observation of this type of intranuclear inclusions in the neurons of the retrochiasmatic area of any mammalian, and also the first report of NR in the hypothalamus of the rat.

Methods. The ultrastructure of the NR of the hypothalamic retrochiasmatic area was studied in normal adult Wistar rats of both sexes. The rats were fixed by perfusion with 3% glutaraldehyde and the hypothalamic blocks were post-fixed in 2% osmium tetroxide. Both fixatives were maintained at pH 7.4 with 0.12 M phosphate buffer. The hypothalamic blocks were dehydrated in acetone and embedded in Durcupan (Fluka). Ultrathin sections were stained with 1% aqueous uranyl acetate and then lead citrate, and examined in a Philips EM-201 electron microscope.

Results. The neurons of the retrochiasmatic area display an irregular nucleus with extensive infoldings of the nuclear membrane. The electron-lucid cytoplasm contains mitochondria, free polyribosomes and isolated cisternae of granular endoplasmic reticulum (figure 1).

In some neurons of this area, both in male and female rats, microfilamentous spindle-shaped rodlets are observed. The rodlets have an indeterminate length and they are 0.1–0.3 µm width (figures 1–3). These structures are composed of a bundle of numerous closely packed microfilaments oriented along its log axis (figures 1 and 4). These microfilaments, of about 60–70 Å in diameter, are generally straight. They are arranged in parallel with a centre-to-centre spacing of 140–150 Å (figure 4).

The position of the rodlets within the nucleus is variable, but they are never related to any other nuclear features, such as the nucleolus or the nuclear envelope. They are not surrounded by a narrow-zone free of chromatin granules, and frequently contacts between the rodlets and chromatin granules can be observed (figures 1 and 4).

Discussion. This study shows that NR are present in neurons of the retrochiasmatic area of the hypothalamus of the healthy adults rats. Although in some instances its presence in the central nervous system has been related to pathological processes[10–12], they must be considered as normal nuclear inclusions in the neurons of this area, both in male and female rats. Since NR are widely distributed in various types of neurons, it has been proposed that they should be considered as a normal cellular organelle[6,9,14]. Our report of these structures in an area where they have not been previously observed support this suggestion.

The structure and shape of NR observed in this area correspond to the microfilamentous spindle-shaped inclusions described in other neuronal locations[2–7]. Other types of NR such as microfilamentous-microtubular spindle-shaped or crystalloids[8] have not been found.

The origin of NR has been studied in detail in embryonic material by Masurovsky et al.[5]. In our study, in the adult rat, we found NR of different thickness, suggesting that they are dynamic structures of the neuron formed by progressive aggregation of protein subunits. This observation is in agreement with the results of Seite et al.[14,15]. According to these authors, it could be suggested that their formation is related with the level of neuronal activity. The irregular distribution of these structures we have observed within the neurons of this retrochiasmatic area supports this hypothesis.

Seite et al.[9] have demonstrated recently that the cyclic AMP induces the formation of NR in the neurons of the sympathetic ganglion. They suggest that this effect is a consequence of the increase of the neuronal activity mediated by the modulating effect of the cyclic AMP on the synaptic transmission. The facts that cyclic AMP plays a prominent role in the catecholaminergic transmission[16], and that this kind of transmission is specially important in the retrochiasmatic area we have studied[17], may suggest a relation between the presence of NR and catecholaminergic neurons. The occurrence of NR in other hypothalamic areas rich in catecholaminergic neurons, like arcuate nucleus[7], supports the above hypothesis.

14 R. Seite, N. Mei and J. Vuillet-Luciani, Brain Res. *50*, 419 (1973).
15 R. Seite, N. Mei and S. Couineau, Brain Res. *34*, 277 (1971).
16 P. Greengard, Adv. cyclic nucl. Res. *5*, 585 (1975).
17 M. Hyyppä, Z. Zellforsch. *98*, 550 (1969).

The effect of tolbutamide on early embryos of *Xenopus laevis*

M. Stanisstreet[1]

Department of Zoology, University of Liverpool, P.O. Box 147, Liverpool L69 3BX (England), 28 April 1977

Summary. The effect of tolbutamide on the early development of Xenopus laevis has been studied. The results suggest that continuous exposure to 3×10^{-4} M tolbutamide causes abnormal development.

Tolbutamide, N′-4-methylbenzenesulphonyl-N″-butylurea, is a therapeutic agent useful for the control of diabetic hyperglycemia. Although its side effects in adults are minimal, there is evidence that such hypoglycemic drugs are teratogenic in mammals[2,3]. However, it is not clear whether its teratogenic effects in mammals are due to tolbutamide itself or to the metabolic state of the pregnant female induced by tolbutamide. As part of a series of experiments designed to assess whether sea-urchin embryos could be used as a test system for pharmacological agents, Hagström and Lönning[4] studied the effects of tolbutamide on the gametes and early embryos of Paracentrotus lividus. Although tolbutamide did not affect fertilization, it did have rather specific effects on early development. Embryos exposed to tolbutamide showed inhibition of the formation of the endoderm, a greater number of yolk platelets than controls, and a change in the ultrastructural appearance of the yolk platelets[4]. Any agent which effects specifically one embryonic tissue might prove a powerful tool for the analysis of early differentiation, and it is important

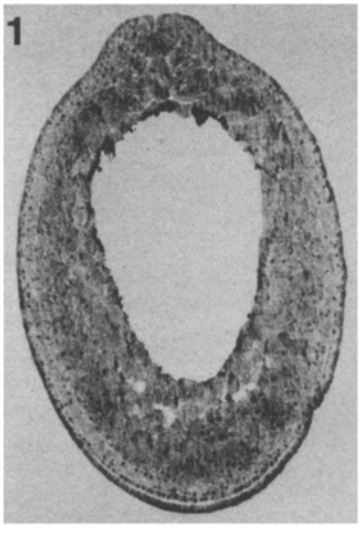
1 Acknowledgments. I wish to thank Mr F. Schaefer who prepared the histological material, and Hoechst U.K. Ltd for their gift of tolbutamide.
2 M. Smithberg and M. N. Runner, Am. J. Anat. *113*, 479 (1963).
3 J. G. Wilson, Teratology *7*, 3 (1973).
4 B. E. Hagström and S. Lönning, Experientia *32*, 744 (1976).

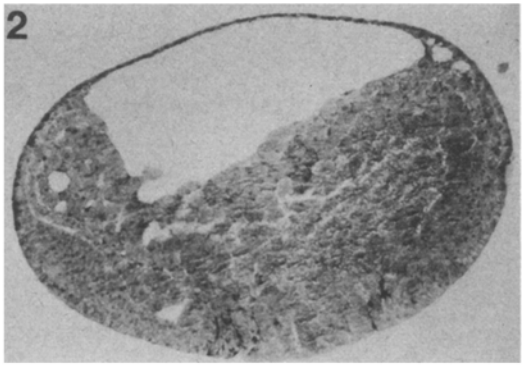
therefore to determine whether tolbutamide has similar effects on other embryos. This paper reports the results of experiments designed to test the effect of tolbutamide on the early embryos of the South African clawed toad, Xenopus laevis.

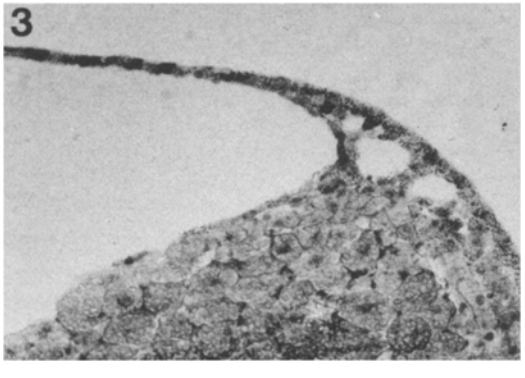
Materials and methods. Embryos of Xenopus laevis were obtained by hormone injection of the adults, and were staged according to Nieuwkoop and Faber[5]. The jelly coats were removed chemically[6] and the embryos were cultured in 10% Steinberg's saline[7] at 20 ± 0.5 °C. Tolbutamide is practically insoluble in water, and so a stock 5×10^{-2} M solution was prepared in ethanol, and dilutions were made by adding different volumes of this stock to 10% Steinberg's saline. Controls contained an equivalent volume of ethanol. Embryos were tested in groups of 10, and each experiment was performed in triplicate using embryos from different batches. Embryos were observed after 3 h, and scored after about 18 h, when the controls had reached the neurula stage. For the purpose of statistical analysis, embryos were classed as normal, abnormal or dead, and each group was compared with its control by χ^2 analysis. 3 types of experiment were performed; to test the effects of continuous exposure to different concentrations of tolbutamide; to test the effects of different times of exposure to one concentration of tolbutamide; and to test the effect of the ages of embryos when first exposed to tolbutamide. Some embryos were fixed in Smith's formol-bichromate fixative[8], sectioned according to routine procedures, and stained with modified Mallory's stain[9].

Results and discussion. Effect of different concentrations. The first experiment was performed to test the effects of continuous exposure of Xenopus early embryos to different concentrations of tolbutamide. Early cleavage stage embryos (stage 5) were exposed to 5×10^{-5} M, 10^{-4} M or 3×10^{-4} M tolbutamide. Judged by external appearance of the embryos, 5×10^{-5} M and 10^{-4} M tolbutamide did not affect early development, and statistical analysis confirmed that the treated embryos did not differ from their controls ($\chi^2 = 0.64$ and 1.68 respectively). At 3×10^{-4} M tolbutamide had a significant teratogenic effect since 77% of the embryos were abnormal ($\chi^2 = 40$, $p < 0.001$). The type of abnormality produced was reasonably consistent: when the controls were early neurulae (figure 1) most of the abnormal embryos resembled midgastrulae (stage 10) except that a large area of the ectoderm opposite the yolk plug appeared translucent. Microdissection showed that this layer was very thin and histological examination showed that it was one cell thick (figures 2 and 3). Gastrulation, judged by the appearance of the lip of the blastopore, appeared to have started normally (figure 4). In a few cases, treated embryos had formed neurulae with similar ectodermal vesicles. Thus at the highest concentration tested, tolbutamide caused abnormal development of Xenopus embryos.

5 P. D. Nieuwkoop and J. Faber, in: A Normal Table of Xenopus laevis Daud. North-Holland Publishers, Amsterdam 1956.
6 I. B. Dawid, J. molec. Biol. *12*, 581 (1965).
7 M. Steinberg, see J. D. Ebert, Carnegie Inst. Wash. Yearbook *56*, 1957.
8 H. F. Steedman, in: Section Cutting in Microscopy. Blackwell, Oxford 1960.
9 J. E. Cason, Stain Technol. *25*, 225 (1950).







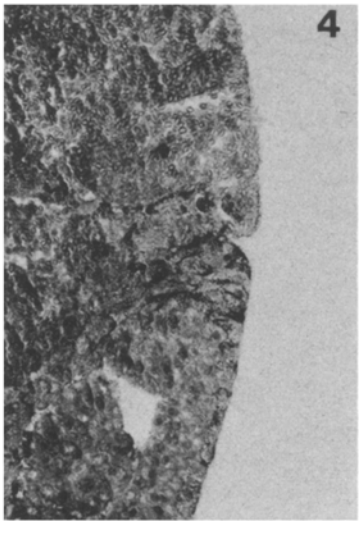


Fig. 1. Transverse section of control neurula of Xenopus laevis.

Fig. 2. Transverse section of Xenopus embryo exposed to 3×10^{-4} M tolbutamide from 16-cell stage (stage 5) showing characteristic abnormality.

Fig. 3. Detail from figure 2 showing ectoderm one cell thick.

Fig. 4. Detail from Figure 2 showing lip of blastopore.

Effect of different times of exposure. Some teratogens produce abnormalities after exposure has ceased[10,11], and in an attempt to analyse whether there is a critical period when embryos were vulnerable to tolbutamide, an experiment was conducted in which embryos were exposed for different lengths of time to a teratogenic concentration of tolbutamide. Early cleavage embryos (stage 5) were exposed to 3×10^{-4} M tolbutamide for 1 h, 3 h or continuously. Following exposure, embryos were washed in 2 changes of 10% Steinberg's saline and cultured in fresh saline. Exposure for 1 h or 3 h had no effect ($\chi^2 = 2.02$ and $\chi^2 = 2.02$), but as in the previous experiment, continuous exposure produced abnormal development ($\chi^2 = 37$, $p < 0.001$). The types of abnormalities were the same as those found previously. Thus it would appear that either continuous exposure is required for tolbutamide to be teratogenic, or that the vulnerable period is after the mid- to late blastula stage (3 h after stage 5). Effect of age of embryos. In the last experiment, early cleavage embryos (stage 5), midblastulae (stages 7–8) and late blastulae (stage 9) were exposed continuously to 3×10^{-4} M tolbutamide. When exposure was commenced at stage 5, 77% of the embryos were abnormal ($\chi^2 = 43$, $p < 0.001$); when exposure was commenced at the mid-blastula stage, 73% of the embryos were abnormal ($\chi^2 = 38.5$, $p < 0.001$) and when exposure was commenced at the late blastula stage, 53% of the embryos were abnormal ($\chi^2 = 15.8$, $p < 0.001$). The types of abnormalities found were similar to those found previously, except that there was a tendency for embryos exposed at the blastula stage to form more neurulae with vesicles. These results show that under certain conditions exposure of early embryos of Xenopus laevis to tolbutamide leads to abnormal development. The type of abnormality produced is apparently not analogous to that produced in echinoderm embryos, but it is reasonably consistent. It is not known whether the action of tolbutamide in mammals is due to the drug or to the metabolic state it induces. The present results confirm that tolbutamide itself may be teratogenic[4], and further experiments to analyse its mechanism of action in amphibian embryos are being undertaken.

10 M. Stanisstreet, Experientia *30*, 1081 (1974).
11 J. L. Smith, J. C. Osborn and M. Stanisstreet, J. Embryol. exp. Morph. *36*, 513 (1976).

Dietary restriction and fetal development[1]

B. Durst-Živković

Department of Histology and Embryology, Medical Faculty, Vijenac 4, 41000 Zagreb (Yugoslavia), 4 April 1977

Summary. The effect of deficient nutrition of pregnant Wistar rats on the fetal weight has been studied. It has been established that the fetal weight of the group of rats fed with a restricted amount of stock diet lags behind the fetal weight of the group receiving unrestricted amounts of the same stock diet. The differences in weight between the 2 groups were, on each day of the observation period, significant at the level of $p < 0.05$ and $p < 0.01$, respectively.

Nutrition of the pregnant female certainly plays an important part in fetal development[2]. The effect of restriction of some food components, especially proteins, has been experimentally studied[3]. So far the studies have been mainly directed towards the effect of malnutrition on reproduction[4] and weight of the offspring[5] but little attention has been paid to the consequences of restricted total daily quantity of stock diet. The intention of this study was to gain an insight into the effect of malnutrition on the fetal development of rats. Restricted daily quantity of stock diet was applied only during the gestation.

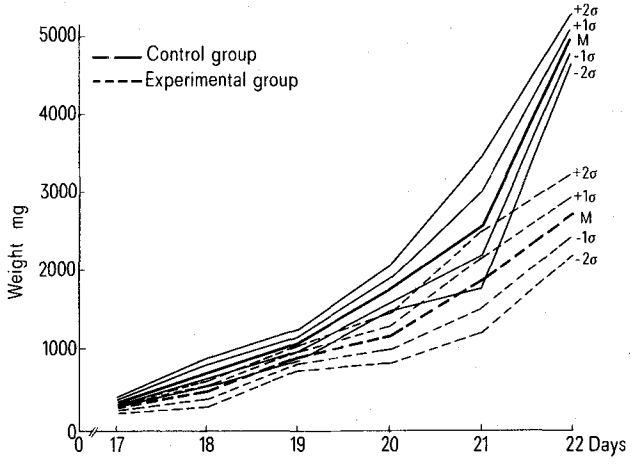


Fig. Mean weight values curves and curves indicating SE in the experimental and the control group.

Material and methods. Experimental ad libitum feeding has shown that a female Wistar rat consumes 12 g of pelleted food, a produce of the pharmaceutical firm 'Pliva', composed of: crude proteins (min. 18%), crude fats (min. 4.5%), crude fibres (max. 3%), Ca (max. 1.10%), P (min. 0.65%), ash (max. 4%), moisture (max. 13%), vitamin A (22,000 IU), vitamin D_3 (2000 IU), Fe (400 mg) and I (0.37 mg) per kg of pelleted food.

Virgin female rats of Wistar strain were mated. The day of insemination was determined by the finding of the sperm in the vaginal smear and was taken as the first day of embryonic development. On that day the females were placed into single cages and were put on the restricted diet consisting of 6 g of the commercially prepared pellets besides unrestricted water consumption. The pregnant femals of the control group continued to receive both

1 This work was supported by a grant of the Republic Research fund of Croatia No. 18-04-06/19-1975.
2 M. Saitoh and S. Takahoshi, J. Nutr. *103*, 1652 (1973).
3 M. M. Nelson and H. M. Evans, J. Nutr. *51*, 71 (1953).
4 B. N. Berg, J. Nutr. *87*, 344 (1965).
5 A. M. Hsueh, C. E. Augustin and B. F. Chow, J. Nutr. *91*, 195 (1967).