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care se bazează pe:

A. **Nota de constatare şi confirmare a indiciilor de plagiat** prin fişa suspiciunii inclusă în decizie.

Fişa suspiciunii de plagiat / Sheet of plagiarism's suspicion	
Opera suspicionată (OS)	**Opera autentică (OA)**
Suspicious work	**Authentic work**
OS	ARDELEAN, A.; PRIBAC, G.C.; BODO, E.; CZAPAR, M.; MOS, L. and COTORACI, C. Protective effects of mild and moderate hypothermia on cultured neuroblastoma-glioma hybrid NG108-15 cells. *Annals of RSCB*, 2009, **14**(1), p.45-50.
OA	SHIBANO, T.; MORIMOTO, Y.; KEMMOTSU, O.; SHIKAMA, H.; HISANO, K. and HUA, Y. Effects of mild and moderate hypothermia on apoptosis in neuronal PC12 cells, *British Journal of Anaesthesia. 2002.* **89**(2). p.301-305.
Incidenţa minimă a suspiciunii / Minimum incidence of suspicion	
p.45:a05 - p.45.a08	p.301:a2 – p.301:a6
p.45:a09 - p.45.a14	p.301:a10 - p.301:a12
p.45:01s – p.46:09s	p.301:05s - p.301:08s; p.301:03d – p.302:16s
p.47:Fig.1	p.303:Fig.1
p.48:Fig.2	p.303:Fig.2
p.48:Fig.3	p.304:Fig.3
Fişa întocmită pentru includerea suspiciunii în Indexul Operelor Plagiate în România de la Sheet drawn up for including the suspicion in the Index of Plagiarized Works in Romania at www.plagiate.ro	

Notă: Prin „p.72:00" se înţelege paragraful care se termină la finele pag.72. Notaţia „p.00:00" semnifică până la ultima pagină a capitolului curent, în întregime de la punctul iniţial al preluării.

Note: By „p.72:00" one understands the text ending with the end of the page 72. By „p.00:00" one understands the taking over from the initial point till the last page of the current chapter, entirely.

B. **Fişa de argumentare a calificării** de plagiat alăturată, fişă care la rândul său este parte a deciziei.

Echipa Indexului Operelor Plagiate în România

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Fişa de argumentare a calificării

Nr. crt.	Descrierea situaţiei care este încadrată drept plagiat	Se confirmă
1.	Preluarea identică a unor pasaje (piese de creaţie de tip text) dintr-o operă autentică publicată, fără precizarea întinderii şi menţionarea provenienţei şi însuşirea acestora într-o lucrare ulterioară celei autentice.	✓
2.	Preluarea a unor pasaje (piese de creaţie de tip text) dintr-o operă autentică publicată, care sunt rezumate ale unor opere anterioare operei autentice, fără precizarea întinderii şi menţionarea provenienţei şi însuşirea acestora într-o lucrare ulterioară celei autentice.	
3.	Preluarea identică a unor figuri (piese de creaţie de tip grafic) dintr-o operă autentică publicată, fără menţionarea provenienţei şi însuşirea acestora într-o lucrare ulterioară celei autentice.	✓
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5.	Republicarea unei opere anterioare publicate, prin includerea unui nou autor sau de noi autori fără contribuţie explicită în lista de autori	
6.	Republicarea unei opere anterioare publicate, prin excluderea unui autor sau a unor autori din lista iniţială de autori.	
7.	Preluarea identică de pasaje (piese de creaţie) dintr-o operă autentică publicată, fără precizarea întinderii şi menţionarea provenienţei, fără nici o intervenţie personală care să justifice exemplificarea sau critica prin aportul creator al autorului care preia şi însuşirea acestora într-o lucrare ulterioară celei autentice.	✓
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10.	Preluarea identică a unor fragmente de demonstraţie sau de deducere a unor relaţii matematice care nu se justifică în regăsirea unei relaţii matematice finale necesare aplicării efective dintr-o operă autentică publicată, fără menţionarea provenienţei, fără nici o intervenţie care să justifice exemplificarea sau critica prin aportul creator al autorului care preia şi însuşirea acestora într-o lucrare ulterioară celei autentice.	
11.	Preluarea identică a textului (piese de creaţie de tip text) unei lucrări publicate anterior sau simultan, cu acelaşi titlu sau cu titlu similar, de un acelaşi autor / un acelaşi grup de autori în publicaţii sau edituri diferite.	
12.	Preluarea identică de pasaje (piese de creaţie de tip text) ale unui cuvânt înainte sau ale unei prefeţe care se referă la două opere, diferite, publicate în două momente diferite de timp.	

Notă:

a) Prin „provenienţă" se înţelege informaţia din care se pot identifica cel puţin numele autorului / autorilor, titlul operei, anul apariţiei.

b) Plagiatul este definit prin textul legii[1].

> *„ …plagiatul – expunerea într-o operă scrisă sau o comunicare orală, inclusiv în format electronic, a unor texte, idei, demonstraţii, date, ipoteze, teorii, rezultate ori metode ştiinţifice extrase din opere scrise, inclusiv în format electronic, ale altor autori, fără a menţiona acest lucru şi fără a face trimitere la operele originale…".*

Tehnic, plagiatul are la bază conceptul de **piesă de creaţie** care[2]:

> *„…este un element de comunicare prezentat în formă scrisă, ca text, imagine sau combinat, care posedă un subiect, o organizare sau o construcţie logică şi de argumentare care presupune nişte premise, un raţionament şi o concluzie. Piesa de creaţie presupune în mod necesar o formă de exprimare specifică unei persoane. Piesa de creaţie se poate asocia cu întreaga operă autentică sau cu o parte a acesteia…"*

cu care se poate face identificarea operei plagiate sau suspicionate de plagiat[3]:

> *„…O operă de creaţie se găseşte în poziţia de operă plagiată sau operă suspicionată de plagiat în raport cu o altă operă considerată autentică dacă:*
> *i) Cele două opere tratează acelaşi subiect sau subiecte înrudite.*
> *ii) Opera autentică a fost făcută publică anterior operei suspicionate.*
> *iii) Cele două opere conţin piese de creaţie identificabile comune care posedă, fiecare în parte, un subiect şi o formă de prezentare bine definită.*
> *iv) Pentru piesele de creaţie comune, adică prezente în opera autentică şi în opera suspicionată, nu există o menţionare explicită a provenienţei. Menţionarea provenienţei se face printr-o citare care permite identificarea piesei de creaţie preluate din opera autentică.*
> *v) Simpla menţionare a titlului unei opere autentice într-un capitol de bibliografie sau similar acestuia fără delimitarea întinderii preluării nu este de natură să evite punerea în discuţie a suspiciunii de plagiat.*
> *vi) Piesele de creaţie preluate din opera autentică se utilizează la construcţii realizate prin juxtapunere fără ca acestea să fie tratate de autorul operei suspicionate prin poziţia sa explicită.*
> *vii) In opera suspicionată se identifică un fir sau mai multe fire logice de argumentare şi tratare care leagă aceleaşi premise cu aceleaşi concluzii ca în opera autentică…"*

[1] Legea nr. 206/2004 privind buna conduită în cercetarea ştiinţifică, dezvoltarea tehnologică şi inovare, publicată în Monitorul Oficial al României, Partea I, nr. 505 din 4 iunie 2004
[2] ISOC, D. *Ghid de acţiune împotriva plagiatului: bună-conduită, prevenire, combatere*. Cluj-Napoca: Ecou Transilvan, 2012.
[3] ISOC, D. *Prevenitor de plagiat*. Cluj-Napoca: Ecou Transilvan, 2014.

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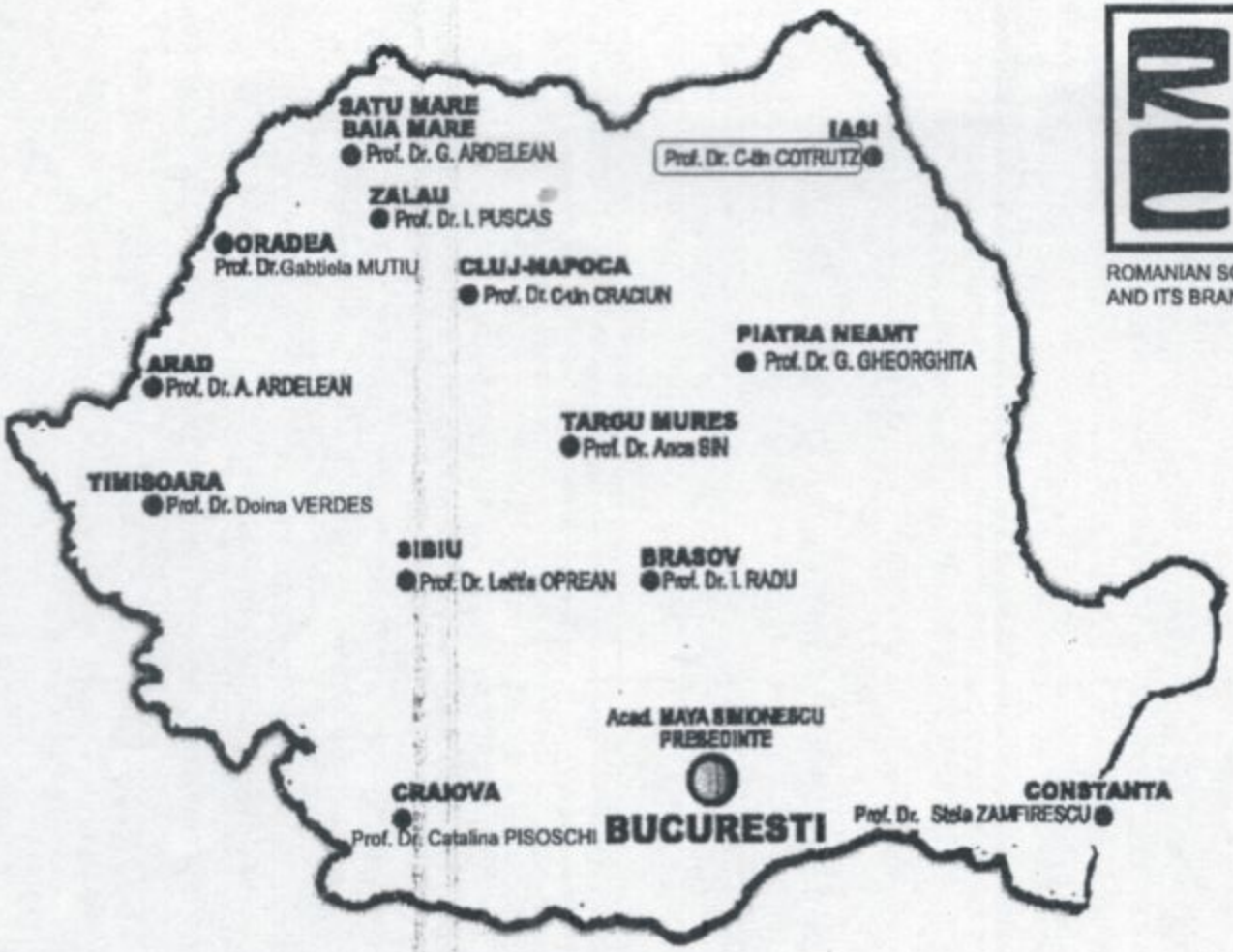

ROMANIAN SOCIETY FOR CELL BIOLOGY
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PROTECTIVE EFFECTS OF MILD AND MODERATE HYPOTHERMIA ON CULTURED NEUROBLASTOMA-GLIOMA HYBRID NG108-15 CELLS

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Summary

We have evaluated if mild hypothermia could have a significant protection against cerebral ischemia by its inhibition on post-ischemic apoptosis. Recent studies on cultured neuroblastoma-glioma hybrid NG108-15 cells used as a neuronal apoptotic model were examined on direct effects of mild hypothermia. Nevertheless, the mechanism of cerebral protection isn't completely known. We hypothesized that mild hypothermia may be protective against cerebral ischemia by inhibiting post-ischemia apoptosis. In this study, we used serum-deprived NG108-15 cells as the neuronal apoptotic model and examined the direct effects of mild and moderate hypothermia. Apoptosis was induced by depriving the cell culture medium of serum, which is one of the most representative methods to induce apoptosis. Cytotoxicity and the percentage of apoptotic cells were evaluated 3 days after induction of apoptosis. With induction at 37°C, cytotoxicity and the percentage of apoptotic cells were over 50 and 80%, respectively. At each temperature examined below 35°C, significant decreases in cytotoxicity and the percentage of apoptotic cells were observed. In the control group, cytotoxicity and the percentage of apoptotic cells were significantly higher at 29°C than at 37°C. We observed that mild and moderate hypothermia inhibited apoptosis.

Key words: hypothermia, apoptosis, NG108-15 cells, cytotoxicity

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Introduction

Studies after 1950s shown that a small reduction of intra-ischemic rat cerebral temperature (from 36°C to 34°C) markedly attenuated ischemic cell damage over 20 min in the four-vessel occlusion model. Even so, the mechanism of cerebral protection by mild hypothermia is still unclear. It is not wholly attributable to metabolic inhibition, because a temperature reduction of 1-2°C decreases the cerebral metabolic rate of oxygen consumption by only 7-14% (Wass et al., 1995). In fact, a group of researchers also reported that cerebral energy metabolites such as ATP were depleted to a similar degree at 33, 34 and 37°C at the end of 20 min of four vessel occlusion (Busto et al., 1987).

It was reported that apoptosis was detected following focal cerebral ischemia, especially in the penumbral region. [3] It has also been reported that delayed neuronal death following global ischemia may be partly attributable to apoptosis (Ardelean A. et al. 2007; MacManus et al. 1999). It is speculated that ischemic cell death is partly due to apoptosis in addition to necrosis. Necrosis occurs due to intracellular energy depletion, after which metabolic depression may inhibit necrosis. Apoptosis occurs due to activation of intracellular cascades.

Therefore, mild hypothermia may protect against cerebral ischemia by inhibiting the intracellular apoptotic cascades activated by ischemia. In this study, we used serum-deprived cultured neuroblastoma-glioma hybrid NG108-15 cells as the neuronal apoptotic model and examined the direct effects of mild and moderate hypothermia on apoptosis.

Other studies were used to treat transitory cerebral hypoxia-ischemia (HI) in newborn animals that appears is as a result of difficulties during labor or during heart attack. Perinatal HI in its most severe form could cause defects of permanent neuronal development that includes spastic tetraparesis, severe cognitive deficiencies and epilepsy (Nelson and Ellenberg, 1981). Advanced methods of obstetrical and neonatal care haven't significantly modified the results of HI encephalopathy (Nelson and Ellenberg, 1988). The treatment of newborn with HI remains symptomatic in spite of the efforts of several researchers to evaluate the neuroprotective interventions (Vannucci and Perlman, 1997). In revived adults after heart attack, mild hypothermia for 12-24 hours is one of the few interventions with neurologic benefits (Bernard et al. 2002). In a study of newborn with cerebral disorders, there are recent encouraging results (Battin et al. 2001).

Material and methods

Cell culture and induction of apoptosis NG108-15 cells were originally derived from a hybrid of neuroblastoma and glioma cells. NG108-15 cells stop dividing and terminally differentiate when treated with nerve growth factor. This makes NG108-15 cells useful as a model system for neuronal differentiation. Cells having undergone up to 11 passages from the original cell line were used in the experiments. NG108-15 cells were maintained on collagen coated dishes in DMEM medium, supplemented with 5% heat-inactivated fetal bovine serum, at 37°C in a 100% humidified atmosphere

Preliminary data of this study reports the safety of mild hypothermia use on affected newborns (Gunn et al. 1998).

In some studies, asphyxic cardiac arrest was chosen as a simulation model of neonatal HI. This model of systemic hypoxia followed by asphyxic cardiac arrest is well physiologically and patologically characterized (Martin et al. 1997; Barkovich et al. 1995), and the model of cerebral lesion is very similar to newborn HI encephalopathy (Barkovich et al. 1995; Low et al. 1989). The mentioned study was focused on the striatum, this being the most vulnerable region and because cell death is the most rapid in these regions, thus requiring a fast and early intervention. The experiment was designed to determine whether whole body hypothermia after HI ameliorates neuronal cell death before rewarming in the putamen and caudate nucleus at 24 h, at which time striatal injury is near maximal (Marint et al. 2000; Brambrink et al. 1999). The hypotheses tested were that sedated piglets subjected to 24 h of whole body hypothermia (34°C) after asphyxic cardiac arrest demonstrate: decreased acute neuronal necrosis in striatum at 24 h in the absence of rewarming, sustained neuroprotection at 7 days of recovery, and improved functional recovery.

containing carbon dioxide and air (5/95). One of the most representative methods of inducing only apoptosis in NG108-15 cells is done by depriving the medium of serum. In previous studies hase been proved that apoptosis, but not necrosis, is induced in NG108-15 cells by serum deprivation using DNA electrophoresis and fluorescence microscopic analyses (Morimoto et al. 2000).

Cytotoxicity evaluation

After two days of normal culture at 37°C, apoptosis was induced in phenol-red-free DMEM without serum. The cells were randomly incubated in chambers, in which the temperature was maintained at 37, 35 or

33°C (mild hypothermia), or 37, 31 or 29°C (moderate hypothermia), in an atmosphere containing carbon dioxide and air 5/95. The temperature of each chamber was accurately controlled. As a control, cells in which apoptosis was not induced were incubated under the same conditions.

Cell death can be assayed by quantifying plasma membrane damage or rupture. The LDH Cytotoxicity Detection Kit offers a simple way to measure plasma membrane damage, based on the release of lactate dehydrogenase (LDH), a stable cytoplasmic enzyme present in most cells. Culture supernatants were collected after the cells were sedimented by centrifugation (cooling centrifuge Sigma 2-16K) and were incubated with a LDH reaction mixture using a cytotoxicity detection kit (Rocher Diagnostics Corporation). At 490 nm were measured changes in absorbance with a Microplate Reader Benchmark (Bio-Rad). Change in absorbance of the LDH standard determines the LDH activity of each sample. LDH activity of the cells was also measured after cells were lysed in 1% Triton X-100. Cytotoxicity (%) can be defined using the following formula:

$$\text{Citotoxicity (\%)} = \frac{\text{LDH leakage activity}}{\text{LDH leakage activity + LDH activity within cells}}$$

Apoptotic cells under hypothermia

After two days of normal culture at 37°C, apoptosis was induced in phenol-red-free DMEM without serum. Cells were randomly placed into mild or moderate hypothermia as described previously. The control, non-induced cells were incubated under the same conditions.

Cells were mixed in 70% ethanol following harvest. Fixed cells were incubated with 0.4 mg/ml RNase A (Sigma) and stained with propidium iodide (Sigma). Cellular DNA content was measured by flow cytometry (FACS Calibur; Becton Dickinson). Calculation of the percentage of apoptotic cells was based on the frequency curves of DNA histograms. Apoptotic cells were regarded as the population that contained less DNA than at the G1 peak (Fig. 1).

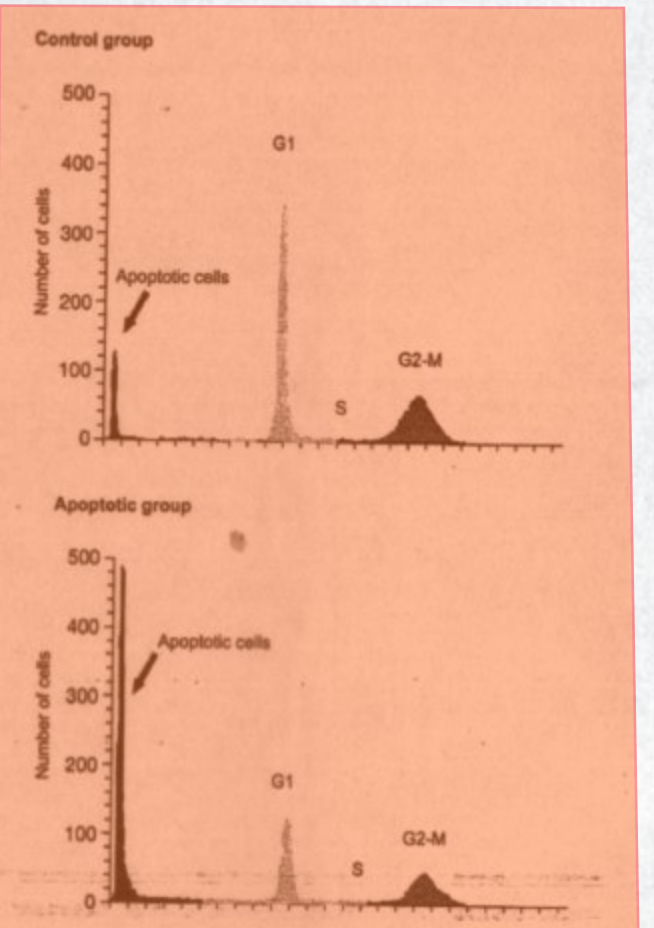

Fig. 1 DNA Histograms. Control group: harvested after 3 days culture in normal medium. Apoptosis group: harvested 3 days after serum deprivation. G1, S and G2/M represent each phase of the cell cycle.

Statistics

To compare cytotoxicity and the percentage of apoptotic cells, was used the ANOVA one-way analyses. Statistical significance was used when $P<0.05$ and values were expressed as mean.

Results and discussion

Cytotoxicity in apoptosis group (Fig. 2), at 37°C was > 55%, 3 days after induction. At each temperature below 35°C, cytotoxicity decreased significantly ($P < 0.01$) compared with 37°C. In control group, we performed some experiments in the mild and moderate hypothermia groups. Cytotoxicity at 37, 35, 33 and 31°C was approximately 9-10%. At 29°C, cytotoxicity was 16.5 (5.4)% and was significantly higher than that at 37°C ($P=0.02$). The percentage of apoptotic cells, in apoptosis group (Fig. 3), at 37°C was >80%. At each temperature below 35°C, this was significantly decreased compared with 37°C ($P<0.01$). At 29°C, this value decreased to 36.0 (7.1%).

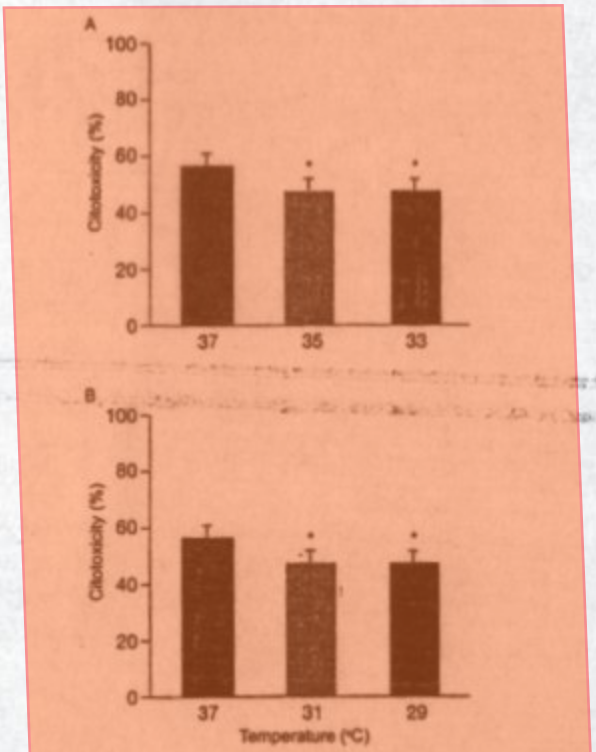

Fig. 2 Hypothermia effect on cytotoxicity (apoptosis group). Mean values indicate cytotoxicity (LDH leakage) in the mild (A) and moderate (B) hypothermia groups. There is a significant difference between mild and moderate hypothermia ($P<0.01$).

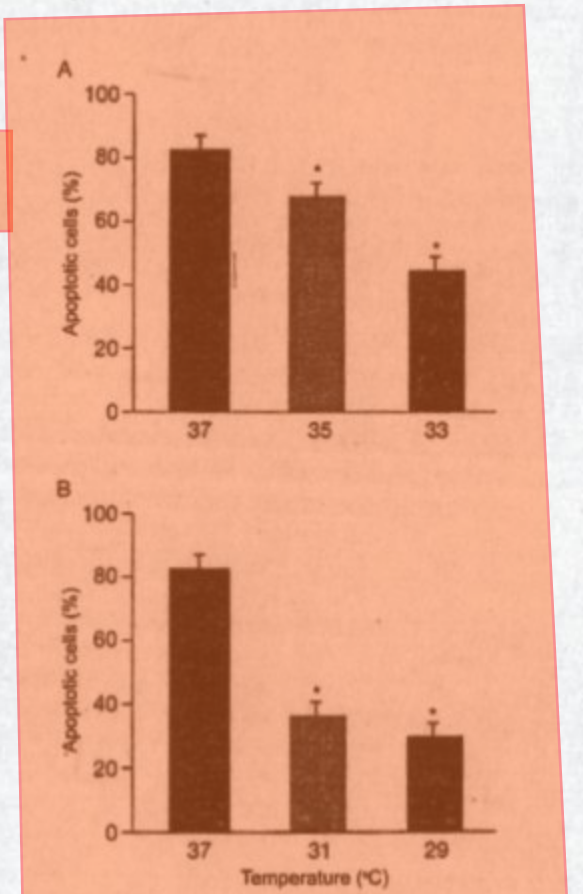

Fig. 3 Hypothermia effect on apoptotic cells (apoptosis group). Mean values indicate percentage of apoptotic cells in mild (A) and moderate (B) hypothermia. There is a significant difference between mild and moderate hypothermia ($P<0.01$).

Percentage of apoptotic cells: control group

In control group, in order to assay the percentage of apoptotic cells we performed a number of experiments in mild and moderate hypothermia. At 37, 35, 33 and 31°C, the percentage of apoptotic cells was less than 10%. This value was 11.2 (3.9%) at 29°C and was significantly higher than that at 37°C ($P<0.01$).