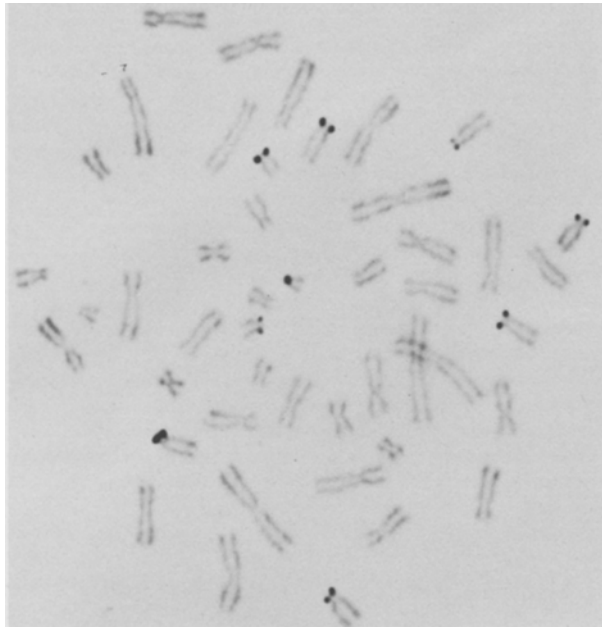

It is obvious that the silver method described here produces quality stained metaphases. The method saves time and money, but more importantly, the procedure has been standardized for the first time. The method should have practical application in laboratories studying nucleolus organizer regions on animal and plant chromosomes.

1 This research was supported by the National Science Foundation, grant Nos PCM-76-82828 and DEB-76-84195 to WMH.
2 W.M. Howell, T.E. Denton and J.R. Diamond, Experientia *31*, 260 (1975).
3 C. Goodpasture and S.E. Bloom, Chromosoma *53*, 37 (1975).
4 S.E. Bloom and C. Goodpasture, Hum. Genet. *34*, 199 (1976).
5 T.E. Denton, W.M. Howell and J.V. Barrett, Chromosoma *55*, 81 (1976).
6 M. Münke and H. Schmiady, Experientia *35*, 602 (1979).
7 D. Schweizer and P. Ambros, Pl. Syst. Evol. *132*, 27 (1979).
8 W.M. Howell and D.A. Black, Hum. Genet. *43*, 53 (1978).
9 R. Liesegang, Kolloidchem. Beih. *3*, 1 (1911).
10 G.W. Bartelmez and N.L. Hoerr, J. comp. Neurol. *57*, 401 (1933).
11 M.L. Silver, Anat. Rec. *82*, 507 (1942).

Fig. 1. Silver-stained human metaphase chromosomes from lymphocyte culture. Ag-NORs appear as black dots above the centromere in 9 of the acrocentric chromosomes.

Effects of anesthesia, surgical manipulation and dehydration on the nucleic acid and protein content of the pituitary and hypothalamus of the frog[1]

L. Iela, M. Di Meglio and R.K. Rastogi

Institute and Museum of Zoology, Faculty of Science, via Mezzocannone 8, I-80134 Naples (Italy), 5 November 1979

Summary. Various types of stress, chemical or surgical, have a negative influence on the protein and RNA content of the hypothalamo-hypophyseal system of *Rana esculenta*. A degree of recovery occurs in these tissues 24 h after MS222 anaesthesia and laparatomy. Decapitation is apparently the most suitable method of sacrificing the animals.

It has been shown that in *Rana esculenta* gonadal activity is integrated with the environment via a neuroendocrine (hypothalamus-pituitary route) link. The hypothalamus and pituitary manifest a seasonal (annual) pattern of metabolic and cytological changes[2,3]. In addition there is also evidence that the hypothalamus controls hypophyseal activity[4-6] and this fact strengthens the idea that these 2 organs exhibit almost concomitant changes.

A vast body of researchers in the filed of neuronal regulation of food and water uptake, temperature regulatory mechanisms and reproductive endocrinology of amphibia practice different killing procedures, surgical manipulation and anesthesia. But so far the problem of the influence of chemical and surgical stimuli and stress on nucleic acid and protein metabolism in the hypothalamo-hypophyseal system of amphibians has been largely ignored. This study was made in order to analyze the effects of different modes of sacrifice, anesthesia, surgical stress and dehydration on the nucleic acid and protein content of the hypothalamus and pars distalis of the pituitary of the green frog, *Rana esculenta*.

Adult male frogs were collected in February. The following treatments were carried out: A. decapitation, B. killing by 0.1% MS222 (m-aminobenzoic acid ethyl ester), C. killing by decapitation 24 h after 0.1% MS222 anesthesia (frogs recover from this anesthesia within approximately 2 h), D. killing by chloroform, E. laparatomy under MS222 anesthesia and killing by decapitation 2 h after awakening, F. laparatomy and killing after 24 h, and G. dehydration for 24 h and killing by decapitation. For the determinations, tissues (pars distalis of the pituitary and hypothalamus) from 5 animals were pooled. Each sample was homogenized in 5 ml cold acetate buffer (pH 5) and analyzed in duplicate for protein[7], DNA[8] and RNA (modification of Scherrer and Darnell's[9] method). 7 tissue pools for groups A, B and C, and 8 tissue pools for groups D, E, F and G were prepared. Results were analyzed for significance using Student's t-test[10].

Results are summarized in the table. Killing by decapitation, because of its rapidity, conserved the maximal quantity of RNA and protein, and the ratios RNA:DNA and protein:DNA in the pars distalis and hypothalamus. These values were significantly higher than those obtained from animals killed under chloroform and MS222 anesthesia and those subjected to dehydration. This last group showed the lowest levels of RNA and protein content, and RNA:DNA and protein:DNA ratios in both tissues. Laparatomy under MS222 anesthesia induced a greater reduction in the macromolecular content of the hypothalamo-hypophyseal system when compared with frogs killed with MS222. However, a great degree of recovery was found to occur in frogs sacrificed 24 h after MS222 anesthesia and those subjected to laparatomy. DNA concentration in the hypothalamus and pars distalis was of the same magnitude in all the experimental groups.

It is well known that the organism responds rapidly to stimuli that could disturb the homeostatic equilibrium with an integrated neuroendocrine reaction and that the hypothalamus represents the controlling channel for this response to any kind of stress. In mammals the hypothalamic neuronal firing rates have been found to differ under Urethane and Brietal anesthesia[11]. In addition to this, the hypothalamus contains abundant noradrenaline, dopamine and catecholamine nerve terminals which have important

Influence of various types of stress and decapitation on the macromolecular content of the hypothalamus and pituitary of *Rana esculenta*

Treatment	RNA (μg/mg wet tissue)	Protein (μg/mg wet tissue)	DNA (μg/mg wet tissue)	RNA:DNA ratio	Protein:DNA ratio
Hypothalamus					
A. Decapitation	1.34±0.04	307±8	6.56±0.94	0.20±0.02	46.9±3.3
B. MS 222	1.06±0.02*	262±9**	6.58±0.86	0.15±0.01**	39.1±2.3**
C. MS 222 (24 h)	1.26±0.07	298±5	6.43±0.75	0.18±0.01	46.1±1.6
D. Chloroform	0.96±0.04*	218±7**	6.09±1.03	0.14±0.01**	35.8±3.1**
E. Laparatomy (2 h)	1.01±0.01*	249±11**	6.16±0.37	0.16±0.01**	39.8±1.4**
F. Laparatomy (24 h)	1.19±0.04	288±5	6.20±0.59	0.19±0.02	46.2±2.6
G. Dehydration	0.68±0.03*	108±9*	5.98±1.06	0.11±0.01*	18.1±2.8*
Pars distalis					
A. Decapitation	3.36±0.08	583±19	12.5±0.17	0.27±0.03	47.0±2.2
B. MS 222	2.96±0.06**	448±9*	13.0±1.06	0.22±0.03	34.6±1.7*
C. MS 222 (24 h)	3.18±0.11	531±12	12.6±0.35	0.25±0.02	42.1±2.9
D. Chloroform	2.81±0.13*	412±9*	12.0±0.28	0.23±0.03	34.3±1.6*
E. Laparatomy (2 h)	2.73±0.05*	426±7*	12.5±0.28	0.21±0.03**	34.0±1.1*
F. Laparatomy (24 h)	2.91±0.11**	477±13**	12.3±0.36	0.23±0.01	38.4±1.2**
G. Dehydration	2.09±0.08*	380±20*	13.0±0.68	0.16±0.05*	29.2±2.4*

* Significantly different from group A ($p < 0.01$); ** significantly different from group A ($< 0.02 < p < 0.05$).

neuroendocrine functions. A particular effect of various types of stress is shown in the noradrenaline levels of nerve terminals due to increased noradrenaline-neuron activity which is usually accompanied by an increase in ACTH secretion from the anterior pituitary gland[12]. In fact it is known that one of the central phenomena of the integrated response to stimuli is the hyperactivity of the adrenal cortex. In the rat, Choudhury[13] described an increased synthetic activity in the neurosecretory hypothalamic nuclei after surgical stress. Our results indicate that in the frog, chemical stimuli, surgical stress and dehydration have a notable influence on the hypothalamohypophyseal system and we suggest that killing by decapitation should be the method of choice, due to its extremely short duration.

1 Work supported by the National Research Council of Italy.

2 R.K. Rastogi and G. Chieffi, Z. Zellforsch. mikrosk. Anat. *111*, 505 (1970).
3 R.K. Rastogi and G. Chieffi, J. Endocr. *58*, 129 (1973).
4 C.B. Jørgensen, in: The hypothalamus, p.649. Ed. L. Martini, M. Motta and F. Fraschini. Academic Press, New York 1970.
5 R.K. Rastogi and G. Chieffi, J. exp. Zool. *181*, 263 (1972).
6 R.K. Rastogi, Boll. Zool. *43*, 151 (1976).
7 O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, J. biol. Chem. *193*, 265 (1951).
8 Z. Dische, in: The nucleic acids, vol. 1, p.285. Ad. E. Chargaff and J.N. Davidson. Academic Press, New York 1955.
9 K. Scherrer and J.E. Darnell, Biochem. biophys. Res. Commun. *7*, 486 (1962).
10 R.A. Fisher, in: Statistical methods for research workers, 12th edn. Oliver and Boyd, London 1954.
11 B.A. Cross and R.G. Dyer, in: The hypothalamus, p.115. Ed. L. Martini, M. Motta and F. Fraschini. Academic Press, New York 1970.
12 K. Fuxe and T. Hökfelt, in: The hypothalamus, p.123. Ed. L. Martini, M. Motta and F. Fraschini. Academic Press, New York 1970.
13 S.R. Choudhury, Experientia *28*, 183 (1972).

Instructions to authors

Experientia is published on the 15th of every month and can be obtained in any country through booksellers or from the publishers. All communications to the editors should be addressed to the publishers. All manuscripts for publication in a given number must be in the hands of the editors 3 months before publication.

Articles of general scientific interest, of interdisciplinary character: briefly stated and hitherto unpublished original reports of sufficient novelty value. In no case will papers of preliminary nature be accepted.

Experiments Papers in which animal experiments have been conducted without using the appropriate anaesthesia will not be accepted.

Manuscripts (including all figures and tables) should be submitted in duplicate.

Text should not exceed 2–3 typewritten pages (50–60 lines). 1–2 relevant figures or tables. English summary of maximum 4 lines. Abbreviations should be properly explained. References should be numbered consecutively and be presented on a separate page. Name and address have to be placed directly under the title. Linguistically inadequate manuscripts will be returned. Manuscripts in languages other than English should be supplemented by an English translation of the title. Footnotes should be avoided.

Figures Illustrations should be separate from the text, with the author's name on the back in soft pencil. The desired labelling should be shown on a second set of figures, which will be used as a model for inscriptions. Drawings for reproductions should be on good paper in Indian ink, photographs should be supplied as glossy positive prints. The illustrations should be at least one and a half times as large as the definitive size desired. Over-large figures can be easily damaged in the mail. Captions should be selfexplanatory, without reference to the text.

Tables should be provided with a title and with selfexplanatory captions.

Headings In submitting their manuscript to Experientia, authors are requested to indicate one of the headings mentioned below, under which they would wish to place their short communication:
1 Mathematics and Physics; 2. Cosmology, Astronautics, Cosmonautics; 3. Mineralogy, Geophysics, Oceanography; 4. Inorganic and Physical Chemistry; 5. Organic Chemistry; 6. Biophysics; 7. Molecular Biology, Cellular Biology; 8. Genetics; 9. Botany; 10. Zoology; 11. Ecology; 12. Biochemistry (analytic and synthetic); 13. Biochemistry (Enzymes, Metabolism); 14. Physiology; 15. Neurology; 16. Pharmacology, Toxicology, Pathology; 17. Experimental Gerontology; 18. Anatomy, Histology, Cytology, Histochemistry; 19. Embryology; 20. Endocrinology; 21. Circulation, Cardiology, Angiology; 22. Nutrition, Gastroenterology; 23. Hematology, Serology; 24. Immunology, Allergy; 25. Microbiology, Parasitology, Chemical Therapeutics; 26. Oncology, Carcinology, Cytostatics; 27. Radiology.

Reprints The authors receive 50 reprints, without cover, free of charge. Price-list for further reprints is available.