing of the freeze-etch particles previously described in gap junctions[8–10]; c) gaps of about 30 Å between the two adjacent cell membranes.

The exact mechanism involved in lymphocyte cell stimulation is not known, but it is widely accepted that the membrane is the site where the mitogen acts. The dependence of lymphocyte stimulation by PHA on cell density and on cell-to-cell contact, and the appearance of intercellular communication in human and bovine lymphocytes after 12 min of PHA treatment[11], suggest a functional role for the low-resistance pathways. Since cellular associations have been observed to occur both in vivo and in vitro in conjunction with the expression of several immune responses, we suggest that lymphocyte-lymphocyte cooperation through gap junctions provide a model system for the study of such cellular interactions. Lymphocyte junctions have some characteristics in common with those described for epithelial cells. An increase in cytoplasmic Ca^{++} concentration causes a marked reduction of the junctional conductance[12] in a way similar to that previously described for salivary gland cell junctions[13].

In a number of different cell systems where intercellular communication has been detected, it has not been possible to establish a correlation between specific morphological structures and the physiological evidence of cellular communication, because of the coexistence of several different types of specialized junctional complexes. As in several other preparations[4,8,10], in human lymphocytes stimulated by PHA only gap junctions have been found. We suggest that these structures are the low resistance channels responsable for intercellular communication in the present cell system. Studies with extracellular space markers are required for a more definitive identification of these junctional complexes and we are at present working on that[14,15].

Résumé. La microscopie électronique et une méthode électrophysiologique ont été utilisées pour étudier les jonctions entre les lymphocytes humains stimulés par la phytohémaglutinine. Le coefficient de couplage électrotonique augmente avec le temps d'incubation. Les seules intéractions cellulaires typiques mises en évidence par la microscopie électronique sont les «gap jonctions».

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Seasonal Variations in the Fertilizing Capacity of *Bufo arenarum* (Amphibia Anura) Spermatozoa[1]

Artificial fertilization of *Bufo arenarum* oocytes can be routinely obtained throughout the year. This species has a continuous spermatogenic cycle with seasonal fluctuations[2]. Testes size changes considerably during the year, maximal size being observed at the beginning of the breeding season, in the spring. During the winter season, when testes are increasing their size, sperm clusters are observed adhered to Sertoli's cells in the seminiferous ampullae[3]. It is likely that spermatozoa remain in the testes until spring, since they have not been observed in urine samples obtained in winter[4].

The fertilizing ability of amphibian spermatozoa, as far as we know, has not been explored and is the subject of this paper. We also studied the decline of the fertile life of spermatozoa incubated in salt solution both in summer and winter season. A further objective was to establish whether soluble products removed from the testes had any influence on the fertilizing capacity of spermatozoa incubated in it.

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	Incubation time		Incubation media	
	Minutes	Hours	Ringer's solution	Testicular supernatant
Summer	0	—	91.5 ± 2.7	81.6 ± 4.9
	15	—	16.0 ± 10.9	40.2 ± 13.1
	30	—	0.5 ± 0.4	3.3 ± 2.2
Winter	—	0	99.3 ± 0.5	97.8 ± 0.9
	—	1	98.0 ± 1.7	62.8 ± 19.1
	—	2	100.0 ± 0.3[a]	58.0 ± 7.8
	—	4	94.8 ± 2.2[a]	27.3 ± 20.3

Sperm concentration was 10^6 spermatozoa/ml in the incubation medium and 10^5 spermatozoa/ml in the insemination medium (10% Ringer's solution). Results represent the mean ± standard error ($N = 8$ in summer and 4 in winter. Each experiment was carried out with different animals. [a] $p < 0.05$. For the remaining results differences are not significant.

Materials and methods. Bufo arenarum (Hensel) oocytes were obtained by injecting one fresh[5] or preserved[6] homologous hypophysis to the female. Sperm suspensions were obtained by mincing testes in 10% amphibian Ringer's solution without bicarbonate or cell-free testis homogenates. Sperm suspension concentrations were standardized by nephelometry[7].

To obtain a cell-free testis homogenate, a sperm suspension obtained in 10% Ringer's solution of about 2×10^7 sperm/ml was centrifugated at 6,000 *g* during 20 min at 0 °C.

In order to estimate the fertilizing capacity, fertilization rate was measured by inseminating the oocyte strings in 10% Ringer's solution during 15 min, before immersing them in 0.1% sodium lauryl sulfate solution for 5 sec, which insures the block of fertilization without affecting the oocytes[8].

Results and discussion. When *Bufo arenarum* oocytes were inseminated with freshly prepared sperm suspensions, a high frequency of fertilization was obtained, irrespective of the season of the year (Table, incubation in Ringer's at time zero). When spermatozoa were incubated in Ringer's solution at different intervals of time, however, their fertilizing capacity was found to drop off faster in summer than in winter.

The results of the experiments carried out to investigate whether cell-free testes homogenate had any influence on the fertilizing capacity of spermatozoa are also given in the Table. In summer, no significant effect was noticed. In winter, however, spermatozoa incubated in the testicular preparation exhibited a lower fertilization rate than those incubated in Ringer's solution. At present, we do not know the meaning of this effect. This loss of fertilizing ability might reflect only some damage suffered by the cells as a result of incubation under this artificial condition. Acrosomic proteinase contained in cell-free testis preparations[9] may be a good candidate to account for this deleterous effect on spermatozoa. It is also possible that this effect was due to the action of some inhibitor present in the testis, protecting spermatozoa against a premature loss of their fertilizing capacity. Further, this interpretation allows us to explain the difference observed between the fertilizing life of sperm in summer and winter seasons. The inhibitor, being much more active during winter, would be capable of extending the limit of fertile life of sperm even when suspended in Ringer's solution.

The seasonal variation in the decline of fertilizing capacity of sperm here reported, besides its theoretical interest, deserves to be taken into account in future researchs about fertilization and sperm metabolism among Amphibia.

Resumen. La capacidad fecundante de los espermatozoides de *Bufo arenarum* decae más rápidamente en verano que en invierno. Ello probablemente sea debido a la presencia, durante el invierno, de factores testiculares de protección.

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Stimulation of Mg^{2+}-Dependent Endonuclease Activity of Rat Testis Nuclei on Incubation with NAD^+ in vitro

Isolated nuclei and chromatin from mammalian tissue possessed poly (adenosine diphosphate ribose) synthetase activity which transfers the ADP-Rib moiety of NAD to form a polymer[1,2]. Paralleled with the formation of poly (ADP-Rib) the template for DNA synthesis of rat liver chromatin was inhibited[3], resulting from a block of the Ca^{2+}, Mg^{2+}-dependent endonuclease activity[4]. The present study was undertaken to determine the effect of poly (ADP-Rib) formation on the endonuclease activities of rat testis nuclei and chromatin.