	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.

Materials and methods. Adult young male rats (150 g) and immature rats (18 days) were obtained from Holtzman Co., Madison, Wis., and killed by decapitation. The testes were excised and the tunica albuginea incised. Preparation of nuclei and chromatin from rat testis and the assay systems for the determination of the template activity treated with and without NAD were described in previous reports[5,6]. To extract the endonuclease from nuclei, appropriate amounts of isolated nuclei from rat testis were centrifuged at 1000 *g* for 15 min. The sediment was resuspended in 10 ml of a medium containing 75 m*M* *Tris*-HCl (pH 7.4), 5 m*M* $MgCl_2$, 2 m*M* 2-mercaptoethanol and with or without 5 m*M* NAD^+ for 30 min at 25 °C. The mixture was centrifuged at 10,000 *g* for 15 min.

Table I. Poly(adenosine diphosphate ribose) synthetase activity of rat testis chromatin

Chromatin	Poly(ADP-Rib) synthetase activity[a]
18 days	720
Adult	254

[a] Incorporation of [^{3}H]ADP-Rib in cpm/10 μg DNA/15 min.

Table II. Effect of various nucleotides on endonuclease activity of rat testis nuclei

Nucleotides added (5 m*M*)	Endonuclease activities			
	Alkaline		Acid	
	counts/min	%	counts/min	%
control	15,500	100	1,600	100
+ NAD^+	1,600	10	13,750	853
+ Nicotinamide	13,120	82	1,380	86
+ AMP	16,320	102	1,560	98
+ ADP	16,480	103	1,550	97
+ ADP-Rib	13,760	86	1,520	95
+ Acetyl-NAD	18,720	117	2,160	135

Isolated nuclei containing 100 μg of DNA obtained from adult male testis were incubated with each nucleotide for 30 min at 25 °C and assayed for endonuclease activity. The values for endonuclease activity were obtained after 60 min of incubation at 37 °C and expressed as cpm/100 μg of nuclear DNA.

The sediment was resuspended in 3.2 ml of a medium containing 30% glycerol, 50 m*M* *Tris*-HCl (pH 8.0), 1 m*M* EDTA, 2 m*M* 2-mercaptoethanol. To the suspension 0.4 ml of 3.5 *M* NaCl and 0.4 ml of 250 m*M* EDTA were added. The mixture was centrifuged and allowed to stand in the cold overnight and centrifuged at 10,000 *g* for 15 min. The supernatant was dialyzed against 5 l of a medium containing 30% glycerol, 2 m*M* 2-mercaptoethanol, 50 m*M* *Tris*-HCl (pH 8.0), 1 m*M* EDTA. The retentate was centrifuged and supernatant assayed for endonuclease activity.

Methods of preparation of [^{3}H]DNA from *E. coli* K38, [^{3}H]DNA gel and the assay systems for the determination of Ca^{2+}, Mg^{2+}-dependent and Mg^{2+}-dependent endonuclease were described in previous reports[7,8]. The endonuclease assay mixtures were incubated at 37 °C for 15, 30 and 60 min. The reaction was stopped by placing the tubes in ice and by the addition of 200 μl of 20 m*M* EDTA (pH 8.0). The mixture was centrifuged at 1000 *g* for 2 min. Radioactivity in 200 μl of the supernatant was measured in a Packard Liquid Scintillation Spectrometer with the use of Aquasol as scintillator.

Protein and DNA were determined by the methods of LOWRY et al.[9] and BURTON[10], respectively. Assay for poly (ADP-Rib) synthetase activity was described in a previous report[5].

Results and discussion. Poly(ADP-Rib) synthetase activity in chromatin prepared from immature rat testis was higher than that associated with adult rat testis chromatin (Table I). Since the template for DNA synthesis

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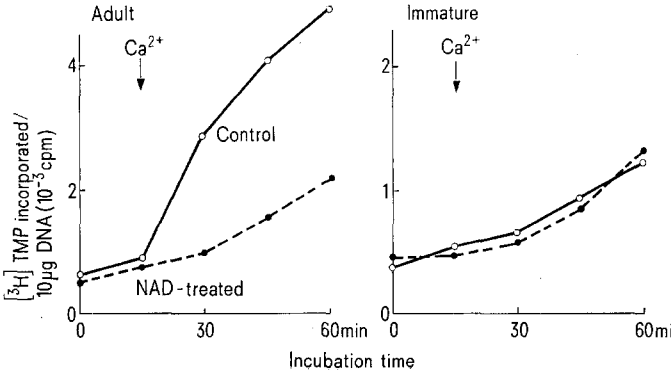

Fig. 1. Effect of ADP-ribosylation on the activation of the template of chromatin obtained from adult and immature rat testis for DNA synthesis.

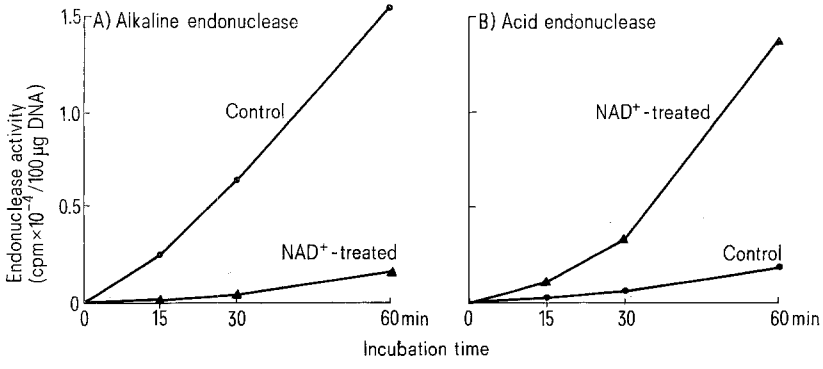

Fig. 2. Effect of ADP-ribosylation on the activities of Ca^{2+}, Mg^{2+}-dependent (A) and Mg^{2+}-dependent endonuclease (B) of adult rat testis nuclei.

of rat liver nuclei and chromatin treated with NAD^+ was inhibited[4], the effect of poly(ADP-Rib) on the template activity was investigated. Ca^{2+} was added to the reaction mixture to activate the template (Figure 1). The template of chromatin incubated with NAD^+ was markedly suppressed compared to that of untreated chromatin (Figure 1). This finding is in agreement with the result obtained with rat liver nuclei[4]. However, the template activity of immature rat testis chromatin incubated with NAD^+ was not suppressed (Figure 1). The basis for the observed differences in template activity between adult and immature rat testis chromatin treated with NAD^+ is not known.

Since it was demonstrated that Ca^{2+}, Mg^{2+}-dependent endonuclease can activate the template of rat liver nuclei for DNA synthesis in vitro[4], the influence of poly(ADP-Rib) on endonuclease activities of rat testis nuclei was investigated. The isolated adult rat testis nuclei possessed high acid and alkaline endonuclease activities (Figure 2). Preincubation of the isolated testis nuclei with NAD^+ affected an inhibition of the Ca^{2+}, Mg^{2+}-dependent endonuclease activity (Figure 2). The finding is in agreement with the result obtained with rat liver nuclei[7,11]. In contrast, preincubation of isolated adult rat testis nuclei with NAD^+ resulted in a stimulation of Mg^{2+}-dependent acid endonuclease activity (Figure 2).

To establish that the observed stimulation of the acid endonuclease activity by NAD^+ treatment was due to poly(ADP-Rib) formation and not to a direct effect of the nucleotide on the enzymic activity, other nucleotides were tested for their capacity to influence endonuclease activity (Table II). Only NAD^+ which formed poly(ADP-Rib) was stimulatory while nucleotides which were not substrates for poly(ADP-Rib) synthetase did not influence acid endonuclease activity (Table II). The present results suggest that the stimulatory effect of NAD^+ is dependent upon its conversion to poly(ADP-Rib).

In a previous study it was demonstrated that the DNA polymerase bound to rat liver chromatin was easily dissociated on incubation of chromatin with NAD^+[5]. Although the molecular mechanism of the interaction of enzymes with chromatin is not known, poly(ADP-Rib) formation might alter the structure and/or electrostatic charge of chromatin and nuclei and influence the interaction of acid endonuclease and DNA polymerase with nucleoproteins. In a previous study it was demonstrated that ADP-ribosylation of Mg^{2+}-dependent endonuclease of rat liver did not occur, suggesting that the effect of poly(ADP-Rib) formation on the enzyme is probably indirect[11].

Zusammenfassung. Inkubation von isolierten Rattenhodenzellkernen mit Nikotinamidadenindinukleotid induzierte die Bildung von Polyadenosindiphosphatribose, eine Hemmung der Ca^{2+}, Mg^{2+}-abhängigen, alkalischen Endonuklease und paradoxerweise eine Stimulation der Mg^{2+}-abhängigen, sauren Endonuklease.

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Glucose Consumption by Early and Late-Passage Diploid Human Fibroblasts During Growth and Stationary Phase

Cultured human fibroblasts are now used extensively to study hereditary disorders[1,2] and since these cells have a finite replicative capacity, they are also useful for research on biological aging[3–7]. Earlier work on carbohydrate metabolism has shown that while human fibroblasts can utilize a variety of hexoses, they have a preference for glucose[8] which is degraded predominantly to lactate[9,10] and CO_2, the latter occurring mainly via the pentose pathway[11,12]. In previous studies on glucose oxidation[11] we were unable to find differences between normal strains at early-passage and those derived from individuals with diabetes mellitus, an age-dependent disorder of carbohydrate metabolism[13]. However, since diabetic cultures have a decreased growth capacity[4,14–16]